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Plenary Lectures

Mechanisms of axon growth and regeneration

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Almost everybody who has seen neurons under a microscope for the first time is fascinated by their beauty and their complex shape. Early on during development, however, neurons look round and simple without signs of their future complexity. How do neurons develop their sophisticated structure? How do they initially generate domains that later have distinct functions within neuronal circuits, such as the axon? And, can a better understanding of the underlying developmental mechanisms help us in pathological conditions, such as a spinal cord injury, to induce axons to regenerate?
Here, I will talk about the cytoskeleton as a driving force for initial neuronal polarization and axon growth. I will then explore how cytoskeletal changes help to reactivate the growth program of injured CNS axons to elicit axon regeneration after a spinal cord injury. Finally, I will discuss whether axon growth and synapse formation could represent mutually excluding processes. Pursuing this developmental hypothesis has helped us to generate a novel perspective on regeneration failure in the adult CNS and to provide new paths to overcome this. Thus, this talk will describe how we can employ developmental mechanisms to induce axon regeneration in the adult after a spinal cord injury.

Unlocking movement: helping paralyzed people with brain-machine interfaces

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Tetraplegia, the loss of movement and feeling in all four limbs, can result from spinal cord injuries at the level of the neck. Brain-machine interfaces (BMIs) can help people with tetraplegia by allowing them to control assistive devices with their thoughts. A BMI consists of tiny electrodes that can record the activity of large numbers of cortical neurons, and machine learning algorithms that can interpret the intent of the participant from the neural activity. Electrical stimulation through electrodes implanted in somatosensory cortex can also restore the sense of touch.

Our lab and collaborating colleagues have used a novel approach of implanting electrodes in a variety of specialized cortical areas rather than just the motor cortex. Using this approach, the participants can control robotics and computers, allowing them to drink a beverage, play a computer piano, use video games and programs like photoshop, drive an automobile, and feel touch to the previously insensate hand and arm. Further, we can decode speech, including silent internal speech, and the actions of others observed by the participants.

For participants in which recording arrays were implanted in both primary motor cortex (MC) and posterior parietal cortex (PPC), we were able to make direct comparisons between the two areas. We find that implants in the hand representation of MC mostly represents attempted movements of the contralateral limb whereas PPC has a highly mixed representation in which movements of all parts of the body can be decoded from a single microelectrode array. MC encodes the execution of current attempted limb movement, whereas PPC encodes both the current movement and the next planned movement of a sequence. These results indicate that recordings from single arrays in PPC are ideally suited for brain-machine interface decoding of movements of the entire body, coordination for multi-effector movements, and sequential movements.
In summary, an approach in which multiple areas of the cortex are implanted with recording arrays allows for a versatile BMI, which can decode motor commands as well as more cognitive and global aspects of motor control.

Odors shape visual categorization in the human brain

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Despite humans being usually considered as overly visual, human vision is actually poor at the beginning of life. In contrast, olfaction is an early-developing sense that promotes adaptive responses and may support the development of visual cognition. This talk will illustrate how the mother's body odor selectively facilitates face categorization in the 4-month-old human brain until the infant's ability to categorize faces improve to become progressively independent of the influence of maternal odor. These findings will be corroborated by studies in adults aligning with the inverse effectiveness principle of multisensory integration. Overall, this body of research highlights the powerful impact of odors on visual categorization, in particular how social chemical cues can drive the categorization of visual inputs in the developing human brain, and suggest that odors bear a disambiguating function following an inverse function of its effectiveness. It underscores a crucial role of multisensory inputs for the acquisition of categories in humans, relying on an ordered sensory development.

Cellular mechanisms of spatial navigation in the human medial temporal lobe

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Spatial navigation is a fundamental cognitive function that relies on neural processes within the medial temporal lobe, including the hippocampus and entorhinal cortex. While extensive research in rodents has identified key neuronal mechanisms underlying spatial coding, such as place cells and grid cells, our understanding of how these mechanisms operate in the human brain remains limited. In this talk, I will present insights from intracranial recordings in human epilepsy patients engaged in virtual spatial navigation tasks. I will discuss how individual neurons in the human medial temporal lobe encode spatial information, including representations of directions and distances. I will also examine how these spatially modulated neurons interact with other types of neurons during hippocampal “ripple” oscillations, which may support the formation and retrieval of complex, associative memories. Combining virtual spatial navigation tasks with intracranial neural recordings in epilepsy patients helps bridge the gap between animal and human navigation research, offering a deeper understanding of the neural basis of spatial cognition.

How interoception shapes cognition

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Interoception refers to how the brain constantly monitors bodily signals such as for instance blood pulsating in vessels. Spontaneous neural activity, as measured during resting state, is partially accounted for by interoceptive processes, including at sometimes unexpected locations such as visual or auditory cortices. In parallel, evidence is growing that the perception of the external world through the classical senses, interoceptive processes, and cognition are intertwined and influence behavior. This happens even in the absence of physiological challenges or emotional contexts. Recent findings in interoception thus call for a revision of the classical paradigm of the perception-action-cognition loop to integrate interoceptive monitoring. They also highlight the need for a refinement of concepts such as arousal or internal state.

Executing, reinforcing and refining actions

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The ability of animals to build individual repertoires based on the consequences of their actions is fascinating, and essential for survival. Understanding this process, i.e. how actions are learned through trial and feedback, requires mechanistic insight into how self-paced actions are initiated, how they can be selected/initiated again, and how feedback can refine their execution and organization. We use behavioral, genetic, electrophysiological, and optical approaches to gain this mechanistic insight. The combination of these approaches allowed us to uncover that dopaminergic neurons are transiently active before self-paced movement initiation. This activity is not action-specific and modulates both the probability of initiation and the vigor of future movements, but does not affect ongoing movement. Dopamine is supposed to have opposite effects on downstream striatal direct and indirect pathways. Contrary to what is classically postulated, we found that both striatal direct and indirect pathways are active during movement initiation. The activity in both pathways is action-specific and has complementary but different roles in movement, which are enabled by specific basal ganglia output circuits. Input from cortex seems to be critical to organize striatal activity, and cortico-striatal plasticity is necessary to select, reinforce and refine the specific neural and behavioral patterns that lead to desirable outcomes. These data invite new models on the mechanisms underlying self-paced movement initiation, and motor dysfunction in Parkinson's disease. They also suggest that cortico-basal ganglia circuits play a generic role in learning to reinforce and refine task-relevant neural activity and behavioral patterns.

From synapse to nucleus and back again – communication over distance within neurons

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The extreme length of neuronal processes poses a challenge for synapse-to-nucleus communication. In response to this challenge several different mechanisms have evolved in neurons to couple synaptic activity to the regulation of gene expression. One of these mechanisms concerns the long-distance trafficking of proteins from postsynaptic sites and here in particular NMDA-receptors to the nucleus. Protein transport from synapse-to-nucleus has been largely neglected but it has the potential to encode information about synaptic signals at the site of origin and to induce sustained changes in gene expression. I will summarize current evidence on mechanisms of transport and consequences of nuclear import of these proteins with special emphasis on a synapto-nuclear protein messenger called Jacob. Finally, I will discuss how long-distance communication via protein transport might allow for precise decoding of NMDA-receptor activity into specific gene transcription and translation.

Successful translation of treatments for higher cognitive disorders from macaques to humans

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The recently evolved dorsolateral prefrontal cortex (dlPFC) subserves high order cognitive abilities including working memory, abstract reasoning, and top-down control of thought, action and emotion. However, these neurons are remarkably vulnerable to stress, inflammation and aging, and degenerate in cognitive disorders such as schizophrenia and Alzheimer's disease (AD). We have found that layer III dlPFC pyramidal cells, which are the focus of pathology in schizophrenia and AD, have unusual neurotransmission and unusual neuromodulation that is needed to generate and sustain mental representations but renders them especially vulnerable to dysfunction. We have found that cAMP magnification of calcium signaling is necessary to sustain representations in working memory but becomes toxic when dysregulated by age and/or inflammation, including hyperphosphorylation of tau in the aged dlPFC. We have also found a constellation of potassium channels concentrated on dlPFC dendritic spines that rapidly alter synaptic efficacy to coordinate cognitive and arousal states, but lead to loss of firing under conditions of stress or inflammation. Treatments that restore regulation of cAMP-calcium signaling are helpful in protecting dlPFC circuits, including a new and expanded postsynaptic role for mGluR3 signaling in primates, and a key role for post-synaptic alpha2A-adrenoceptors on dendritic spines. The alpha2A-adrenoceptor agonist, guanfacine, enhances dlPFC neuronal firing, protects the PFC from dendritic spine loss during chronic stress, and improves dlPFC cognitive function in animals and humans. Guanfacine is FDA approved for the treatment of ADHD and is also in widespread use for the treatment of cognitive disorders, including treating trauma in children and new studies for the treatment of delirium.

Symposia

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S21 Social immunity as defense against diseases: from sensory biology to collective animal behavior

S22 The listening brain: frontiers in auditory cognition and health

S23 Extracellular matrix alterations in aging and neurological diseases

S24 Evolution of behavior: from genes to circuits

S25 Multilevel human brain mapping and atlas as a tool connecting micro- and macro-structures

S26 Neural circuits for flexible social behavior

S27 Brain organoids for modelling immune-neural interactions in epilepsy

S28 Early dysfunction of the locus coeruleus noradrenergic system in neurodegenerative diseases

S29 Neural circuits and decision strategies for behavioral trade-offs

S30 Glia-neuron interactions sculpting functional circuit architecture; insights from genetic animal models

S31 From olfaction to emotions

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S33 Non-canonical contribution of oligodendrocyte precursors in brain circuits

S34 Modelling CNS recovery from autoimmune neurodegeneration

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Symposium

S1: Assessing neuronal excitability and sensory neuron subclasses using Patch-seq

S1-1 Associating ion channel alternative splicing with neuronal intrinsic electrophysiological properties using Patch-seq
Shreejoy Tripathy

S1-2 Molecular identity of sleeping nociceptors revealed by a multimodal Patch-Seq study
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S1-3 Areal specification of excitatory cortical neurons in the human brain
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S1-4 MEA-SeqX: Decoding the Impact of Rich Experience on Multiscale Hippocampal Network Dynamics
Brett Addison Emery, Xin Hu, Diana Klütsch, Shahrukh Khanzada, Ludvig Larsson, Ionut Dumitru, Jonas Frisén, Joakim Lundeberg, Gerd Kempermann, Hayder Amin

S1-5 Patch-Seq in the auditory system – Where ascending and descending neurons meet
Eckhard Friauf, Ayse Maraslioglu-Sperber, Jonas Fisch, Kathrin Kattler, Erika Pizzi, Tamara Ritter

Associating ion channel alternative splicing with neuronal intrinsic electrophysiological properties using Patch-seq

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The molecular basis of intrinsic excitability is highly complex, as illustrated by the sheer diversity of neuronal ion channels. Most ion channel genes are alternatively spliced to produce multiple isoforms, which can differ in a number of functional attributes, including their gating, voltage sensitivity, and intracellular trafficking. The talk by S. Tripathy will cover a novel approach for identifying alternative splicing events that are correlated with intracellular electrophysiological features. We use data obtained from Patch-Seq, a methodology that enables the simultaneous collection of both electrophysiological and transcriptomic features from the same cells. Using this approach, we have identified an association between the relative abundance of isoforms of the Shaw-related potassium channel gene, Kcnc1, with electrophysiological features such as firing rates and action potential widths. These relationships are consequences of the polarized targeting of the two Kcnc1 isoforms to different cellular compartments, which results in the observed alterations in biophysical properties. This example illustrates that the associations we identified using our method could potentially lead to the discovery of novel regulatory mechanisms in neuronal excitability.

Molecular identity of sleeping nociceptors revealed by a multimodal Patch-Seq study

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A hall mark of neuropathic pain is the spontaneous activity of a specific class of sensory neurons, the so-called sleeping nociceptors. To-date their molecular identity in humans remains elusive. We used a multimodal approach including Patch-Seq to transfer their functional responsiveness from human microneurography recordings to dissociated sensory ganglia neurons, and singled out one of 16 identified sensory neuron subclasses which are likely representing sleeping nociceptors.

Areal specification of excitatory cortical neurons in the human brain

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The human brain is parcellated into distinct functional areas tailored to transform specific inputs into usable outputs. How cortical neurons are specialized in different areas to achieve these diverse functional roles is not completely understood. We performed multimodal Patch-seq analysis in primary human tissue from different cortical areas to better understand areal cell type specialization. Our data suggest that, even in adjacent gyri such as the MTG and ITG, excitatory neurons differ in their intrinsic biophysical properties and morphologic features. Leveraging the genome-wide transcriptome data from this multimodal analysis, we can identify candidate molecular pathways and specific genes that may drive these morpho-electric differences between neurons in different cortical areas.

MEA-SeqX: Decoding the Impact of Rich Experience on Multiscale Hippocampal Network Dynamics

Brett Addison Emery, Xin Hu[1], Diana Klütsch[1], Shahrukh Khanzada[1], Ludvig Larsson[4], Ionut Dumitru[5], Jonas Frisén[5], Joakim Lundeberg[4], Gerd Kempermann[2,3], Hayder Amin[1,6]

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Neural information processing in the brain occurs at multiscale, through combinations of transcriptional regulation, neural activity, and their computational dynamics, providing the basis of cognitive functions and behaviors. This intrinsic complexity of diverse information integration necessitates methodological approaches that span multiple modalities and scales. Recent developments in multiscale methodology with patch-sequencing (Patch-seq) and electro-sequencing (electro-seq) techniques have enabled mRNA sequencing of individual neurons following electrophysiological recordings. However, both suffer from low throughput and limited scalability to probe synchronous brain activity and neural computations with the underlying molecular signatures at a high spatiotemporal resolution. To address this gap, the recent advent of high throughput spatially resolved transcriptomics (SRT) provides insights into the spatial distribution of gene expression in different regions across the tissue, highlighting diverse cellular interactions and organization; however, SRT lacks temporal information. The continued development of spatiotemporally resolved high-density microelectrode array (HD-MEA) based on active pixel sensor technology provides non-invasive, multi-site, long-term, and label-free measurements of extracellular activity, both local field potentials and spiking activity, from thousands of neurons simultaneously with single-cell accuracy. However, when SRT and HD-MEA are applied separately, they only provide single-scale information regarding genome and network function, respectively. Here, we implement a novel multimodal multiscale platform combining these technologies with optical imaging and computational frameworks, creating a comprehensive tool capable of recording and analyzing cross-scale transcriptional and functional network readouts mesoscopically.

To validate this combinatorial platform, a mouse model of experience-dependent plasticity - environmental enrichment (ENR) was applied. ENR has been shown to promote neurogenesis, highlighting the mammalian brain’s complexity and adaptability through the integration of new neurons into existing neural networks. Here, we examine the precise impact of experience on network-wide computational dynamics of complex, network-wide brain activity and the underlying molecular infrastructure within the large-scale hippocampal-cortical network. First, spatial patterns of gene expression and their correspondance to functional network features were assessed hrough Spearman’s correlation analysis. This revealed significant enhancement of gene expression patterns corresponding to DG and CA3 hippocampal subregion network function in the ENR compared to the SD. Next, identification of genes with a strong causal link between correlated molecular and functional networks revealed enhanced hippocampal region-specific transcriptional expression in ENR than in SD linked to IEGs, ion channel activity, synaptic function, and neurogenesis. Overall, this study unveils the spatially

resolved causal regulation across molecular-functional features and the coordinated neural activity and gene expression changes resulting from experience. This cross-disciplinary platform provides a foundation for deepening our understanding of the molecular mechanisms and functional dynamics resulting from neurogenesis with applications for uncovering deficit mechanisms underlying brain function and paving the way for targeted therapies.

Patch-Seq in the auditory system – Where ascending and descending neurons meet

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The lateral superior olive (LSO), a prominent integration center in the auditory brainstem, contains a remarkably heterogeneous population of neurons. Ascending neurons, predominantly principal neurons (pLSOs), process interaural level differences for sound localization in a fast and temporally precise manner. Descending neurons (lateral olivocochlear neurons, LOCs) provide feedback into the cochlea and are thought to protect against acoustic overload. The molecular determinants of the neuronal diversity in the LSO are largely unknown. We used patch-seq analysis in mice at postnatal days P10-12 to classify developing LSO neurons according to their functional and molecular profiles. Across the entire sample (n = 86 neurons), genes involved in ATP synthesis were particularly highly expressed, confirming the energy expenditure of auditory neurons. Unsupervised clustering analysis revealed two clusters. Fifty-six of the 86 neurons (2/3) belonged to cluster 1, and 30 (1/3) belonged to cluster 2. The two clusters were distinguished by 353 differentially expressed genes (DEGs), with 254 genes upregulated in cluster 1 and 99 in cluster 2. Most of these DEGs were novel for the LSO. DEGs whose expression was previously described in the LSO clearly affiliated the two clusters with pLSOs and LOCs. We determined 16 electrophysiological parameters prior to scRNA-seq. Analysis of these physiological features confirmed the transcriptomic clustering. To investigate neurotransmitter diversity in LSO neurons, we analyzed gene expression of a subset of neurotransmitter-associated molecules. Our analysis revealed that pLSOs mainly express glutamatergic or GABAergic genes. In contrast, LOCs displayed multi-transmitter properties, most frequently GABA plus two neuropeptides. We focused on genes affecting neuronal input–output properties and validated some of them by immunohistochemistry, electrophysiology, and pharmacology. These genes encode proteins such as osteopontin, Kv11.3, and Kvβ3 (pLSO-specific), calcitonin-gene-related peptide (LOC-specific), or Kv7.2 and Kv7.3 (no DEGs). We also identified 12 “Super DEGs” and 12 genes showing “Cluster similarity.” Collectively, we provide fundamental and comprehensive insights into the molecular composition of individual ascending and descending neurons in the juvenile auditory brainstem and how this may relate to their specific functions, including developmental aspects.

Symposium

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Deema Awad

S2-2 Neural correlates of cyclic vs. stable progesterone levels and their relation with mood
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S2-3 Decoding Hormonal Dynamics: Insights into Mental Health from Oral Contraceptive Research
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S2-4 Predicting risk of postpartum depression using neurophysiological measures
Emma Fransson, Allison Eriksson, Richelle Björvang, Ebba Ancker, Fotios Papadopoulos, Inger Sundström Poromaa, Alkistis Skalkidou

S2-5 Brain changes and stress reaction after menopause - exploring estrogen's role
Anna Franziska Denninger, Melanie Henes, Inger Sundström Poromaa, Birgit Derntl, Lydia Kogler

Contribution of leptin signaling to the sex- and estrous cycle-dependent regulation of adaptive behaviors

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Mammalian reproduction is highly energy intensive, and requires an animal to prioritize feeding or sociosexual interaction, depending on need and opportunity. Such innate behaviors are regulated by hypothalamic circuits which are sensitive to peripheral signals indicative of the physiological need state. An important signal is leptin, an anorectic adipocyte-derived hormone secreted in proportion to fat stores. Although leptin levels strongly differ in males and females, little is known about sex-specific differences in leptin signaling.

In this study, we used pharmacological interventions to investigate the sex-dependent role of leptin signaling for the expression of innate behavior - feeding behavior, social and sexual behaviors, as well as exploration under anxiogenic conditions - both in males and naturally cycling females.

We first evaluated the effects of leptin treatment on the expression of feeding behavior. Leptin treatment reduced food intake in males and in females in the non-receptive estrus cycle stage, but increased food intake in females in the receptive cycle stage, in comparison to animals treated with the control substance (PBS). To test the effect of leptin treatment on sociosexual behavior, we treated mice with leptin or control substance and measured the expression of social and sexual behaviors in pairs of freely interacting mice of opposite sex. In males, leptin treatment enhanced exploratory behavior, but did not strongly affect social or sexual behavior. The same treatment decreased sociosexual behavior in non-receptive females, while increasing sociosexual behavior in receptive females.

In summary, our results demonstrate that leptin signaling mediates the expression of essential innate behaviors in a sex- and cycle-dependent manner.

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Neural correlates of cyclic vs. stable progesterone levels and their relation with mood

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The reproductive life of a female is characterized by cyclical fluctuations in ovarian hormones across the menstrual cycle. Notably, these periods are associated with affective and cognitive symptoms. Such hormonal variations have indeed the potential to modulate neurophysiological and behavioral dynamics, which is of relevance to the field of psychiatry characterized by sex differences emerging during hormonal transitions. Knowledge on the cross-talk between ovarian hormones and the female brain and mental health will be presented. Specifically, progesterone, being highly lipophilic, is a steroid hormone that easily passes through the blood-brain barrier. Animal models revealed various molecular processes to be modulated by progesterone, i.e., neurogenesis, synaptogenesis, myelination, and neurotransmitter signalling. While evidence of its effects on the human brain stems from neuroimaging studies of females during hormone transitional periods, such as the menstrual cycle, premenstrual dysphoric disorder (PMDD) presents itself as the ideal model to study the impact of progesterone on the brain in relation to mental health. Indeed, the temporal relationship with the cyclic occurrence of mood symptoms suggests progesterone as the trigger behind PMDD pathophysiology. Progesterone receptor modulators (SPRM) on low dose regimen exert antagonistic effects on progesterone, while maintaining estradiol levels on low levels. Neural correlates of cyclic vs. stable progesterone levels and their relation with mood will be here presented. Namely, neuroimaging findings on trait vs. state brain properties illustrate whether altered sensitivity to ovarian hormone fluctuations in PMDD is tied to the endocrine variations in progesterone and estrogen. Furthermore, the impact of the SPRM ulipristal acetate on the brain and mood is described within the context of a pharmaco-multimodal, neuroimaging, randomized controlled trial on patients with PMDD. Besides proposing SPRM as a potential new treatment for PMDD, these results provide new insights contributing to advance our understanding of the influence of progesterone on the brain structure and function as well as on mental health in females.

Decoding Hormonal Dynamics: Insights into Mental Health from Oral Contraceptive Research

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Adverse mood symptoms are frequently cited reasons for discontinuing oral contraceptive (OC) use, yet the neuroendocrine mechanisms underlying these symptoms remain poorly understood. This longitudinal study aimed to investigate the relationship between changes in natural and synthetic hormone profiles and both functional brain architecture and mood.

To ensure a diverse sample reflecting various hormonal states, we assessed 88 young healthy women twice: 26 in the early follicular phase, 26 long-term OC users, 25 OC discontinuers, and 11 OC starters before and after discontinuation or initiation, respectively. In addition to traditional mean-based analyses to assess the effects of OC initiation and discontinuation, we employed inter-subject representational similarity analyses (IS-RSA) to explore connections between interindividual patterns of variability in hormone concentrations, parcelwise resting-state functional connectivity (RSFC), and mood.

The results indicated a significant reduction in depressive symptom scores following OC discontinuation. While no direct associations were found between changes in depressive symptoms and sex hormone concentrations or functional brain architecture, alterations in progestogen (progesterone and progestin) levels were generally linked with changes in RSFC in frontal, subcortical, and cerebellar regions. Notably, RSFC patterns in the orbitofrontal gyrus were associated with progestogen concentrations and changes in positive mood.

Overall, women experiencing adverse mood effects may be more inclined to discontinue OC use, potentially leading to their underrepresentation in scientific studies. Although no direct neuroendocrine mechanisms underlying changes in depressive symptoms were identified, concentrations of progestogens, rather than estrogens, were associated with alterations in brain functional architecture in regions relevant for mental health.

Predicting risk of postpartum depression using neurophysiological measures

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Postpartum depression (PPD) is characterised by depressed mood, increased worry, lack of energy, and reduced interest in daily activities. PPD is a major public health issue that increases the risk of sick leave, morbidity and even suicide. Despite routine screening in several countries and increased awareness of the problem, research has shown that only a small proportion of women with PPD are identified and adequately treated. Better prediction, ideally before symptom onset, would enable early preventive interventions, emphasising the need to identify predictive factors that could provide a measure of the risk for future depression onset during pregnancy.

Sensorimotor gating is a mechanism by which sensory information supresses a motor response. One common way to study sensorimotor gating is by measuring the pre-pulse inhibition (PPI) of the startle reflex, the involuntary movement elicited by sudden stimuli (such as increased heart rate and blinking in response to a loud noise). Here we explore (PPI, as a biological tool for prediction of women at risk for PPD.

Using data from the longitudinal BASIC study in Uppsala, Sweden, we used PPI measures from late pregnancy and reports on depressive symptoms assessed 6 weeks postpartum with the Edinburgh Postnatal Depression Scale to determine the association between pregnancy PPI and PPD. Participants were invited in late pregnancy and trials of inhibiting the startle response using pre-pulse noise at different decibels (dB) were carried out. Among participants without depressive symptoms during pregnancy, we found that decreased inhibition of the startle response following a pre-pulse signal was associated with an increased risk of depression at 6 weeks postpartum.

Lower PPI was associated with PPD onset in women who were not depressed during pregnancy. Adjusted statistical modelling showed that for every unit increase in inhibition at 86 dB, the odds of developing PPD decreased by 3%. This finding might be useful for the future development of valid prediction tools for pregnancy care, particularly for women without established risk factors such as pregnancy depression.

Brain changes and stress reaction after menopause - exploring estrogen's role

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Estrogen, a sex hormone traditionally associated with reproductive functions, plays a critical role in modulating psychological well-being including stress reaction even after menopause. After menopause, when estrogen levels have sharply declined, changes in brain activation during affective tasks occur and may specifically influence the stress network. In pre-menopausal women, fluctuating sex hormone levels during the menstrual cycle have been associated with altered brain activation and stress reaction. Long-term estrogen administration during hormone replacement therapy (HRT) has been associated with altered subjective stress reaction in postmenopausal women. Although, by 2025 up to 1 billon women are expected to be experiencing menopause and hormonal fluctuations seem to present a vulnerability window for affective disorders, nothing is known about estrogen's effects on the neural stress reaction after this hormonal transition phase.

The current study explores the intricate relationship between estrogen and neural stress reaction in post-menopausal women. Therefore, we pharmacologically increased estrogen levels (E2) in postmenopausal women to levels comparable with ovulation during the natural menstrual cycle in a double-blinded, placebo-controlled design. Estrogen-levels were therefore increased rapidly within 24h, independent of fluctuations in other sex hormones such as progesterone. Women further underwent a psychosocial stress paradigm. Neural, subjective hormonal and physiological stress reactions were assessed. Stress induction was successful as shown by higher subjective stress levels after compared to before the stress induction independent of the drug condition (E2 vs. placebo) as well as significantly increased mean heart rate (HR) and decreased heart rate variability (HRV) during stress induction compared to rest. Additionally, preliminary analyses showed a tendency towards differences between the drug conditions (E2 vs. placebo) in subjective and physiological (HR & HRV) stress reaction. We will further present data on alterations in neural activation within the neural stress network, including regions such as the hippocampus, the amygdala, the basal ganglia, as well as medial and lateral frontal and temporal areas.

With the study, we can elucidate the effects of a short-term increase of estrogen levels in post-menopausal women, after metabolic changes and changes in receptor density occurred due to transitions in availability of sex hormones. Estrogen is considered to have a protective effect on dysfunctional stress reactions in pre-menopausal women. Initial research on HRT suggests that this association is different in post-menopausal women. Considering the close relationship between the stress reaction and mental disorders such as depression or anxiety disorders, it is particularly important to clarify the effects of sex hormones on neural stress reaction after menopause. Although gathering research data for pre-menopausal women is increasing, women before and after menopause differ in various bodily, cognitive, environmental, and affective aspects which must be taken into account in contemporary, future-oriented neuroscientific research to build a bridge from basic research to precision medicine for millions of women.

Symposium

S3: Prefrontal mechanisms of adaptive cognitive behaviors in health and disease

S3-1 Organization of Task Elements into Functional Modules in Prefrontal Cortex
Claudia Böhm

S3-2 Prefrontal orchestration: a cortical network for rodent motor inhibition
Zoe Jäckel, Niels Schwaderlapp, Ahmed Adzemovic, Florian Steenbergen, Stefanie Hardung, Katharina Fuchs, Christian Leibold, Maxim Zaitsev, Ilka Diester

S3-3 Prefrontal-hippocampal neural dynamics as useful biomarkers of cognitive impairment and rescue in schizophrenia: Role of serotonin receptors
M. Victoria Puig, Thomas Gener, Cristina López-Cabezón, Sara Hidalgo-Nieves

S3-4 Synaptic development of prefrontal and sensory cortical circuits
Paul George Anastasiades, Luca Discepolo, Sarah Apilado, James McAllister, Seth Grant, Cian O`Donnell, Michael Ashby

S3-5 Prefrontal Cortex and Cognitive Dysfunctions in Schizophrenia: The Role of Neural Oscillations and E/I-Balance Parameters
Peter J. Uhlhaas

Organization of Task Elements into Functional Modules in Prefrontal Cortex

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Prefrontal cortex (PFC) is critically important for the organisation and execution of flexible behavior, including sensory integration, decision making, and cognitive control. Its functions are believed to be supported by networks of cells coding for information that is currently relevant for the execution of the task at hand. This information can include sensory or behavioral variables, such as specific motor actions, and more abstract information, such as task-relevant categories, task phase or rules.
Despite the well-recognized role of PFC in flexible behaviour, the organization of these different types of information and learned concepts, and how they are flexibly accessed during task execution, is not fully understood. In a multi-phase spatial working memory task that required rats to navigate flexibly from start to goal locations via unpredictable routes, we found that the neural representation of task elements were structured according to their meaning in the task. Key locations, actions and task phase dependent direction of movement were organized into functional motifs that collectively reflected the core conceptual elements of the task. Additionally, the structured representation of task elements was supported, at least in part, by functional preferences of subsets of cells. These organized activity patterns in PFC might provide a cohesive framework for understanding a task, facilitate the correct sequence of task-phase dependent actions and, together with the nonrandom selectivity of individual cells, may enable modularized computation across different behaviors and contexts.

Prefrontal orchestration: a cortical network for rodent motor inhibition

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Goal-directed action control and adaptive behavior rely on the prefrontal cortex (PFC), where distinct subsections collaborate in functional networks. The extent to which this network exhibits a hierarchical system, characterized by early convergence within the PFC, remains elusive. We combined optogenetic modulation—facilitating reversible cell excitation for within-animal effect comparison—with behavioral and fMRI measurements to investigate the impact of PFC-subarea stimulation on motor control and evoked neuronal responses. We quantified error rate and reaction time as behavioral indicators of motor inhibition in rats performing a response-preparation task during optogenetic stimulation of the prelimbic (PL), infralimbic (IL), or ventro-orbital (VO) cortices. Our previous work revealed discrete behavioral effects of inhibiting specific PFC subsections; in contrast, excitation of each area impacted behavior in a similar manner. Identifying potential network nodes underlying this effect via opto-fMRI, we revealed a common activation volume among the subsections, spanning the prefrontal cortex, olfactory bulb, basal forebrain, and secondary motor cortex. Notably, IL excitation led to the smallest but most precisely localized activation volume, and produced the most robust behavioral effect. We further investigated functional synchrony within this region through multisite in-vivo recordings, uncovering performance-specific increases in PL-IL delta phase locking. Principal component analysis revealed varied neural activity patterns in distinct subsections, challenging the common practice of treating these regions as a single entity under the term medial PFC (mPFC). As the PL is the most relevant subsection relating to mPFC motor control, the results suggest that PL exerts the biggest influence on action control, with IL as a modulator. We summarize our findings in a simple model of PFC subsections that suggests PL as an input dependent switch between motor inhibition and execution modulated by an IL-dominated network allowing goal-directed action while keeping the behavior flexible.

Prefrontal-hippocampal neural dynamics as useful biomarkers of cognitive impairment and rescue in schizophrenia: Role of serotonin receptors

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The Cognition, Neural Networks and Neurotechnology laboratory (www.puiglab.org) investigates dysfunctional neural networks and circuits underlying intellectual disability in preclinical models of schizophrenia. We focus on prefrontal and hippocampal neural activities, paying special attention to the circuit's connectivity. Recent studies by the group unravelled a functional disconnection between the medial prefrontal cortex and the dorsal hippocampus during psychosis and memory impairment associated with NMDAR hypofunction (Delgado-Sallent C et al., 2022, 2023), a pathophysiological mechanism relevant for schizophrenia. More specifically, during psychosis induced by the NMDAR antagonist phencyclidine, aberrant gamma (~60 Hz) and high frequency (~160 Hz) bands emerged within prefrontal microcircuits whereas overall activity in the dorsal hippocampus decreased. We are currently mapping the emergence of these abnormal neural networks within the distinct areas of the medial prefrontal cortex and investigating the underlying cellular mechanisms via chemogenetics. The prefrontal-hippocampal disconnection also correlated with short- and long-term memory impairment assessed by the novel object recognition test following subchronic administration of phencyclidine. Together, our results suggest that dysfunctional prefrontal-hippocampal communication plays critical roles in cognitive impairment observed in schizophrenia. Another main aim of the laboratory is to find novel therapeutical targets for cognitive amelioration produced by new generation antipsychotic drugs. Interestingly, among highly prescribed antipsychotic medication, the ones showing the most pro-cognitive abilities bind strongly to one or more serotonin receptors. To gain further insight into the distinct roles of serotonin receptors in cognition and intellectual disability, we have investigated at a cellular and functional levels the contribution of serotonin 5-HT_{1A}, 5-HT_{2A}, 5-HT_4 and 5-HT_7 receptors to the modulation of prefrontal-hippocampal circuits and to the pro-cognitive actions of several antipsychotic compounds, including risperidone and lurasidone. Finally, the group also contributes to the development of state-of-the-art neurotechnologies to advance personalised treatment for brain disorders. These include the *in vivo* testing of new generation neural probes based on graphene (Viana D et al., 2024), that allow simultaneous recording and stimulation of brain tissue, and novel photoswitchable neuroinhibitors that restrain pathological hyperexcitability with light illumination on demand (Matera C et al., 2022).

Synaptic development of prefrontal and sensory cortical circuits

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During postnatal development, the brain undergoes a series of processes that bring about its mature architecture. These include neuronal migration, apoptosis, synapse formation and maturation. The timeline of cortical maturation is thought to be hierarchical, with primary sensory areas maturing earlier than higher-order areas, such as the prefrontal cortex (PFC). The cortex is a laminar structure with distinct synaptic inputs targeting specific layers. Within each cortical area there is also evidence for layer-specific development. For example, sensory critical periods in the mouse somatosensory barrel cortex (S1BF) follow an “outside-in” pattern, occurring first in thalamo-recipient layer (L)4 followed by superficial L2/3. The maturation of the PFC is thought to be delayed to that of S1BF. However, much less is known about the timeline of this maturation, particularly with respect to the development of synaptic connectivity within specific layers. Determining this may provide insight into when different inputs or cell types undergo synapse maturation. This project aims to determine similarities and differences in the synaptic maturation of a primary sensory barrel cortex (S1BF) and the PFC. To do so we have developed a pipeline for high-throughput synaptic puncta analysis using transgenic mice that express the synaptic protein PSD-95 tagged to GFP. We compare the maturation of synapses between layers and regions to determine both common rules and region-specific differences of synapse development between cortical areas. We find that PFC and S1BF not only develop at different times, but also via distinct layer-specific trajectories. We also highlight how inputs to superficial layers of sensory areas undergo protracted maturation during adolescence, potentially representing the final phase of top-down circuit maturation in the neocortex.

Prefrontal Cortex and Cognitive Dysfunctions in Schizophrenia: The Role of Neural Oscillations and E/I-Balance Parameters

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Schizophrenia (ScZ) is a severe psychiatric syndrome which involves pronounced cognitive deficits that have been linked to the integrity of prefrontal cortex (PFC), such as impaired working memory, executive functions and attention. Importantly, these deficits are present prior to the onset of psychosis, suggesting the contribution of aberrant brain development towards the manifestation of cognitive dysfunctions.
Recent work has attempted to identify circuit mechanisms underlying PFC-mediated cognitive deficits in ScZ, highlighting alterations in excitation/inhibition (E/I) balance parameters. Among the circuit mechanisms that are involved in the maintenance of E/I-balance during normal brain functioning, parvalbumin-expressing (PV+) γ-Aminobutyric acid (GABA)ergic interneurons are of particular interest as inhibition of pyramidal cell activity regulates the output of cell-assemblies and leads to rhythmic fluctuations in excitability or neural oscillations. Moreover, there is consistent evidence that α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)- and N-methyl-D-aspartate Receptor (NMDA-R)-mediated activation of PV+ interneurons.
In my presentation, I will give an overview of current studies that have used computational, cognitive and electrophysiological approaches towards identifying circuit mechanisms of PFC-deficits in schizophrenia. I will highlight the role of low- and high-frequency oscillations during PFC-mediated cognitive processes during normal brain functioning and summarize studies in schizophrenia patients that have utilized electro- and magnetoencephalography (EEG/MEG) to identify oscillatory correlates of cognitive deficits. Moreover, I will present evidence that oscillatory activity is impaired in participants at clinical high-risk of psychosis (CHR-P), suggesting that EEG/MEG-measures could be used as diagnostic and prognostic biomarkers.
Furthermore, I will highlight the importance of aberrant adolescent brain development for the emergence of cognitive deficits in schizophrenia. Drawing on data from EEG/-MEG studies during normal brain maturation, I will show that neural oscillations at low- and high-frequencies fully emerge during the transition from adolescent to adulthood which are consistent with the ongoing modifications in executive functions. Accordingly, these findings suggests that adolescence may constitute a sensitive period for the maturation of PFC-circuits which could be involved in the emergence of psychosis during this developmental period.

Symposium

S4: Current advances of extracellular vesicles in CNS-cell interaction and brain-periphery communication

S4-1 Microglial EVs travelling at the neuron surface: implication in the delivery of eat-me signals to the synapse
Claudia Verderio, Giulia D`Arrigo, Giulia Cutugno, Maria Teresa Golia, Francesca Sironi, Sara Colombo, Caterina Bendotti, Rosa Chiara Paolicelli, Martina Gabrielli

S4-2 Proteomic profiling of tau interacting molecules in brain derived extracellular vesicles uncover key molecules contributing to tau pathology spread in Alzheimer's disease
Tsuneya Ikezu, Zhengrong Zhang, Yang You, Kaiwen Yu, Arun Reddy Ravula, Seiko Ikezu, Michael DeTure, Dennis Dickson, Junmin Peng

S4-3 High Purity Fluorescence-activated Vesicle Sorting for Enrichment of Extracellular Vesicle (Brain) Specific Populations
Isabel Graf, Anne Rissiek, Jochen Behrends, Santra Brenna, Christina Krüger, Christopher Urbschat, Bente Siebels, Franz Ricklefs, Amanda Salviano-Silva, Anke Diemert, Petra Arck, Tim Magnus, Berta Puig

S4-4 Scope and function of extracellular vesicle-based communication between periphery and CNS in vivo
Stefan Momma

S4-5 Extracellular vesicles in the communication between periphery and brain
Roosmarijn Vandenbroucke

Microglial EVs travelling at the neuron surface: implication in the delivery of eat-me signals to the synapse

Claudia Verderio[1], Giulia D`Arrigo[1], Giulia Cutugno[1], Maria Teresa Golia[1], Francesca Sironi[2], Sara Colombo[1], Caterina Bendotti[2], Rosa Chiara Paolicelli[3], Martina Gabrielli[1]

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Microglia remove synapses tagged by complement factors, but how complements mark synapses is not fully understood. Our previous studies indicate that extracellular vesicles (EVs) released by microglia carry C1q and C3 and move at the neuron surface, more efficiently along axons than dendrites, where EVs often stop at preferential contact sites. On this basis we investigated whether microglial EVs can interact with synapses, tag them with C1q and promote synaptic pruning. By optical manipulation we placed single EVs on dendrites receiving Vglut-1-RFP positive puncta and by live imaging we found EVs stopping their motion at Vglut-1+ puncta. We next exposed neurons in bulk to mCLING-labelled fluorescent EVs and found that most EVs localized at pre- and/or post-synaptic sites 3 hours after addition and increased the fraction of C1q puncta interacting with synapses. When EVs-treated neurons were cultured with microglia both pre- and post-synaptic density decreased compared to neurons cultured alone and microglial synaptic engulfment augmented. Conversely, only post-synaptic density was reduced in EVs-untreated neurons, implicating EVs in microglial-mediated pre-synaptic removal. Co-culturing neurons with C9orf72 knockout microglia, characterized by elevated EV production but normal phagocytic activity, reduced both pre- and post-synaptic density and mutant microglia engulfed more synapses compared to WT cells. Moreover, treatment of C9orf72 knockout microglia with GW4869, a pharmacological inhibitor of EV biogenesis that normalized EV production, restored normal pre-synaptic density, implicating EVs and EVs-mediated C1q deposition at synapses in microglia-mediated pre-synaptic engulfment. Analysis of synaptic density and microglia-mediated synaptic pruning in CA1 hippocampal region at P17, a time of intense synaptic pruning, confirmed enhanced C1q deposition, decreased pre-synaptic density and higher microglial synaptic engulfment in knock-out mice, associated with higher microglial EVs production, as revealed by Western Blot (and SimoA analysis) of EVs isolated from brain interstitial fluid. Finally, quantification of brain EVs across postnatal development revealed peak production of Iba-1+ EVs and C1q+ EVs during the period of intense pruning in the hippocampus. Taken together, these data unveil a novel role for microglia-derived EVs in instructing microglia-mediated synapses engulfment during postnatal brain development.

Proteomic profiling of tau interacting molecules in brain derived extracellular vesicles uncover key molecules contributing to tau pathology spread in Alzheimer's disease

Tsuneya Ikezu[1], Zhengrong Zhang[1], Yang You[1], Kaiwen Yu[2], Arun Reddy Ravula[1], Seiko Ikezu[1], Michael DeTure[1], Dennis Dickson[1], Junmin Peng[2]

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Aims
Brain cells secrete extracellular vesicles (EVs) containing signaling- and pathological proteins relevant to Alzheimer's disease (AD) and AD related disorders including tauopathies. Recent studies demonstrate brain-derived EVs (BDEVs) contain misfolded tau and are highly transmissible of tau pathology in the central nervous system. However, molecular interplays between pathological tau and BDEV cargo proteins underpinning EV-mediated tau transfer is still largely unexplored.
Methods
We conducted the immuno-affinity purification of tau in human BDEVs isolated from age and sex-matched 14 AD and 14 CTRL cases and performed tandem mass-tag mass spectrometry to profile unbiased tau interactome in BDEVs. We applied the thermophoresis (Nanotemper Monolith) to validate the interaction of potential EV associated molecule with tau and super-resolution microscopy to further visualize their colocalization in a single-EV level. We next designed siRNAs to silence EV-tau interactors in SH-SY5Y cells overexpressing human P301L tau (SH-SY5Y-P301Ltau) and assessed tau loading to EVs at single EV resolution by Flow Nanoanalyzer. In addition, these EVs were tested for their uptake and tau seeding by human iPSC-derived neurons (iNeurons) using IncuCyte. Finally, the efficacy of targeting identified molecules on tau propagation was determined in PS19 mice expressing P301S tau mutant in vivo.
Result:
A total of 764 proteins were identified in BDEV-associated tau interactome from CTRL and AD patients, which are related to exocytic vesicle, transmembrane transport and cytoskeleton. Sixty-five proteins were significantly downregulated in AD BDEVs; whereas 5 proteins were significantly upregulated in AD BDEVs compared to CTRL BDEVs. The most enriched EV-tau interacting proteins were significantly positively correlated with Braak stage. We validated the direct binding of candidate proteins to tau protein using purified proteins with micromolar binding affinities by Monolith. Furthermore, we confirmed colocalization of candidate molecules with tau in human BDEVs at single EV level using super-resolution microscopy (ONI). Silencing of candidate molecules in human SH-SY5Y-P301Ltau cells results in reduced loading of tau in secreted EVs, their reduced uptake by iNeurons, and their seeding activities. Finally, intracerebroventricular injection of neutralizing antibody against one of the target molecules results in reduction of tau accumulation in PS19 mice expressing P301S human tau mutant.

Conclusion:
Our study identified tau-interacting molecules highly enriched in AD BDEVs compared to CTRL BDEVs. Silencing of candidate molecule reduced tau loading to the EVs and their uptake by iNeurons and neutralizing antibody suppressed tau dissemination, highlighting them as promising therapeutic targets to halt tau pathology in AD and related tauopathies.

High Purity Fluorescence-activated Vesicle Sorting for Enrichment of Extracellular Vesicle (Brain) Specific Populations

Isabel Graf[1], Anne Rissiek[2], Jochen Behrends[3], Santra Brenna[4], Christina Krüger[4], Christopher Urbschat[1], Bente Siebels[5], Franz Ricklefs[6], Amanda Salviano-Silva[6], Anke Diemert[7], Petra Arck[1], Tim Magnus[4], Berta Puig[4]

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Background:
To date, the options for a minimal invasive tissue-based evaluation of the brain features and functions in diseased patients are still limited and are largely based on imaging techniques. These limitations underpin the need for novel in-depth investigation tools of brain tissue for prognostic and diagnostic purposes. In this context, the enrichment of extracellular vesicles (EV) from peripheral blood samples is a promising approach to gain minimal invasive, but biologically highly relevant insights into the brains' function. The aim of this study was to develop a highly reproducible and validated protocol for sorting cell-specific EV populations of interest across different samples, from human to mice by employing different high-end fluorescent sorter cytometers to ensure feasibility.

Methods:
Using density gradient ultracentrifugation or differential ultracentrifugation, we isolated two types of samples: I) EVs from human blood samples II) brain-derived EVs (BDEVs) from Cre-loxP transgenic mice expressing td Tomato in all the cell membranes but EGFP replacing the tdTomato fluorescence in brain endothelial cells (under the Slco1 promoter). Nanoparticle Tracking Analysis, Transmission Electron Microscopy and Western Blots were performed to validate the isolation. Human EVs were stained with antibodies for several proteins of interest to sort for tissue-specific EVs. The sorting was tested with BD FACSAria Fusion Sorter, BD FACSAria III Sorter and BD FACSDiscover S8 Cell Sorter. Subsequently, the sort was validated by Imaging Flow Cytometry (IFCM), Liquid-chromatography coupled Tandem-Mass-Spectrometry and Transmission Electron Microscopy.

Results:
EVs from human as well as murine cross-tissues were successfully sorted by either Aria Fusion or Discover S8 cell sorter. Optimal parameters for nozzle size, detection thresholds, setting gates and sample collection were identified. IFCM of the sorted fractions revealed an enrichment of the populations of interest up to 90%, even of low frequent EV populations (under 10%) in the source samples. This was further confirmed by the detection of tissue-specific proteins in quantitative proteomic analyses.

Conclusion:
We show the successful sorting and enrichment of specific EVs from different source (brain tissue, blood), species (human, mouse) posing a powerful tool for basic science and translational applications.

Scope and function of extracellular vesicle-based communication between periphery and CNS in vivo

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Communication between peripheral organs, inclusive of the microbiome, plays a pivotal role in regulating brain functionality during both healthy and diseased states. Conventionally, the primary pathways considered for this neuroimmune interaction involve individual molecules such as cytokines transported via the bloodstream, neural transmission, or, in more severe conditions, the infiltration of peripheral immune cells into the brain. Through the utilization of a transgenic mouse model employing the Cre-LoxP system, we demonstrate the transfer of functional mRNA via extracellular vesicles released from blood cells to neural cells within the brain. While this mode of communication is infrequent under normal physiological circumstances, instances of peripheral inflammation and pathological as well as physiological neuronal activity stimulate the uptake of extracellular vesicles from the bloodstream, overcoming the blood-brain barrier. Employing the same methodology, we have also demonstrated the transfer of functional molecules from gut bacteria to cells in various organs, including neurons in the brain. These findings shed light on a widespread communication network connecting the periphery and the brain, regulated by pathological and physiological stimuli.

Extracellular vesicles in the communication between periphery and brain

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Growing evidence indicates that peripheral inflammation affects the brain and contributes to the onset and progression of neurological disorders such as Alzheimer's and Parkinson's disease, despite the protective presence of the brain's tight barriers. Extracellular vesicles (EVs), which are secreted by both eukaryotic cells and bacteria, are believed to play a key role in transmitting inflammatory signals across long distances. Our research showed that the release of EVs from the choroid plexus epithelial cells that form the blood-cerebrospinal fluid barrier increases in response to peripheral inflammation, transmitting these inflammatory signals to the brain parenchyma. Additionally, bacterial EVs from Helicobacter pylori (called outer membrane vesicles or OMVs) were found to induce brain inflammation and accelerate Alzheimer's disease pathology in a mouse model of AD. Overall, our findings highlight the critical role of both eukaryotic and bacterial extracellular vesicles in mediating communication between the peripheral systems and the brain.

Symposium

S5: The role of co-proteinopathies in neurodegenerative diseases: bystander or disease driver?

S5-1 From biology to classification: understanding Parkinson's disease and related synucleinopathies
Tiago Outeiro

S5-2 Four-repeat tau in atypical parkinsonisms; strategies for combat
Yun Kyung Kim

S5-3 Cellular mechanisms driving tau and TDP-43 aggregation in neurodegenerative diseases
Josephine Labus

S5-4 Alpha-synuclein and tau in Parkinson's disease, bystanders or partners-in-crime?
Franziska Richter

S5-5 Characterization of Lewy Body-like Structures in Cellular System and Patient Samples
Asima Nayak, Roberto Sansevrino, Jian-Hua Chen, Christian Hoffmann, Aleksandr A. Korobeinikov, Paula Brosius, Axel Ekman, Joshua Jackson, Gerard Aguilar Pérez, Han Wang, Johannes Vincent Tromm, Mark A. Le Gros, Daniele Bano, Pallavi Gopal, Carolyn Larabell, Dragomir Milovanovic

From biology to classification: understanding Parkinson's disease and related synucleinopathies

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Since the original description by James Parkinson, Parkinson's disease (PD) has intrigued us for over 200 years, and remains an unsolved progressive condition that affects millions of people worldwide. The accumulation of alpha-synuclein in pathognomonic inclusions known as Lewy bodies and Lewy neurites is used as the ultimate confirmation of PD during postmortem evaluation of brain tissue. Over the years, our knowledge of the molecular alterations taking place during disease has expanded tremendously. In particular, we now know that multiple co-pathologies are present in a large fraction of PD cases. Although we have been using a range of criteria to classifiy distinct forms of PD, it is still not consensual how to diagnose and classify a disease that manifests with diverse features, and that responds differently to existing therapies and to those under development. We are now living a time when 'biological' information is becoming more abundant and precise, enabling us to attempt to incorporate different sources of information to classify different forms of PD. This is extremely important for basic science, as it will enable us to develop improved models for studying PD, and also in clinical practice, as this will be the path towards effective personalized medicine.

Four-repeat tau in atypical parkinsonisms; strategies for combat

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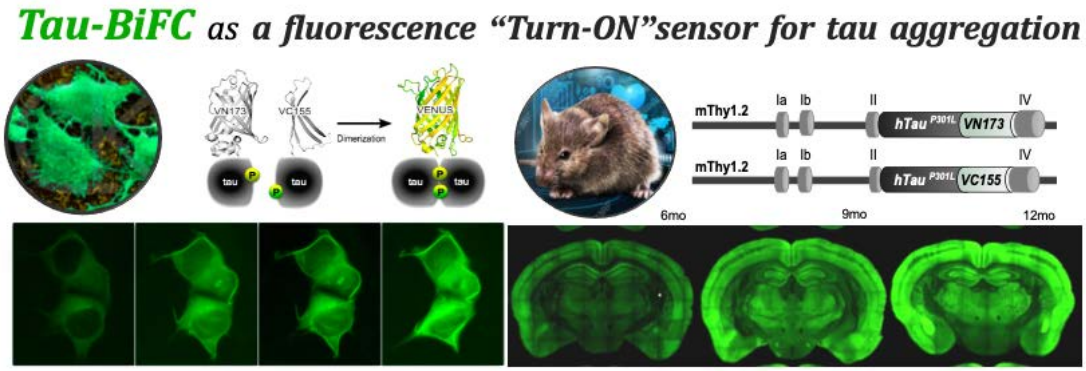
Tau protein aggregation is a hallmark of numerous neurodegenerative diseases. In atypical Parkinsonisms, particularly progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD), the pathological accumulation of 4-repeat (4R) tau isoforms drives rapid disease progression. These tauopathies are marked by severe neuronal dysfunction and accelerated neurodegeneration, with tau oligomerization representing a critical stage that promotes neuronal death. The urgent need to understand and target these specific tau species has driven efforts to develop effective therapeutic strategies. However, monitoring tau aggregation and targeting these toxic species has proven challenging, as they remain elusive, much like invisible agents of neurodegeneration.

To address this, we developed an advanced sensor platform capable of real-time visualization of tau self-assembly. Using bimolecular fluorescence complementation (BiFC), we "tagged" tau to track its transition from soluble monomers to large, harmful aggregates. In their basal state, our tau-BiFC cells emit minimal fluorescence, indicating monomeric "benign" tau. However, upon inducing tau hyperphosphorylation, a surge in fluorescence reveals a dramatic increase in tau-tau interactions.

We further established a tau-BiFC transgenic mouse model, offering unprecedented insights into tau oligomerization in vivo. This model enables the detection of early-stage soluble tau oligomers from 3 months of age, followed by the gradual development of tangle-like aggregates, mirroring the progression of human tauopathies.

Leveraging this model, we conducted high-throughput screening of FDA-approved and Phase I drugs, leading to the discovery of levosimendan, a heart failure drug with significant potential to inhibit tau oligomerization. However, recognizing its limitations for neurodegenerative diseases, we developed DA-7503, a potent tau-targeting candidate now in Phase I clinical trials in 2024.

In this presentation, I will introduce the tau-BiFC platform and discuss our journey in developing effective anti-tau therapeutics, specifically targeting 4R tau in atypical Parkinsonisms. This work illuminates the microscopic battlefield of neurodegeneration and offers new hope in combating these devastating diseases.



Cellular mechanisms driving tau and TDP-43 aggregation in neurodegenerative diseases

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Intracellular aggregates of hyperphosphorylated microtubule-associated protein tau are a common hallmark of Alzheimer's disease and other tau-related neurodegenerative diseases. In addition to tau, cytoplasmic inclusions of the RNA/DNA-binding protein TDP-43 are also frequently found in these patients, although TDP-43 pathology is more commonly associated with amyotrophic lateral sclerosis. Research over the last decades has significantly improved our understanding of the molecular mechanisms underlying pathological protein aggregation, however the cellular receptors involved are only poorly characterized.

We have previously shown that the serotonin receptor 7 (5-HT7R) induces tau hyperphosphorylation and tau aggregate formation through direct interaction with the tau kinase cyclin-dependent kinase 5, leading to its activation. Interestingly, activation of this signaling pathway does not require serotonin binding but is mediated by the constitutive activity of the 5-HT7R. Recent studies from our laboratory indicate that the 5-HT7R is also involved in the mislocalization and aggregation of TDP-43. Although the underlying 5-HT7R-induced cellular mechanisms seems to be distinct, the constitutive activity of the receptor is crucial for the aggregation of both tau and TDP-43. Blocking the constitutive activity by specific 5-HT7R inverse agonists ameliorated tau and TDP-43 pathology in various disease-related cell and animal models.

In summary, the 5-HT7R might be involved in the development of co-pathologies and emerged as a novel promising target for the treatment of neurodegenerative diseases.

Alpha-synuclein and tau in Parkinson's disease, bystanders or partners-in-crime?

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Neurodegenerative proteinopathies are categorized by the protein that dominates the histopathological picture, upon post-mortem examination of human brain tissue, accumulating as misfolded, aggregated species. Among these, alpha-synuclein (aSyn) is the main component of Lewy pathology in Parkinson's disease (PD). However, apart from pathology that characterizes PD as synucleinopathy, Tau pathology develops frequently in neurons across different brain regions most prominent in PD patients with cognitive decline (mild cognitive impairment or dementia). Despite these overlaps in the pathological distribution, the detailed and potentially toxic interplay of aSyn and Tau for neuronal uptake and spreading remains elusive. This talk will highlight current state of knowledge and future perspectives on co-pathologies in PD pathophysiology and therapeutic implications.

Characterization of Lewy Body-like Structures in Cellular System and Patient Samples

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The presence of proteinaceous inclusions known as Lewy bodies (LBs) is a hallmark of Lewy body disorders, characterized by abnormal protein deposits primarily composed of α-synuclein. These deposits disrupt neuronal function, leading to cognitive and motor deficits and ultimately cause cell death. Despite its direct implication in disease, the biogenesis of LBs remains unclear. Recently, we established a minimal system for reverse engineering of Lewy body-like inclusions (LBLs) in cellular system. Immunohistochemical studies of patient LBs and LBLs demonstrate that both share a core-shell architecture, where & α-synuclein forms a dense shell surrounding a core containing various proteins and membrane-bound organelles. This structural similarity is further supported by soft X-ray tomography and live-cell imaging, which reveal that both LBs and LBLs accumulates mitochondria at their interface. Additionally, the dynamic interactions between & α-synuclein and membrane-bound organelles in LBLs mimic those observed in patient LBs, indicating that the processes governing their formation and maturation are likely conserved. These findings highlight the potential of LBLs as cellular models for studying LB architecture and developing novel therapeutic strategies.

Symposium

S6: Sensing LOOPS: cortico-subcortical interactions for adaptive sensing, perception and learning

Context-dependent corticofugal control by the somatosensory cortex of thalamic information processing

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In vibrissae-possessing mammals, tactile information is transmitted from the whiskers, via the brainstem and thalamus, to the somatosensory cortex (S1). The thalamus supports somatosensory information transfer via the leminiscal , extraleminiscal and paraleminiscal pathways. The paraleminiscal pathway provides information about kinematics of both single whiskers and whisker populations that is relayed by the posterior medial thalamus (Pom) to layers 1 and 5A of S1.

The Pom has been proposed by others to integrate corticofugal motor and tactile information, and serve to optimize tactile signal-to-noise ratios thereby distinguishing suprathreshold from subthreshold stimuli. Corticofugal pathways project from layer 6 of S1 to the thalamus to fine tune its activity during ongoing somatosensory information processing. Layer 6 cell populations can be differentiated into dopamine D1 receptor (Drd1R)-expressing that projects to Pom, and neurotensin receptor 1 (Ntsr1)-expressing that project to both VPM and Pom.

Here, we report that corticofugal projections from these cell populations in S1 layer 6 engage in functionally distinct feedback modulation of context-dependent somatosensory information processing in Pom that subserve experience- and context-dependent modulation of information transfer from the thalamus to the somatosensory cortex.

Hierarchical and reciprocal connections for visual cognition in the primate brain

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Perceptual decisions are shaped in an intricate network of cortico-cortical and cortico-subcortical circuits. For decisions about 3D-motion stimuli in the macaque monkey, this network involves extrastriate visual cortical area V5/MT and its input from earlier visual cortical areas, lateral intraparietal area LIP and the pulvinar nuclei. For eye movement responses, these areas send signals to the frontal eye fields and the superior colliculus. While cortico-cortical projections are thought to provide the major feedforward pathway for perceptual processing, it has been suggested that connections through the pulvinar provide a reference signal for adapting perceptual signalling in changing behavioural contexts like dynamic changes in eye position. The contribution of individual nodes has been established through electrophysiological recordings of single neurons during active performance of behavioural tasks and importantly also by causal methods, especially electrical microstimulation, which can be shown to alter behavioural performance.

More recently, using small focal anatomical tracer injections, we can identify the precise topographic nature of this connectivity, both from one structure to another and also intrinsically within cortical areas. For instance, focal injections of retrograde tracer Cholera Toxin B into dorsal parts of area LIP (LIPdorsal) reveals a precise point-to-point projection from neurons in the ventral portion of LIP (LIPventral), which in turn receives direct input from retinotopically organised area V5/MT. LIPdorsal and LIPventral receive distinct, topographically organised input from medial and anterior parts of the pulvinar. These projections to LIP arise from different subdivisions of the pulvinar nuclei than those reaching visual area V5/MT, which originate mainly from lateral and inferior pulvinar. Taken together, these pathways form several distinct processing loops that act together to dynamically shape and adapt perceptual decisions to changing contexts.

Multisensory integration and modality-specific decision-making in frontal cortex and superior colliculus

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The integration of sensory inputs from different senses is a crucial aspect of sensory perception and the generation of corresponding behavioral decisions. However, whether such multisensory integration occurs at specific stages of neural processing, for example after the initial processing of unisensory information but preceding the formation of a behavioral choice, remains unclear. Two brain regions, the anterolateral motor cortex (ALM) and the superior colliculus (SC), have been implicated as particularly important structures for both multisensory integration and decision-making, suggesting that they are part of a cortico-subcortical loop that transforms multisensory inputs into behavioral decisions. To study the role of these areas in multisensory integration and decision-making, we trained mice in a multisensory discrimination task, where animals had to integrate visual and tactile information over time to identify the target stimulus side. We then performed simultaneous neural recordings in ALM and SC, using high-density Neuropixels probes, in task-performing animals. We found robust visual and tactile responses in ALM and SC, with a clear separation of modalities between superficial and deep SC layers (dSC). To ensure that multisensory responses were not driven by correlated movements, we used a generalized linear model that included rich behavioral information to separate stimulus-related from movement-related neural activity. Aside from sensory responses, both ALM and dSC showed strong choice-predictive activity during stimulus presentation and a subsequent delay period. Interestingly, visual and tactile choices were encoded in ALM through different neuronal populations. Moreover, neurons encoding multisensory choices were not simply a combination of these populations. In contrast, choice signals in dSC neurons were largely independent of the sensory modality. ALM thus showed modality-specific choice-tuning, possibly contributing to the transformation of unisensory information into modality-independent choices. To causally confirm these results, we performed optogenetic inactivation in each area during simultaneous Neuropixels recordings. Optogenetic inactivation of both ALM and dSC strongly reduced animals' choice performance during the stimulus and delay period and disrupted choice-related dynamics in both regions. This suggests a hierarchical transformation of multisensory information into behavioral decisions, where the SC sends multisensory information to ALM, which creates modality-specific decisions that are then returned to the SC to create motor outputs.

A computational framework for subcortical-cortical interactions in cognition

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Computational modeling of cognitive processes has largely focused on cortical areas, but recent studies suggest that multiple subcortical areas contribute to different cognitive computations. Theoretical frameworks are needed to clarify why and how subcortical structures are engaged during cognitive tasks, as well as to guide and interpret future experimental studies on subcortical-cortical interactions.

In the first part of my talk, I will present a computational framework that introduces the concept of 'dynamical modes': population-level neural activity patterns in the cortex that can be interpreted as basic cognitive building blocks. I use this framework to examine how differences in synaptic spine density across frontal and parietal areas result in complementary dynamical modes related to working memory and decision-making computations. I then consider the pulvinar, the largest part of the visual thalamus that is reciprocally connected to multiple visual and association cortical areas. I put forward a framework of pulvino-cortical interactions based on computations on (cortical) dynamical modes to clarify the pulvinar's involvement in attention, confidence, and communication. Next, I present a circuit model of subcortical-cortical interactions during motor planning, constrained by multisite recordings in the mouse. I propose that subcortical inputs to the thalamus selectively gate dynamical modes relevant for movement planning and execution. Finally, I discuss the generation and control of spindles in the thalamocortical network, a hallmark oscillation during sleep that is thought to be crucial for the consolidation of episodic memories. Overall, the modeling results support the existence of computational principles for large-scale subcortical-cortical interactions in the brain that will prove useful in the present era of large-scale data collection and analysis.

The sound of noise in cortico-subcortical LOOPs

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The sound of noise in cortico-subcortical LOOPs

Symposium

S7: The 4th dimension of plasticity: extracellular matrix interplay with neurons and glia at the synapse

S7-1 Extracellular matrix remodeling in the ischemic brain
Dirk M. Hermann, Egor Dzyubenko

S7-2 Polarized microtubule remodeling transforms the morphology of reactive microglia and drives cytokine release
Casper Hoogenraad

S7-3 Microglial-Neuronal Interactions in the Recovery Phase of Ischemic Stroke
Charlotte Catharina Oldenburg

S7-4 The matricellular protein hevin in reward-related plasticity
Vincent Vialou

S7-5 Structure-function analysis of PNN in health and diseases
Liliia Kushnireva, Sapir Havusha-Laufer, Irit Sagi

Extracellular matrix remodeling in the ischemic brain

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Stroke remains one of the leading causes of long-term disability worldwide, and the development of effective restorative therapies is limited by an incomplete understanding of intrinsic brain recovery mechanisms, especially those involving multicellular interactions and the remodeling of the brain extracellular matrix (ECM). Ample research in stroke has focused on cellular components, the role of the complex ECM network, which dynamically responds to stroke and shapes brain cell behavior, has often been overlooked. Emerging evidence suggests that the remodeling of ECM and glial cells is crucial for neuroplasticity and neurological recovery after stroke [1], making it a promising but underexplored therapeutic target.

Our data demonstrate that Tenascin-C (TnC), a juvenile matrix glycoprotein normally confined to stem cell niches in the adult brain, is significantly upregulated in reactive astrocytes following experimental stroke in mice [2]. This de novo production of TnC plays a key role in post-stroke neuroinflammation, modulating astrocyte-microglial interactions and limiting post-ischemic astrogliosis [3].

In parallel with the altered ECM synthesis, we observed structural remodeling of perineuronal nets (PNNs)—highly condensed, mesh-like aggregates of mature ECM that enwrap fast-spiking neurons. Using advanced 3D superresolution microscopy techniques (3D SR-SIM and 3D STED), coupled with our in-house computational analysis, we quantified PNN nanostructure and discovered a transient increase in PNN facet size during the first two weeks after stroke [4]. This temporary loosening of PNNs facilitated the dynamic reorganization of GABAergic inputs to motor cortical layer 5 interneurons and preceded the recovery of motor coordination.

Interestingly, the observed morphological changes in PNNs were closely associated with increased contact between activated microglia and PNN-coated neurons. Following stroke, microglial cells preferentially targeted PNN-enwrapped fast-spiking interneurons, and even under CSF1R inhibition, microglia maintained their proximity to PNNs. These findings reveal a novel neuroplasticity mechanism involving tripartite interactions between microglia, ECM, and synapses, which plays a critical role in stroke recovery.

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Polarized microtubule remodeling transforms the morphology of reactive microglia and drives cytokine release

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Microglial reactivity is a pathological hallmark in many neurodegenerative diseases. During stimulation, microglia undergo complex morphological changes, including loss of their characteristic ramified morphology, which is routinely used to detect and quantify inflammation in the brain. However, the underlying molecular mechanisms and the relation between microglial morphology and their pathophysiological function are unknown. Here, proteomic profiling of lipopolysaccharide (LPS)-reactive microglia identifies microtubule remodeling pathways as an early factor that drives the morphological change and subsequently controls cytokine responses. We find that LPS-reactive microglia reorganize their microtubules to form a stable and centrosomally-anchored array to facilitate efficient cytokine trafficking and release. We identify cyclin-dependent kinase 1 (Cdk-1) as a critical upstream regulator of microtubule remodeling and morphological change in-vitro and in-situ. Cdk-1 inhibition also rescues tau and amyloid fibril-induced morphology changes. These results demonstrate a critical role for microtubule dynamics and reorganization in microglial reactivity and modulating cytokine-mediated inflammatory responses

Microglial-Neuronal Interactions in the Recovery Phase of Ischemic Stroke

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Stroke remains one of the most common cause of death world-wide. Morphologically, the stroked area can be divided into a necrotic infarct core and a periphery, called the penumbra, where cells survive the initial stroke, but may die later if blood flow is not re-established fast enough. During and after stroke, microglia – the immune cells of the central nervous system – sense a perturbance in the brain parenchyma, migrate into the affected area and release all kinds of pro- and anti-inflammatory cytokines, growth factors and matrix-metalloproteases. Mediation of these ambivalent processes makes it difficult to investigate what role microglia play in the recovery phase of stroke.

We utilize a Cre-dependent DREADD (Designer Receptor Exclusively Activated by Designer Drugs) system to manipulate G protein signaling exclusively in microglia. We show that Gq activation in microglia triggers Ca^{2+} transients and leads to a retraction of cellular processes. The DREADD system allows effective manipulation of microglia without side effects or co-activation of other cell types, helping to decipher mechanisms of stroke recovery directly related to microglia. To study the effects of microglia activation on neurons and synapses, we use oxygen-glucose deprivation (OGD) of organotypic hippocampal slice cultures to mimic stroke conditions *in vitro*. OGD (20 min) affects both microglia morphology and the density of spines on CA1 pyramidal cell dendrites, and these effects are modulated by pre-activation of Gq. In summary, our methodological approach helps us to understand the impact of microglia on neuronal survival and synaptic function in the stroke penumbra.

The matricellular protein hevin in reward-related plasticity

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Astrocytes in the nucleus accumbens (NAc) play a dynamic role in regulating synaptic plasticity induced by drugs of abuse through modulation of glutamatergic neurotransmission. Astrocyte-secreted factors may also contribute to the reprogramming of brain circuitry leading to drug-seeking behavior. Here we investigated the role of astrocyte Ca2+ signals in vivo and of the astrocyte-secreted matricellular protein hevin in the rewarding properties of cocaine.

The response of NAc astrocytes to cocaine in freely-moving mice was measured by in vivo fiber photometry during conditioned place preference (CPP) to cocaine. Depletion of Ca2+ using human plasma membrane Ca2+ ATPase and chemogenetic activation were employed to evaluate the contribution of astrocyte Ca2+ signals to CPP to cocaine. The effects of cocaine in hevin-null mice and after RNAi-mediated hevin knockdown in NAc astrocytes were evaluated by imaging of medium spiny neuron spines after gene-gun DiI delivery, electrophysiology and CPP. Hevin secretion by astrocytes upon chemogenetic stimulation was monitored by light-sheet imaging of hevin-SEpHluorin in brain slices.

Cocaine exposure increased the amplitude of Ca2+ signals in astrocytes in vivo during CPP conditioning. Attenuating Ca2+ signals in astrocytes prevented cocaine CPP, whereas augmenting these signals potentiated this conditioning. Astrocyte activation induced a surge in hevin secretion ex vivo. Hevin knockdown in NAc astrocytes led to a decrease in cocaine CPP and in structural and synaptic plasticity in medium spiny neurons.

These findings reveal a fine-tuning by cocaine of in vivo Ca2+ signals in NAc astrocytes. Astrocyte Ca2+ signals are sufficient and necessary for the acquisition of cocaine-seeking behavior. Hevin can be released upon astrocyte activation, and is a major effector of the action of cocaine and calcium signals on reward and neuronal plasticity.

Structure-function analysis of PNN in health and diseases

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The Perineuronal Net (PNN) is a specialized form of the extracellular matrix (ECM) with a diverse composition that includes proteoglycans, glycoproteins and hyaluronic acid, and is notably rich in chondroitin sulfate proteoglycans. PNNs play a crucial role during the formation of and the development the nervous system. However, after the critical period, the functions of the PNN and its localization on certain neurons in the cortex remain unclear.

Studies based on indirect evidence suggest that PNNs facilitate the onset and closure of the critical period, and are necessary for long term memory formation, neuronal isolation, protection from free radicals and limiting synaptic plasticity. However, there is a dearth of data on the mechanisms by which specific combinations of matrix components participate in plasticity and memory retention. Additionally, the reason only certain neurons are covered by PNN while others are not is not well understood.

Many studies have focused on parvalbumin-expressing neurons, as they are considered the largest population of neurons enveloped by PNNs. However, this is true only for specific brain regions such as the visual and somatosensory cortex, and the CA3 region of the hippocampus. This pattern does not apply to the CA1 region of the hippocampus, the amygdala, and other regions. Contrary to previous reports that considered the matrix as a uniform structure for neurons, it has been demonstrated that PNNs exhibit distinct populations with characteristic differences across various brain regions. Using expansion microscopy (ExM) coupled with confocal microscopy of the somatosensory cortex, restrosplenial area and hippocampus, distinct PNN subpopulations differing in composition were identified. In combination with specialized image analysis pipelines, it was possible to generate detailed 3D models of PNN-covered neurons, revealing structural heterogeneity within single neurons.

We wish to expand the current HLT model (Hyaluronan, Lecticans, Tenascins) describing the structural hierarchy of the PNN to include PNN microenvironments. We propose that the matrix components surrounding synaptic contacts with different receptor compositions have a differential structure, forming a "mosaic."

It is a well-known fact that PNNs degrade or modulate in various of brain pathologies due to changes in the expression levels of various metalloproteinases responsible for matrix remodeling. Among them are Alzheimer's disease and fragile X syndrome. We found that degradation selectively affects certain components of the matrix, underscoring the functional dependence of individual PNN components.

The ongoing task is to elucidate the fundamental principles governing matrix assembly. By utilizing unique inhibitors synthesized in our group, we aim to modulate the restoration of perineuronal net (PNN) components and, consequently, influence neuronal synaptic functions. These investigations have the potential to inform therapeutic strategies targeting PNNs, with the goal of mitigating neurodegenerative conditions and enhancing cognitive function.

Symposium

S8: A neurobiological and computational framework for understanding the complex sensory symptoms of autism

S8-1 Do early auditory processing disruptions associated with autism cause hyperreactivity to sound?
Susanne Schmid, Ella Doornaert, Alaa El-Cheikh Mohamad, Ala Seif

S8-2 Neural alterations in the neocortex underlie tactile perception changes in a mouse model of autism
Andreas Frick, Ourania Semelidou, Théo Gauvrit, Célien Vandromme, Yves Le Feuvre, Anna Saint-Jean, Alexandre Cornier, Melanie Ginger

S8-3 *In vivo* investigation of spontaneous neuronal activity during zebrafish development using light-sheet microscopy
Gesine Fiona Müller, Thomas Offner, Thomas Frank, Jan Huisken

S8-4 Aberrant updating of internal models in autism
Jean-Paul Noel

S8-5 Alterations in subcortical sensory pathways in autism
Katharina von Kriegstein

Do early auditory processing disruptions associated with autism cause hyperreactivity to sound?

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Autism spectrum disorder (ASD) is a neurodevelopmental condition affecting one in 160 children world-wide. A core symptom of ASD is hyperreactivity to sensory stimuli, including to sound. Both autistic humans and Cntnap2 KO rats, a rodent model for neurodevelopmental disorder, show hyperreactivity to sound, measurable e.g. by a highly exaggerated startle response. However, measurements of auditory brainstem responses (ABRs) in autistic humans have shown that auditory signals in the brainstem are attenuated and delayed after birth and only catch up in amplitude and speed by reaching adulthood, which is in stark contrast with the increased sound reactivity.

We measured the ABRs and acoustic startle responses within the same individual autistic humans and Cntnap2 KO rats of both sexes and during different age stages. The results confirmed that the same individuals express attenuated ABR responses and exaggerated startle, with the ABR normalizing by adulthood while the startle responses are increasingly exaggerated. This leads us to hypothesize that the lower ABR responses early in development might trigger some compensatory upscaling of auditory signalling in sensorimotor pathways during a critical period of sensory development that becomes maladaptive when the auditory brainstem eventually normalizes, leaving the individual with the highly exaggerated auditory reactivity. If true, early intervention during the critical period of sensory plasticity should be able to change this developmental trajectory and potentially impede the compensatory changes in reactivity. We test this in Cntnap2 rats by two interventions during the critical period: a highly enriched sensory environment that provides ample of auditory and other stimulation, and a treatment with the GABAB agonist R-baclofen through systemic injections restricted to postnatal days 14 -21, which is during the critical period of auditory development. Animals are tested as juveniles and as adults. Preliminary data show that the environmental enrichment has only a very mild effect on startle responsiveness and other ASD symptoms. R-Baclofen treatment reversed the increased startle phenotype in a first cohort, however, further verification of these results is ongoing.

In summary, our study shows that hyperreactivity to sound associated with autism does not indicate higher sensitivity to sound, but higher reactivity due to potentially compensatory upscaling of auditory signals during a critical period of sensory development of sensorimotor pathways.

Neural alterations in the neocortex underlie tactile perception changes in a mouse model of autism

Andreas Frick[1], Ourania Semelidou[1], Théo Gauvrit[1], Célien Vandromme[1], Yves Le Feuvre[1], Anna Saint-Jean[1], Alexandre Cornier[1], Melanie Ginger[1]

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Touch is fundamental for our interaction with the world, and atypical tactile experience is one of the core characteristics of autism, significantly affecting daily life. However, we do not know the neural underpinnings of low-level tactile perception and how they change in autism. Using a translational perceptual task, we reveal that concomitant neuronal activation and inhibition in the primary somatosensory cortex encode tactile stimuli and determine their detection. We recapitulate the multifaceted tactile features of autistic individuals in the Fmr1-/y mouse model of autism, showing tactile hyposensitivity, interindividual variability, and unreliable responses. Weak stimulus encoding in Fmr1-/y-hyposensitive mice renders perception vulnerable to the ongoing network state and impedes reliable response decoding. Strengthening stimulus encoding by decreasing neuronal hyperexcitability in Fmr1-/y -hyposensitive mice improves tactile perception. Our work shows an evolutionarily conserved role for the primary somatosensory cortex in tactile detection and presents a highly translational approach for probing neuronal-perceptual changes in neurodevelopmental conditions.

In vivo investigation of spontaneous neuronal activity during zebrafish development using light-sheet microscopy

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The emergence of spontaneous neuronal activity during central nervous system development remains enigmatic. With its small size, high transparency, and rapid development, the zebrafish has evolved into a powerful model system to investigate these dynamic neural processes. However, capturing whole-brain activity at sufficiently high temporal resolution remains challenging. Light sheet microscopy has the potential to fill this gap particularly due to its ability to acquire fast volumetric stacks, low phototoxicity, and high speed [1], [2]. We leverage the custom-built Flamingo light sheet microscopy platform [3] to investigate neural activity patterns in developing zebrafish embryos by high speed and long-term imaging of its entire brain.
Using transgenic zebrafish lines expressing the genetically encoded calcium indicator GCaMP6s [4], we apply time-lapse imaging to capture and analyze neuronal activity across the entire brain during early developmental stages [4], [5]. Specifically, our focus is on identifying the critical time points and neuronal ensembles associated with the emergence of distinct, brain-wide patterns of spontaneous activity and their subsequent development.
To further understand how the activity of specific neuronal populations relates to early behavior, we will specifically examine the development of both activity and morphology of sparser groups of neurons, such as reticulospinal neurons and motoneurons [6]. These neuronal groups are known to play key roles in initiating motor behaviors, and tracking their structural changes and functional activity over time could provide new insights into the mechanisms that drive spontaneous behavior in the developing nervous system [7].
By relating locomotor circuit activity to global patterns of neuronal activity, we aim to elucidate how neuronal ensembles are involved in spontaneous behavior emergence during zebrafish development.

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Aberrant updating of internal models in autism

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It is well established that individual on the autism spectrum disorder (ASD) show anomalies in (multi)sensory processing. In this talk I will show that these anomalies are not due to a dysfunction in multisensory integration per se, but a broader computational anomaly in causal inference - inferring what hidden sources caused sensory signals. I will then describe initial work we have conducted over 3 different monogenetic models of ASD to understand the neurobiological and genetic underpinning of bayesian inference and it's dysfunction in autism.

Alterations in subcortical sensory pathways in autism

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The majority of neuropsychological theories and experiments on autism spectrum disorders (ASD) focus on emotional, motivational and cognitive alterations and the integrity of cerebral cortex or the limbic system. ASD is, however, associated with sensory alterations and their role for the autism phenotype is unknown. In my talk, I will present functional magnetic resonance imaging (fMRI) studies showing that ASD is associated with alterations already in the sensory pathways. Using fMRI with high spatial resolution we showed that the so-called magnocellular section of the lateral geniculate nucleus (mLGN) is altered in adults with ASD in contrast to controls. This finding confirmed a longstanding hypothesis on a ‘magnocellular deficit’ in ASD and could explain why people with ASD have difficulties in several aspects of visual motion perception. Visual motion perception is critical for recognition of many social signals e.g., facial motion revealing emotions.

Symposium

S9: Neuronal circuits, energy state and eating disorders

S9-1 Melanocortin 3 receptor neuron activity across night and day, fed and fasted
Robert Chesters, Selma Yagoub, Katrin Ritter, Rachel Lippert

S9-2 Lateral hypothalamic neurotensin-expressing neurons shape the balance between drinking, feeding and socializing
Chantal Wissing, Anne Petzold, Hanna Elin van den Munkhof, Tatiana Korotkova

S9-3 Alterations in neural activity and dopamine release induced by specific nutrients during times of need
James Edgar McCutcheon

S9-4 Food reward thresholds and binge-eating vulnerability are epigenetically determined by *Tet1* dosage in dopamine neurons
Tim Gruber, Robert Chester, Luca Fagnocchi, Stefanos Apostle, Josef Gullmets, Melanie Huber, Lisa DeCamp, Brooke Grimaldi, Ilaria Panzeri, Rachel Lippert, Andrew Pospisilik

S9-5 A main role for the Nucleus accumbens in energy balance: relevance for eating disorders
Pierre Trifilieff

Melanocortin 3 receptor neuron activity across night and day, fed and fasted

Robert Chesters[1], Selma Yagoub[1], Katrin Ritter[1], Rachel Lippert[1,2]

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The brain exerts control over energy homeostasis by regulating food intake through signaling within the melanocortin system. Whilst we understand the role of the hypothalamus within this system, how extra-hypothalamic brain regions are involved in the control of energy balance, and how the development of these systems might be perturbed due to early influences, remains under investigation.
The melanocortin-3 receptor (MC3R) is implicated in modulating feeding behavior and body weight changes under different nutritional challenges, and MC3R deficient animals show a defective fasting response. The MC3R is highly expressed in the paraventricular nucleus of the thalamus (PVT): a brain region that integrates information about internal energy state with environmental stimuli to determine feeding and reward behaviors. Understanding the role and the development of MC3R neuronal activity within the PVT could present as an interesting nexus for food intake regulation in various contexts.
In this study we show that, in adult ad-libitum fed mice, MC3R-PVT neuronal activity follows a circadian pattern of activity. Moreover, this fluctuation of activity is dependent on food availability, as a 16-hour overnight fast alters this rhythm. Upon refeeding, however, this activity significantly increases to that seen under fed conditions. To further explore these effects in the PVT, we identified a role of early maternal overnutrition in impacting the development and maintenance of projections to the PVT and assessed changes in neuronal response to fasting established in the non-maternal diet manipulated paradigm outlined above.
In conclusion, we have identified circadian fluctuations in PVT-MC3R neuronal activity. These fluctuations are significantly impacted by energy state, as fasting results in changes to the activity pattern. Further in-depth analysis of PVT MC3R neurons may yield advanced understanding of feeding-related behaviors.

Lateral hypothalamic neurotensin-expressing neurons shape the balance between drinking, feeding and socializing

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In the lateral hypothalamus (LH), distinct cell populations play critical roles in regulating innate behaviors that support survival. Here, we investigated the role of neurotensin-expressing (Nts) neurons in the LH in coordinating feeding, drinking, and social behaviors across different internal states.
Using in vivo calcium imaging, we observed that Nts-LH neurons exhibit distinct activity patterns in response to water and food approach as well as consumption. Notably, Nts-LH neuron responses to water are markedly enhanced following prolonged food deprivation, suggesting that Nts-LH neurons help maintain a balance between hydration and food intake under hunger pressure.
Activation of Nts-LH neurons counteracted hunger pressure by promoting water intake and rapidly increasing water consumption. While the activation of Nts-LH neurons also moderately increased food intake, the relative consumption of water was higher compared to food, indicating that Nts-LH neurons maintain the balance between water and food intake.
Remarkably, Nts-LH neurons also responded to social stimuli, preferentially to unfamiliar conspecifics. Activation of Nts-LH neurons decreased social exploration, particularly of unfamiliar conspecifics.
Using anterograde tracing, we identified the anterior thalamic output targets of Nts-LH neurons. Optogenetic stimulation of these neurotensinergic projections modulated food-seeking behaviors dynamically, depending on hydration status and food availability. This stimulation enhanced responsiveness to food-related cues and influenced social interactions, highlighting the potential role of thalamic output targets in regulation of innate behaviors by Nts LH neurons. These findings indicate that Nts-LH neurons and their outputs are critical for balancing internal states by modulating competing behaviors such as feeding, drinking, and social interactions, thereby providing new insights into how these neurons contribute to survival through the integration of internal and external stimuli.
We gratefully acknowledge support by the ERC Consolidator Grant (772994, FeedHypNet, T.K.) and DFG (Project-ID 431549029 – SFB 1451 T.K., EXC2030 CECAD T.K., EXC 2030 – 390661388, A.P.).

Alterations in neural activity and dopamine release induced by specific nutrients during times of need

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Acquiring the necessary balance of nutrients in one's diet is a compelling problem faced by many animals including humans. For the macronutrient protein, this process is particularly pertinent as essential amino acids cannot be stored so must be constantly sourced through dietary choices. Thus, behavioural and physiological mechanisms likely exist to help compensate for any deficiency. Accordingly, we and others have shown that animals fed a low-protein diet develop a strong preference for protein over carbohydrate. This preference develops rapidly and is also associated with increased motivation for protein in an operant-responding paradigm.

To explore the neural basis of this shift in behaviour we have been using a combination of calcium imaging, voltammetry, and activity-dependent "trapping" of neural populations in rodents. Using fibre photometry and voltammetry, we have shown that mesolimbic circuitry is modulated by the state of protein restriction with activity in ventral tegmental area and forebrain dopamine release elevated when animals are in need. In addition, with single cell multiphoton microscopy we have shown that an important projection to the VTA – GABAergic Vgat neurons in lateral hypothalamus – is similarly modulated by the state of protein restriction. Furthermore, we are using transgenic FosTRAP mice to identify how the state of protein restriction alters whole-brain patterns of neural activity evoked by consumption of protein and infusion of protein directly into the stomach.

Food reward thresholds and binge-eating vulnerability are epigenetically determined by *Tet1* dosage in dopamine neurons

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Midbrain dopamine (mDA) neurons are required for the formation of reward-associated memories, a vital mechanism allowing animals to adapt to their environment. On the flipside, dopaminergic dysfunction caused by repeated exposure to unnaturally strong reinforcers can trigger addiction-like behaviors. Here, we show that mice given intermittent access to hyperpalatable foods (HPF) develop compulsive, binge-style eating behaviors. In stark contrast to conventional obesity models with continual HPF access, this was associated with a dramatic hyperactivation of mDA neurons during food intake, which when chemogenetically inhibited prevented binge-eating. Intriguingly, humans as well as other animals display striking inter-individual differences in their mDA reward circuitries and by extension in their vulnerability to develop addiction-like behaviors like binge-eating disorder, which can even be observed despite identical genetics and environment. Thus, we here sought out to explore the role of epigenetic modifications as potential drivers of individuality in mDA reward circuitries and binge-eating susceptibility, respectively. To accomplish this, we purified fluorescently tagged nuclei of mDA neurons from control mice versus mice trained to binge-eat on HPF and performed DNA profiling using Enzymatic Methyl (EM)-seq. The genome-wide assessment of both 5-methylcytosine (5mC) and 5-hydroxymethylcytosines (5hmC) landscapes revealed a striking reorganization of DNA modifications partially overlapping with molecular changes observed upon cocaine exposure. The nature of this reorganization suggested to us a prominent role of demethylating enzymes such as Ten-Eleven Translocation 1 (TET-1) dioxygenase, which was highly enriched in mDA neurons. Consistently, global and mDA-specific *Tet1* haploinsufficient mice ($Tet1^{\Delta FL/wt}$ and mDA:$Tet1^{\Delta/wt}$) exhibited marked differences in mDA neurocircuit architecture and in motivated, reward-related behaviors including binge-eating. Notably, bistable segregation into either binge-prone versus binge-resistant animals was significantly amplified in these mice, which was linked to gene set enrichments regulated by the transcription factor Early Growth Response 1 (EGR-1), a known TET-1 binding partner. Therefore, we virally overexpressed an EGR1-TET1 fusion protein specifically in mDA neurons and found that Egr1 motif-specific epigenome editing was sufficient to accentuate binge-eating in *Tet1* haploinsufficient mice. In sum, our results suggest that individual food reward threshold in binge-eating disorder are determined by *Tet1* dosage in mDA neurocircuits and hints at the fact that a substantial fraction of addiction-like vulnerability is epigenetically, not genetically, defined.

A main role for the Nucleus accumbens in energy balance: relevance for eating disorders

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Accumulating evidence points to dysregulations of the Nucleus Accumbens (NAc) in eating disorders (ED), however its precise contribution to ED symptomatic dimensions remains unclear. Using chemogenetic manipulations in male mice, we found that activity of dopamine D1 receptor-expressing neurons of the NAc core subregion facilitated effort for a food reward as well as voluntary exercise, but decreased food intake, while D2-expressing neurons have opposite effects. These effects are congruent with D2-neurons being more active than D1-neurons during feeding while it is the opposite during running. Chronic manipulations of each subpopulations had limited effects on energy balance. However, repeated activation of D1-neurons combined with inhibition of D2-neurons biased behavior toward activity-related energy expenditure, whilst the opposite manipulations favored energy intake. Strikingly, concomitant activation of D1-neurons and inhibition of D2-neurons precipitated weight loss in anorexia models. These results suggest that dysregulations of NAc dopaminoceptive neurons might be at the core of EDs.

Symposium

S10: Sex, glia and disease: understanding sex-specific glia biology in health and disease

S10-1 Sex differences impact an astrocyte-mediated synaptic elimination in health and major depressive disorder
Barbara Di Benedetto

S10-2 The feMale epigenome: sex-specific epigenetic profiles in health and disease
Julia Schulze-Hentrich, Samantha Schaffner, Michael Kobor, Thomas Hentrich

S10-3 A role of prefrontal inputs to lateral hypothalamus and their noradrenergic modulation in coping with stress
Alisa Bakhareva

S10-4 Mind the Gender Gap: Charting New Territories with Computational Glial Models
Kerstin Lenk

S10-5 Computational analysis of sex differences in omics data for Alzheimer's and Parkinson's disease
Enrico Glaab

Sex differences impact an astrocyte-mediated synaptic elimination in health and major depressive disorder

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Sex differences have long been recognized as a variable affecting many brain disorders in terms of predisposition, rates of incidence, age of onset, symptomatology and outcomes. However, research on sex-dependent disorders and their neurobiological molecular and cellular causes is still scarce, thereby limiting the development of sex-specific diagnoses and treatments.
Studies on the asymmetric development of psychopathologies has revealed sex-dependent differences in volume and tissue density in areas implicated in sex-biased pathological conditions. Among them, the cortex showed a high sex-dependent diversification in synaptic densities, with an aberrant pruning of weaker synapses being recently regarded as a mechanism relevant for the onset of brain disorders. Most of these remodeling events take place during the postnatal critical periods of brain development, when astrogenesis parallels synaptogenesis to guide the formation/elimination of neuronal synapses and when the masculinization of the brain is induced by the neonatal surge of male gonadal activity. Astrocytes respond to circulating gonadal hormones, which influence their relative sex-dependent maturation rates and possibly impact the sex-dependent astrocyte-mediated synaptic elimination of weaker synapses. Astrocytes regulate synapse elimination prevalently through the multiple EGF-like domains 10 (MEGF10) phagocytic pathway, mostly directed toward glutamatergic synapses.
Using a combination of ex vivo and in vivo model systems together with high-sensitive tissue labelling techniques and STED microscopy with IMARIS software-assisted 3D reconstruction analysis, we revealed early sex- and age-dependent different patterns of astrocyte-mediated synaptic elimination in the cortex of rat brains from postnatal day (P) 7 through P32, which especially impact glutamatergic synapses. These differences may accont for sex-dependent onset and course of brain pathologies and responses to pharmacological treatments in women and men and might be helpful to develop alternative personalized treatment regimens.

The feMale epigenome: sex-specific epigenetic profiles in health and disease

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Parkinson's disease (PD), a progressive neurodegenerative disorder, exhibits a higher prevalence and severity in males compared to females. This disparity suggests underlying sex-specific biological mechanisms that influence disease onset and progression in this rather multifactorial disorder which is seemingly based on an interplay of genetic predispositions, aging and environmental influences. In this complex interplay along the gene-environment axis, the epigenome increasingly emerges as a central nexus that integrates environmental variables into the genetic program and its regulation in a sex-dependent manner.

However, although sex, genetics, and exposures can individually influence risk for sporadic PD, the joint contributions of these factors to the epigenetic etiology of PD are still unclear. Together with the DIGPD-study group, we profiled sex-stratified genome-wide blood DNA methylation patterns, SNP genotype, and pesticide exposure in agricultural workers and found more associations of blood DNA methylation with PD in females than in males.

In order to extend these descriptive observations and to understand basic mechanisms underlying the described differences, we are using transgenic rodent models to profile their sex-dependent brain transcriptomes and epigenomes at pre-symptomatic as well as symptomatic stages. Here, we will present an overview of our current findings and discuss next steps required to decipher the feMale epigenome in health and disease.

A role of prefrontal inputs to lateral hypothalamus and their noradrenergic modulation in coping with stress

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Animals experience various forms of stress - such as hunger, thirst, social isolation and aggression - throughout their lifetime. To successfully cope with such stressors, animals need to flexibly adapt behaviour. The impact of stress on behavioural adaptation depends on the type and duration of stress, as well as on the sex and age of the animal, and individual vulnerability.

The medial prefrontal cortex (mPFC) is involved in the stress response and in adapting behaviour to a certain context. One of the output targets of the mPFC is the lateral hypothalamus (LH), a brain region that regulates innate behaviours. Yet, little is known about whether and how the prefrontal-hypothalamic circuit mediates the influence of stress on innate behaviours. Both norepinephrine (NE) and dopamine (DA) dynamics respond to stressful experiences and strongly modulate neuronal activity in mPFC and LH. However, the dynamics of neuromodulator release in mPFC and LH during stress experiences is still elusive.

To address these questions, we first optogenetically stimulated mPFC inputs to LH and analysed innate behaviours of mice following physical, metabolic or social stress. We found that this circuit promotes behaviours that alleviate stress, such as food seeking following fasting or seeking out conspecifics following restraint. Further, we employed dual-site, dual-colour fibre photometry of neurotransmitter sensors for NE and DA in mPFC and LH of freely-moving mice. We identified the release patterns of these neuromodulators during innate and anxiety-related behaviours in response to different stressors.

Taken together, our data highlights the role of the mPFC-LH circuit and its neuromodulation in state- and context-dependent behavioural adaptation to stress.

We gratefully acknowledge support by the ERC Consolidator Grant (772994, FeedHypNet, to T.K.) and the DFG (Project-ID 431549029 – SFB 1451, to T.K., EXC2030 CECAD, to T.K., EXC 2030 – 390661388, to A.P.).

Mind the Gender Gap: Charting New Territories with Computational Glial Models

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Computational modeling has become an invaluable tool in neuroscience research. However, besides many other aspects, gender differences in experimental data are not reflected in these models. The talk will address this disparity in the development and analysis of sex-specific biophysical models of glial cells, focusing on astrocytes and microglia. I will explore the current landscape of biophysical glial research regarding sex differences. This highlights the need for greater inclusion of sex-specific factors in future modeling efforts.

I will outline a framework for constructing detailed biophysical models of male and female glial cells, incorporating sex-specific membrane properties, ion channel distributions, and subcellular compartmentalization. These models will be based on emerging data from electrophysiological studies and morphological analyses highlighting sex differences in glial cell function.

I argue that developing these models is essential for advancing our understanding of sex differences in neurological and psychiatric disorders. Sex-specific biophysical models, thus, represent a crucial step towards more comprehensive and accurate computational models of the brain. By stepping into new territories in sex-specific biophysical modeling of glial cells, I aim to stimulate further research and collaboration in this important yet understudied area of computational neuroscience.

Computational analysis of sex differences in omics data for Alzheimer's and Parkinson's disease

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Sex differences can play an important role in the risk, manifestation, progression, and treatment of complex neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD). While epidemiological studies have long established sex-specific patterns in disease incidence and progression, the molecular mechanisms underlying these differences remain poorly understood. Here, we present a comprehensive computational analysis of sex differences in omics data for AD and PD, with the aim of uncovering disease-specific molecular factors that contribute to the observed clinical differences.

We used a meta-analytic approach to examine transcriptomic data from multiple human studies for both AD and PD. By testing for statistical interactions between sex and disease status, we categorized sex differences in disease associations as sex-specific (occurring in only one sex), sex-dimorphic (occurring in both sexes, but with opposite directions of change), or sex-modulated (occurring in both sexes, with the same direction of change but significant differences in effect size). This approach allowed us to identify differentially expressed genes, enriched pathways and cellular subnetworks that show different patterns of significant sex-dependent changes in each disease.

For both PD and AD, our analyses revealed numerous sex-dependent differentially expressed genes, many of which have well-established associations with neurodegenerative disorders. Pathway analysis highlighted significant sex-specific changes, for example in hallmark aging processes related to mitochondrial function and inflammation. We also performed gene regulatory network analyses, identifying transcription factors with sex-specific changes and differences in their downstream target genes.

To understand changes in AD during early, pre-symptomatic stages, which are difficult to assess in humans due to the predominance of data from more advanced stages, we extended our investigation to include single-cell RNA sequencing data from mouse models of early AD-like pathology. This analysis revealed significant sex-specific and sex-dimorphic changes in multiple cell types, including findings at the gene, pathway and network levels that overlap with those obtained in human AD.

In conclusion, our computational analyses of sex differences in omics data for AD and PD reveal complex patterns of molecular alterations that may underlie the observed clinical differences. We have identified significant sex-dependent changes at the level of gene expression, pathways, and regulatory networks, including a subset of changes that are also detectable in mouse models of early AD-like pathology. These findings may contribute to the study of early disease mechanisms and inform the development of more patient-tailored, sex-specific diagnostic and therapeutic strategies.

Symposium

S11: Wired for motion: perspectives on motor control

S11-1 Molecular blueprints for spinal circuit modules controlling locomotor speed in zebrafish
Irene Pallucchi, Maria Bertuzzi, David Madrid, Pierre Fontanel, Abdeljabbar El Manira

S11-2 Electrophysiological characterization of central brain neurons controlling walking in *Drosophila*
Sirin Liebscher

S11-3 Multilayer Circuit Processing for Self-Motion Estimation in *Drosophila*
Corinna Gebehart, Tomás Cruz, Claire Rusch, M. Eugenia Chiappe

S11-4 Sensation to action: a spinal perspective
Graziana Gatto

S11-5 Restoring touch through a brain interface: spatio-temporal patterning of microstimulation of human somatosensory cortex
Giacomo Valle

Molecular blueprints for spinal circuit modules controlling locomotor speed in zebrafish

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Locomotion is an essential motor function and is driven by spinal circuits. Despite a growing understanding of the functional heterogeneity of neuronal populations within the locomotor circuit, a molecular characterization of this diversity is lacking. In mouse, recent studies have shown that the molecular diversity of motoneurons (MNs) reflects their muscle innervation pattern rather than electrophysiological subtypes, while no information is available at present regarding premotor interneurons (INs). In zebrafish, swimming is produced by axial muscles, which are spatially segregated into slow, intermediate and fast fibers MN pool with distinct electrophysiological properties. Similarly, the rhythm-generating V2a interneurons are organized into three subtypes that are selectively connected to the MN pools to form three speed circuit modules. These modules are sequentially recruited to increase the speed of swimming. However, it is still unclear if the functional diversity of MNs and V2a INs, and their modular circuit organization, are molecularly encoded. Here we use single-cell RNA sequencing to the detailed molecular diversity of MNs and V2a INs in adult zebrafish. Cluster analysis revealed molecular subtypes within both the MN and V2a IN populations, which were validated using RNAscope, neuronal tracing and whole-cell patch-clamp recordings during swimming. Our results reveal clusters that correspond to the known functional subtypes of MN and V2a IN (slow, intermediate and fast). Specifically, the slow and fast MNs have distinct gene expression profiles while that of the intermediate MNs displays overlapping gene expression. The rhythm-generating V2a IN population clusters into three subtypes. One cluster of V2a INs expressing esrrga display pacemaker firing and are recruited during slow swimming. A second cluster expressing shox2 sareA third cluster includes neurons expressing vachta which display electrophysiological features of fast V2a INs. Furthermore, we show that the MN and V2a IN subtypes are defined by the expression of specific transcription factors. , our analysis uncovers markers, including transcription factors, that are selectively expressed in MNs and V2a INs belonging to the same speed circuit modules, suggesting a shared molecular identity across CPG classes for neurons that are electrophysiologically and functionally similar. Thus, our results reveal the molecular signatures of functional neuronal subtypes within and across different populations of the spinal locomotor circuit.

Electrophysiological characterization of central brain neurons controlling walking in *Drosophila*

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Walking is a fundamental, yet surprisingly complex behavior many animal species rely on. The initiation, direction, and speed of walking must be finely orchestrated for an animal to successfully negotiate complex, dynamic environments. This requires continuous adjustments based on sensory cues. Despite their importance, the neuronal pathways enabling animals to adjust their walking behavior to changing environmental demands are not fully understood.

The fruit fly, *Drosophila melanogaster*, can serve as an excellent model organism for studying the neural circuits that govern locomotion. Despite their name, flies spend a large portion of their lives walking through complex environments. The combination of their compact nervous system, with only approximately 130.000 neurons in the brain and 15.000 neurons in the ventral nerve cord (the fly's version of the spinal cord), an extensive genetic toolkit, and available connectomes, offers the opportunity to systematically investigate the role of different neuronal populations in the control of walking.

We performed an optogenetic activation screen to identify central brain neurons involved in specific aspects of walking, and identified several cell types involved in forward walking, backward walking, and turning. Using intracellular recordings from individual neurons in tethered flies, we measured the neuronal activity of identified neurons during spontaneous locomotion. Thus, we were able to correlate the activity of individual neurons with distinct phases of spontaneous locomotion. Walking-related activity in these neurons aligned with their optogenetic activation phenotypes, and often preceded the behavior. Additionally, we examined how sensory inputs from different modalities shape neuronal activity and, ultimately, the corresponding motor outputs.

In summary, our detailed characterization of neurons involved in walking direction and speed control provides novel insights into the neuronal circuits underlying adaptive locomotion. This work offers an entry point for understanding how sensorimotor pathways control adaptive walking in complex environments.

Multilayer Circuit Processing for Self-Motion Estimation in *Drosophila*

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Coordinated movements depend on the brain's ability to monitor self-motion in real time. An internal estimate of self-motion is essential for various aspects of walking control, from rapid, moment-to-moment postural adjustments to motor planning and action selection. Circuits involved in self-motion estimation provide a unique opportunity to understand how multiple signals are integrated and coordinated within sensorimotor networks to support this multilevel control of locomotion.

Using the small brain of *Drosophila melanogaster* as a model, we have identified a compact network sensitive to the angular movements of an adult walking fly. This multilayered network projects to both the ventral nerve cord (VNC)—the insect equivalent of the spinal cord—and to higher brain regions involved in decision-making during turns. Notably, at intermediate layers, this network receives ascending inputs from the VNC and feedback signals from higher brain areas, suggesting that it serves as a key integration point for angular motion estimation.

To understand the interactions of these signals, we combine whole-cell patch clamp recordings, optical imaging, anatomical connectivity mapping, and cell-type-specific perturbations in the context of exploratory locomotion. Whole-cell recordings from central elements of the intermediate layers, combined with two-photon calcium imaging of various inputs show that the network receives angular motion information at different timescales.

Altogether, these findings uncovered a multi-layer network encoding self-motion information, likely integrating multiple signals at different timescale for state estimation. We are currently analysing how these signals are transformed across different layers of the network and their functional contributions on this transformation. Our goal is to provide a detailed functional map of circuits involved in state estimation for continuous movement control and action selection.

Sensation to action: a spinal perspective

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Behaviors effortlessly performed by animals, such as navigating complex dynamic environments, are extremely challenging for artificial intelligence (AI) systems, given AI limited ability to continuously update decisions and actions based on a sparse representation of the external world. They lack the “sensorimotor intelligence” inbuilt in the animal nervous system by the evolutionary necessity to flexibly adjust to a varying and unpredictable environment for survival. Animals constantly adapt their behavior to minimize threats, reduce metabolic consumption, and satisfy their internal needs, with sensations and actions becoming inevitably interdependent. Thus, the dynamic interplay between sensing and acting renders the process of turning decisions into actions a complex choreography of movements, assessments, and re-tuning rather than a hierarchical sequence of events. The neuronal underpinnings generating this complex choreography are far from being understood, even in simpler behaviors, like locomotion.

My lab aims at understanding how the spinal neural circuits that encode sensations and actions cooperate to shape adaptive motor behaviors. The spinal cord comprises networks of interconnected neuron types, dedicated to the generation of movement and the processing of sensory input. Sensory information is conveyed by peripheral afferents to the dorsal horn of the spinal cord, where it is processed and relayed to the ventral premotor network to elicit reflex responses or to supraspinal nuclei for perception and long-term adaptation. The ventral premotor network comprises many different classes of excitatory and inhibitory interneurons that contribute to set the timing (rhythm) and sequence (pattern) of muscle contractions. Despite the great advances in identifying the heterogeneity of the populations making up the spinal networks, we are still far from understanding how distinct cell types and their synergistic or antagonistic interactions contribute to sensorimotor transformation. We use an intersectional genetic approach to dissect at cellular resolution the spinal circuits underlying sensorimotor integration, and their modulation by descending afferents and sensory feedback. We identified modular networks of excitatory neurons in the dorsal horn, whose differential recruitment dictates the emergence of sensory modality-appropriate reflex responses. These networks are highly heterogenous, comprising many cell types, receive unique combinations of cutaneous sensory input and are differently connected to the ventral premotor network. Within the ventral network, we identified a core circuit composed of excitatory and inhibitory interneurons that synergistically cooperate to set the timing and sequence of ipsilateral body movements across different rhythmic behaviors, e.g. locomotion and scratching. Since some of our experimental findings seemingly contradicted the predictions of the classic half-center models for rhythmic behaviors, we developed a biomechanical model to explain how the complex interactions among diverse neuron types drive rhythm and pattern. Current work is aimed at understanding and modeling the flexibility and robustness of the interactions between the dorsal sensory modules and the ventral premotor circuits during sensorimotor adaptation.

In summary, we identified and modeled a premotor neuronal network that acts as a convergence point for diverse motor responses, functioning as a malleable substrate under the modulation of sensory and descending inputs.

Restoring touch through a brain interface: spatio-temporal patterning of microstimulation of human somatosensory cortex

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Intracortical microstimulation (ICMS) of somatosensory cortex (S1) evokes vivid touch sensations, the properties of which can by systematically manipulated by varying the parameters of stimulation. However, natural touch conveys much richer information about objects and our interactions with them, which supports dexterous manipulation. We seek to expand the repertoire of ICMS-based artificial touch, by judiciously designing spatiotemporal patterns of ICMS inspired by our understanding of tactile coding in S1, and thus to confer greater dexterity to brain-controlled bionic hands.

Symposium

S12: Breaking News

S12-1 All-optical investigation of the role of CaMKII on long-term plasticity in the hippocampus
Rui Wang

S12-2 Visual encoding by retinal ganglion cells in optogenetic models for vision restoration
Varsha Ramakrishna

S12-3 Criteria for Identification and Accurate Quantification of Spinal Motor Neurons in Healthy and Disease Mouse Models
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All-optical investigation of the role of CaMKII on long-term plasticity in the hippocampus

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Synaptic plasticity, inducing long-lasting changes in synaptic efficacy and structure, is a major mechanism of information storage in the brain. Calcium–calmodulin-dependent protein kinase II (CaMKII) is one of the most important memory molecules that, through its autophosphorylation feature, transforms transient activation due to synaptic activity-related increases in calcium into longer-lasting changes in synaptic strength. Whether CaMKII is essential for both induction and/or maintenance of synaptic plasticity has been controversial. We took advantage of optogenetic tools to investigate the role of CaMKII in synaptic plasticity, by inducing synaptic plasticity and manipulating relevant signaling pathways at the same time. Specifically, we induced spike-timing-dependent plasticity (STDP) at Schaffer collateral synapses in rat hippocampal slice culture by independently stimulating the pre- and postsynaptic neurons expressing spectrally separated channelrhodopsins with violet and red light, respectively. The all-optical protocol induced timing-dependent long-term potentiation (tLTP) increased synaptic strength both acutely (about 30 minutes, early tLTP) and more interestingly, chronically (3 days, late tLTP). When we co-expressed a photoactivatable inhibitor of CaMKIIα (paAIP2) only in postsynaptic neurons to inhibit CaMKIIα during the during the 60 s induction protocol, acute tLTP was abolished. Unexpectedly, 3 days later the late tLTP was apparent and indistinguishable from neurons with active CaMKIIα during induction. STDP-induced CA1 neurons received significantly stronger input than their neighbors 3 days after stimulation, a delayed potentiation that appears to be independent of CaMKIIα activity during induction of early tLTP. Coincidently, expression of the immediate early gene c-Fos following induction was also independent of CaMKIIα activity. Interestingly, an inhibitor of protein kinase Mζ, applied 3 hours after STDP induction prevented late tLTP.
Rather than inhibiting CaMKII, 100 s illumination of photoactivatable CaMKII (paCaMKII) in CA1 neurons was sufficient to induce functional early LTP, structural and ultrastructural synapse alterations but no late LTP. By continuous longitudinal large-scale tracking of excitatory synapse and spine dynamics for about 14 hours after paCaMKII activation, we noticed that the structural plasticity developed in a spatially clustered manner. Together, these data suggest that activity-dependent potentiation of synaptic inputs has two phases: CaMKIIα is necessary and sufficient for the induction of early LTP. A second, CaMKIIα independent mechanism, dependent on the persistent activity of protein kinase Mζ, is responsible for the selective strengthening of inputs days later.

Visual encoding by retinal ganglion cells in optogenetic models for vision restoration

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Retinal degeneration is one of the leading causes of blindness and optogenetics as a potential therapeutic measure has garnered much attention. Naturally light-sensitive molecules like Channelrhodopsin (ChR2) and other engineered ion channels are inserted into the neurons in the inner retina to play the role of light-sensing elements after the loss of photoreceptors. Previous studies have shown responses of retinal ganglion cells (RGCs) in blind animal models with optogenetically modified retinas, mostly based on simple light stimuli. Our study aims to directly compare encoding by RGCs under photoreceptor and optogenetic stimulation in response to spatiotemporally complex and natural stimuli. Furthermore, we would like to estimate an optimal stimulation of such modified retinas to elicit responses like that in a normal retina.

Preliminary experiments using multielectrode array recordings with retinal ganglion cells expressing ChR2 showed light-dependent optogenetically driven responses in the RGCs. Temporal filtering, as assessed via a linear-nonlinear model fitted to ganglion cell responses under flickering stimulation, was much faster under ChR2 activation compared to photoreceptor activation. This is in line with the direct stimulation of RGCs by ChR2 with no photoreceptor or bipolar cell processing. The estimated receptive field sizes of the ganglion cells based on photoreceptor-evoked and ChR2-evoked responses to spatiotemporal white noise had a significant difference in size possibly due to the absence of canonical center-surround receptive field activation under ChR2 stimulation.

We also compared responses of RGCs to natural images under photoreceptor and optogenetic stimulation. We saw that the responses of RGCs under the two conditions were characteristically different which reflected the role of both ChR2-channel dynamics and cellular spiking machinery (cell-type specificity) in generating responses to natural stimuli. Based on these differences, the natural images were modified by methods such as thresholding, scaling and blurring and presented to the RGCs under ChR2 activation to elicit responses like that under photoreceptor activation. We found that certain modified images elicited stronger and also more similar responses to the photoreceptor-evoked responses than unmodified images under ChR2 activation in a cell-type dependent manner.

These findings will help in generating better optogenetic therapies for patients by targeting cell-type specific stimulation and expression of optogenetic constructs in the retina to achieve more natural vision.

Criteria for Identification and Accurate Quantification of Spinal Motor Neurons in Healthy and Disease Mouse Models

Aaron Lorenzo Norman, Leonie Sowoidnich[1], Florian Gerstner[1], Josianna Kelly Sime-Longang[1], Jannik Maximillian Buettner[1], John G. Pagiazitis[2,3], Konstantin Pilz[1], Katharina Sophie Apel[1], George Z. Mentis[2,3], Christian Marc Simon[1]

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Introduction. Motor neuron (MN) death is the hallmark of MN diseases such as spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS). Quantification of MN loss in mouse models is an important readout for disease progression and therapeutic assessment. However, there is a large variability in MN death reported by different groups, even within identical mouse models, which makes interpretation of therapeutic assessments difficult. This variability of MN numbers may depend on different technical approaches to label MNs as well as investigating distinct areas of the spinal cord with differential vulnerability.

Materials and Methods. First, a meta-analysis of MN death in SMA mouse models was conducted using PubMed search results from which 77 papers were included in the analysis. Data from experiments done on the three commonly used SMA mouse models were collected and compared. Next, MNs of selected lumbar spinal cord segments of C57/BL6 mice were labelled via ventral-root backfills, immuno-stained subsequently for choline acetyltransferase (ChAT), cleared and imaged via confocal microscopy. Selected spinal segments of SMNΔ7, SOD1-G93A and control animals were prepped similarly. Additionally, spinal sections of C57/BL6 mice were stained and imaged in groupings for ChAT, SMI-32 and Nissl or ChAT and Hb9. Lastly, we developed a novel approach for automated MN counting in the intact spinal cord by combining clearing with confocal imaging and open-source image analysis software.

Results. Here, we show in the meta-analysis a lack of dissection specificity, the use of unspecific neuron markers, and a wide distribution of reported MN loss within the same mouse models underlining the importance of a consistent pipeline for MN quantification. To address this inconsistency, morphological criteria were determined to ensure consistent quantification of MNs. First, we describe an anterior spinal corpectomy, allowing segment specific MN isolation and counting. In combination with ex vivo ventral-root backfills and immunohistochemistry, we concluded that ChAT and HB9 are reliable markers for ventral horn MN pool identification. In contrast, Nissl and SMI-32 immunoreactivity are neither selective to MNs nor to the ventral pool. Second, ventral-root backfills of MNs within select lumbar segments combined with tissue clearing and ChAT immunoreactivity revealed that different spinal segments contain different numbers of MNs. Third, comparisons of MNs within the lumbar intumescentia of SMA mice exhibit selective MN death restricted to specific spinal segments while global spinal MN loss was evident in the ALS mouse model, SOD1-G93A. Finally, MNs marked with HB9 in select spinal segments were counted by an automated open-source counting software, Cellpose, to provide an unbiased quantification of MNs.

Conclusion. Our detailed procedural account demonstrates that a select set of criteria is required for the

valid identification of motor neurons and their accurate quantification of MNs in normal and diseased mouse models. Furthermore, our results can be used as a reference for future studies requiring accurate assessment of MN counts as part of therapeutic assessment.

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Experience- and state-dependent adaptation of eating behavior by BDNF-expressing lateral hypothalamic populations

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In modern societies, we live in environments that are highly conducive to eating – with high food palatability, easy food availability, and omnipresence of food cues – leading to increased caloric intake, weight gain and associated health disorders. Therefore, the identification of anorectic mechanisms is of paramount importance for public health. Eating behaviour is regulated through neurochemically distinct neural subpopulations of the lateral hypothalamus (LH). In this study, we focused on an LH subpopulation that secretes the neurotrophin brain-derived neurotrophic factor (BDNF) and may thereby influence the formation of eating patterns over time through synaptic plasticity mechanisms.

Using single-cell Ca^{2+} imaging in freely moving mice with free access to nutritional and social rewards, we found that $BDNF^{LH}$ neurons are excited by food more than by any other rewards. The prevalence of food-encoding among $BDNF^{LH}$ neurons increased over time. To investigate whether the activity of $BDNF^{LH}$ cells affects feeding behaviour, we selectively activated $BDNF^{LH}$ cells in freely behaving mice using a chemogenetic approach. Chemogenetic activation of $BDNF^{LH}$ neurons acutely decreased the consumption of unhealthy, but palatable, high fat food after three days of activation of $BDNF^{LH}$ neurons, without affecting food intake over 24 h, body weight or glucose tolerance compared to control group. Conversely, the activation of $BDNF^{LH}$ neurons did not impair homeostatic re-feeding after overnight food deprivation. Thus, chemogenetic activation of $BDNF^{LH}$ neurons acutely affects food intake in a state-dependent manner without shifting the homeostatic body weight setpoint. To test whether $BDNF^{LH}$ neurons are involved in the experience-dependent adaptation of eating behavior, we performed a context-conditioned overconsumption task. Here, hungry animals are allowed to feed in one particular context, leading to overconsumption of sated animals when presented again with the conditioned context previously associated with satiation. We found that chemogenetic activation of $BDNF^{LH}$ neurons during consolidation of the conditioned context led to overconsumption across contexts. Satiation is mediated by peripheral hormones such as leptin, which is released by adipose tissue in response to a meal and reaches hypothalamic control centers through the brain's ventricular system. To evaluate the degree of leptin sensitivity among $BDNF^{LH}$ neurons, we performed calcium imaging and patch clamp experiments and found that $BDNF^{LH}$ neurons tend to be activated by leptin. Overall, our findings suggest an important role of $BDNF^{LH}$ neurons in the experience- and state-dependent adaptation of eating behavior.
Thereby, we provide an entry point for the identification of neural mechanisms that shape eating patterns and that could potentially be harnessed to limit food overconsumption in the modern obesogenic environment.

Appetite for Aggregation: How starvation fuels locust social life

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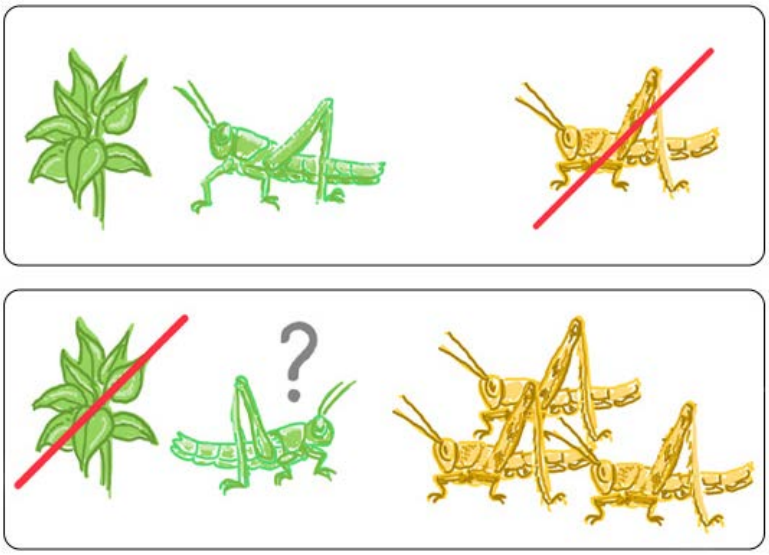
Desert locusts (*Schistocerca gregaria*) undergo a dramatic phase change, between cryptic (solitarious) and swarming (gregarious) forms, culminating in locust outbreaks that threaten global agriculture. With rising temperatures, recurrent extreme weather events, and droughts, climate change is expected to exacerbate the frequency and intensity of outbreaks. Drought conditions reduce food availability, driving locusts to aggregate around scarce resources, which can trigger a cascade of behavioural and physiological adaptations, leading to crowding and swarming. While much research has focused on gregarious locusts, less is known about the solitarious phase and the mechanisms that drive gregarisation, particularly in relation to hunger.

In this study, we explore how starvation affects locust behaviour, especially focusing on aggregation tendencies. Through a combination of behavioural experiments and functional calcium imaging, we investigate how the locust's internal states influence social preferences and neural responses to conspecifics and food-related odours. Our behavioural assays reveal that hungry solitarious locusts exhibit a heightened tendency to aggregate, highlighting the role of hunger in triggering locust aggregations, and potentially swarming.
Calcium imaging of the antennal lobe allowed mapping the neural processing of social and food odours in both fed and starved locusts. Our findings suggest differential odour responses based on feeding state, offering insights into the neural mechanisms driving behavioural changes during gregarization.
The study highlights the importance of internal states and environmental factors in regulating locust phase change and contributes to the global effort to manage the increasing risks of future locust outbreaks.



Schematic representation of how internal state affects social preferences in solitarious locusts. While fed solitarious animals avoid conspecifics (top), starvation seems to promote aggregation (bottom).

A Novel AI-based Tool for Real-Time USV Detection as Unbiased Markers of Distinct Social Interactions

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Ultrasonic Vocalizations (USVs) are integral in the complex world of rodent communication especially during social interactions. Unfortunately, these interesting communicative cues have remained elusive for a long time, hidden beyond the human range of hearing. Only in the mid-1950s USVs were discovered and became the subject of extensive research.

Notably, researchers identified USVs as reliable biomarkers for neurological conditions, including Autism Spectrum Disorder and Parkinson's Disease. Yet, the process of manually identifying USVs proved challenging and demanded significant human effort. Recent advances in Digital Signal Processing and Machine Learning allowed the development of novel tools offering automatic and semi-automatic identification and analysis of USVs.

Within this context, we will introduce DeepFisFis, a novel end-to-end solution for real-time detection of USVs utilizing a 1-Dimensional Convolutional Neural Network (1D-CNN). Here, we demonstrate the functionality of DeepFisFis by analyzing the USV emission pattern of mice during a social familiarization task monitored in a purpose-built multi-modal experimental arena.

We show that DeepFisFis was able to reliably detect ultrasonic vocalizations of mice. Interestingly, the vast majority of USVs were emitted during periods of intense social contact, classifying USVs as a facile indicator of close social interactions. Notably, the number and total duration of USVs decreased as a function of repeated social interactions, identifying them as ethologically relevant and unbiased biomarkers for social habituation. These findings are corroborated by A-SoiD- guided automated postural analysis, underlining the strong correlation between USV emission during close social contact and suggesting the association between USVs with different types of social behavior in mice.

Functional characterization of target-defined MTCs in olfactory information processing

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Parallel processing of sensory information is a crucial mechanism to decode complex information from the environment, which is well studied in most sensory modalities except chemical senses. The two main types of output neurons of the olfactory bulb (OB), mitral and tufted cells (MTCs) convey odor information to different but partially overlapping regions of the olfactory cortex. However, the question of whether these different regions of the olfactory cortex also receive differential input from the OB remains elusive, yet.

This study aimed to investigate target-specific odor coding of MTCs in the olfactory bulb during odor information processing by the use of adeno-associated virus (AAV)- mediated retrograde tracing from the anterior olfactory nucleus (AON) and the anterior piriform cortex (APC). Using widefield microscopy, the activity of different MTC populations was imaged with virally expressed GCaMP6f. Odor responses of AON- and APC-traced MTC populations showed significant differences in respiration-linked activity but response onset times and spatial response maps were similar between the two populations. Subsequently, we measured single-cell level responses of these populations during odor stimulation using two-photon microscopy. We found that MTCs projecting to AON respond to a wider range of odorants compared to APC projecting cells. On the other hand, MTCs targeting APC were more sensitive to concentration changes. In summary, tracing of MTC populations separated by their target regions showed significant differences in their selectivity and sensitivity, providing support for a role in parallel processing of olfactory information.

Effects of adolescent stress on synaptic transmission and plasticity in the adult mouse dentate gyrus

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Neuropsychiatric disorders are rising globally, notably among adolescents who are particularly vulnerable to stressful life events. Adolescence represents indeed a critical period of late brain maturation marked by cognitive and emotional changes, where excessive stress is a significant risk factor for the development of neuropsychiatric disorders in adulthood, including first and foremost major depression. However, the interplay between environmental stressors and genetic predispositions in driving long-term cognitive and affective impairments remains poorly understood. Here, we asked whether adolescent stress (AS) has a persistent impact on basic features of signal processing in the dentate gyrus (DG). This region of the hippocampal formation is of particular interest as it emerged as a crucial site for the therapeutic action of antidepressant drugs and electroconvulsive therapy alike. With regard to their molecular underpinnings, activin A, a member of the TGF-β family with a pronounced neuromodulatory profile, is increasingly recognized as a significant player in this field.

We used an animal model of AS, in which corticosterone (CORT) was added to the drinking water from postnatal days (PND) 30 - 45. After a CORT-free interval of 45 - 90 days, we recorded excitatory transmission at the medial perforant path - granule cell (mPP-GC) synapse in the DG in *ex vivo* hippocampal slices from CORT-exposed and control wild-type (wt) mice and likewise treated transgenic mice expressing a dominant-negative mutant of activin receptor IB (dnActRIB), which disrupts activin signaling.

As a necessary prelude, we examined first whether endogenous activin modulates the properties of the mPP-GC synapse in adult stress-naïve mice. Whereas the mice did not not exhibit genotype-specific differences in basic synaptic properties and short-term plasticity (STP), long-term potentiation (LTP) was impaired in dnActRIB mice. Lending strong support to the notion that severe stress in early ages leaves a pathogenetic stamp on the brain, we report here that AS led to enhanced synaptic transmission and reduced STP in both groups, with the key difference that wt mice, unlike dnActRIB mice, displayed these effects only after $GABA_A$ receptor-mediated inhibition was blocked with picrotoxin. This finding implicates activin A in re-adjusting the balance between excitation and inhibition after AS exposure. Importantly, LTP-inducing high-frequency stimulation uncovered aberrant hyperexcitability in AS-treated dnActRIB mice, while LTP in adult wt mice remained unaffected by AS.

Western blotting showed that AS causes a reduced expression of stress hormone receptors in the hippocampus of adult wt mice. This apparent down-regulation of CORT signaling may explain why, in preliminary experiments on adult wt mice with AS experience, acute application of CORT (modeling recurrent stress in adulthood) strongly biased synaptic functions.

In conclusion, our study demonstrates that AS has an impact on essential features of synaptic transmission and plasticity that persists into adulthood and is likely associated with alterations in glucocorticoid signaling pathways. Our study also supports the view of activin A as an endogenous antidepressant that is recruited by mood-elevating therapies, since, in our hands, genetic disruption of activin receptor signaling made the adolescent hippocampus much more vulnerable to the detrimental consequences of stress on brain function later in life.

Identification, Organization, and Connectomics of Monoaminergic Neurons in the Adult *Drosophila* Brain

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Remarkable progress in electron microscopy (EM) connectomics provided us with single neuron morphology and synaptic connectivity in the brain of fruit fly, *Drosophila melanogaster*. Machine learning-enhanced algorithms added prediction on neurotransmitter identity to those connectomes with high confidence on GABA, glutamate, and acetylcholine, but with low confidence on monoamines.

In this work, we developed a solid pipeline to identify monoaminergic neurons in two connectomes of adult fly brains (FlyEM hemibrain and FlyWire). We collected single neuron images of putative monoaminergic neurons from light microscopy (LM) datasets (e.g. FlyCircuit and FlyLight) and searched for morphologically matching neurons in the EM connectomes using ColorDepth MIP mask search. EM matches were then carefully compared with original LM data in 3D rendering software (VVDviewer). Selected EM candidates were further examined in detail with immunohistochemistry labeling specific for each monoamine using COVISE (the COllaborative VIsualization and Simulation Environment) in a five-panel three-dimensional virtual reality system (CAVE Automated Virtual Environment). Using this pipeline, we identified 92% (23 out of 25) serotonergic, 98% (48 out of 49 non-PAM) dopaminergic, and 95% (84 out of 88) octopamine/tyraminergic, and 100% (11 out of 11) histaminergic neurons in the EM connectome data.

To understand how these precisely identified neurons would modulate brain activity, we clustered them based on their innervation patterns and found that neurons of each monoaminergic system are organized into seven to about 30 groups, which we call the modulatory blocks.

Monoaminergic neurons within a modulatory block have mostly overlapping innervation patterns, and those of different blocks are essentially segregated. Neighboring modulatory blocks can communicate at the small overlapping volumes between them. In addition, we found a few specific brain parts where multiple modulatory blocks overlap extensively.

Our results show that synapses located within each block are modulated by a specific set of monoaminergic neurons, so that activities in different parts of the brain can be modulated independently. As neurons in different brain parts are likely to control different aspects of behavior, our results suggest that each modulatory block might modulate one or certain aspects of behavior.

Optogenetic Control of Mitochondria in PV+ Interneurons Alters CA1 Function

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Parvalbumin-positive (PV+) interneurons are crucial for maintaining spatial memory functions within the hippocampus that are otherwise impaired in neurodegenerative diseases. To maintain such an extensive control, PV+ interneurons require a high energy demand, which requires highly functional mitochondria to produce ATP quickly and efficiently. Previous studies have observed mitochondrial dysfunction within the hippocampus (HPC) in early cases of Alzheimer's Disease (AD), even preceding the major pathological hallmarks: tau aggregation, amyloid-beta plaques, and impairments in memory. Optogenetic tools have been instrumental in discovering and understanding the function of specific neuronal subtypes within various different brain networks. Recently developed optogenetics tools have allowed for light-control of intracellular organelles, such as mitochondria. To understand how the function of mitochondria within PV+ interneurons contributes to the learning and memory processes regulated by the hippocampal CA1 circuit, we packaged a previously developed optogenetic construct, mitoChR2, into an adeno-associated vector (AAV) and performed viral vector injections in PV-Cre mice targeting the CA1 region of the hippocampus. The mitoChR2 construct targets the inner membrane of the mitochondria (IMM), and in the presence of light, the channel opens and causes a disruption of the proton motive force that drives ATP production, which in turn decreases the amount of ATP produced. Through the use of this technique, in conjunction with performing electrophysiological recordings while the mice are freely moving, we discovered stimulation of mitoChR2 impaired the firing activity of both interneurons and pyramidal cells and consequentially altered spatial properties of place cells within the HPC during exploration of a familiar environment. Our findings emphasize the importance of mitochondria in learning and memory mechanisms, and suggest mitochondria be considered for potential therapeutic targets for the treatment of AD.

Symposium

S13: Breaking News

S13-1 Cognitive biases influence numerosity judgments in macaques and crows
Lena Jannasch

S13-2 Primary Neuronal Cell Culture in Ambient CO_2
John Carl Begley

S13-3 Decreased Synaptic Density in Sleep Deprived Mice with [^{18}F] SynVest-1 PET Imaging
Jing Ma, Alexandra Drechsel, Angela Oskamp, Nadja Hermes, Ulrike Holz, Sabine Jakobs, Sabina Klein, Stefan Stüsgen, Philipp Krapf, Bernd Neumaier, Alexander Drzezga, Andreas Bauer, Astrid Rollenhagen, Björn Kampa, David Elmenhorst

S13-4 Characterization of Somatostatin-Expressing Neurons in the Anterior Olfactory Nucleus: Morphological Diversity and Functional Implications
Kaoutar Elhabbari, Daniela Brunert, Markus Rothermel

S13-5 Behavioral algorithms of ontogenetic switching in larval and juvenile zebrafish phototaxis
Maxim Quirijn Capelle, Katja Slangewal, Armin Bahl

S13-6 Transcriptomic decoding of the Locus Coeruleus region identifies differential vulnerability in an early stage mouse model of Parkinson's Disease
Vera Evander, Pauline Jakobs, Diana Municchi, Katharina Dragendorf, Miquel Vila, Matthias Prigge

S13-7 Retinal input integration in excitatory and inhibitory neurons in the mouse superior colliculus *in vivo*
Carolin Gehr, Jérémie Sibille, Jens Kremkow

S13-8 Spatiotemporal Deep Learning Pipeline for Decoding Stimulus-Driven Whole-Brain Calcium Imaging
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S13-9 Extraembryonic source of Serotonin involved in Neurodevelopment
Niccolò Milani

S13-10 Defining the Roles of PV and VIP Neurons in Texture Discrimination of Mice via Chemogenetics
Aybeniz Cetin, Martin Möck, Jochen F. Staiger

Cognitive biases influence numerosity judgments in macaques and crows

Lena Jannasch

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Our judgments are influenced by prior outcomes or extreme values, to which our cognitive processes tend to adjust or normalize over time. Two examples of such cognitive biases are a "regression to the mean" and a "serial effect". Regression to the mean refers to a statistical tendency for extreme values to move closer to the average of the tested range, whereas serial effects occur when previous judgments influence subsequent ones, creating a systematic bias toward similarity between successive estimates. In human estimation tasks, both regression to the mean and serial effects impact individual estimates. We hypothesized that animals might also be subject to the same cognitive biases during numerosity judgments. We trained two rhesus macaques and two carrion crows – both species known for their advanced numerical abilities – to discriminate the number of dots (numerosities) in visual displays. By presenting a wide range of target numerosities and analyzing detailed behavioral performance functions, we investigated potential deviations in performance caused by cognitive biases. We found that both macaques and crows exhibited systematic biases in their responses that had previously gone undetected. The animals demonstrated a regression to the mean effect by biasing their responses toward the numerical center of the tested range. Additionally, both species displayed a serial effect: when subjects had previously seen a large numerosity, they tended to overestimate the current numerosity, and vice versa. This strong relationship between quantity judgement of the current trial and the magnitude of the previous trials diminished over time as a function of task history. Our results indicate that cognitive biases in magnitude estimations, previously demonstrated in humans, extend to numerosity judgments in nonhuman primates and corvid songbirds – two species with entirely different endbrain structures. The underlying biasing processes resemble active inference or predictive coding mechanisms, which may offer an efficient solution to the common computational challenge posed by sensory and cognitive noise. Simulating such behavior with a Bayesian model that incorporates task history as a prior may explain the observed signatures of both effects. An integrative approach that combines internal representations with context-dependent predictions could also help resolve the long-standing debate in numerical cognition regarding the scaling scheme of internal numerosity representations.

Primary Neuronal Cell Culture in Ambient CO_2

John Carl Begley

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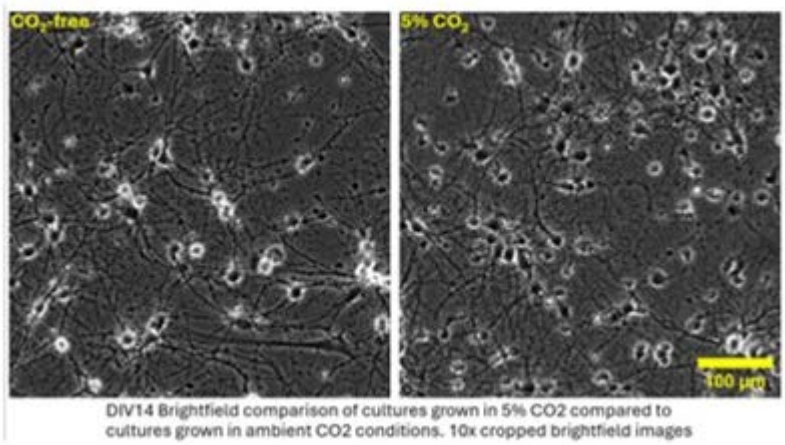
Dissociated neuronal cell culture is a well-established method that is universally performed in warmed, humidified incubators with 5% CO_2. Cultures in these conditions require a suitable culture medium that is buffered with a bicarbonate-, HEPES-based buffer system. Removing cells from the incubator and placing them in atmospheric conditions leads to significant cell death, which is a major limitation of this culture system.

We have developed and tested a method for growing primary, dissociated cells outside of CO_2 environments. Using a modified culture medium, we can grow primary neuronal cultures for up to two weeks in ambient CO_2 conditions. These neurons develop axons and dendrites, allowing them to form synaptic connections and develop active networks. By using a combination of calcium imaging, electrophysiology, and immunocytochemistry, we are characterizing these cells functionally and morphologically.

A CO_2-free system presents several advantages. Our culture system enriches for neurons rather than glia which could be beneficial for certain applications. Not requiring a continuous supply of CO_2 lowers the barrier to establishing culture systems in labs around the world and removes the need for CO_2 supply, which is a toxic, potentially lethal gas. Furthermore, the role of bicarbonate in CNS cell survival and development could be selectively studied in a controlled system, and CO_2-free cultures could potentially be used for biological studies in space, where CO_2 supply is expensive and problematic. Beyond culturing, this modified culture medium could be used in the future to transport living cultures without CO_2 either immediately after plating or once they reach maturity without significant changes to neuronal structure and function.



DIV 14 10x brightfield comparison of cultures grown in ambient CO2 (left) and in 5% CO2 (right)

Decreased Synaptic Density in Sleep Deprived Mice with [^{18}F] SynVest-1 PET Imaging

Jing Ma, Alexandra Drechsel[1], Angela Oskamp[1], Nadja Hermes[1], Ulrike Holz[1], Sabine Jakobs[1], Sabina Klein[1], Stefan Stüsgen[2], Philipp Krapf[2], Bernd Neumaier[2], Alexander Drzezga[1,3], Andreas Bauer[1], Astrid Rollenhagen[5], Björn Kampa[4,5], David Elmenhorst[1,3]

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Background: Synaptic plasticity, particularly long-term plasticity, is considered a key mechanism underlying brain functions related to learning and memory. The synaptic homeostasis hypothesis (SHH) proposes that overall synaptic strength increases during wakefulness and decreases during sleep. However, evidence shows both increases and decreases in synaptic density following sleep. Currently, studies examining synaptic density across the brain during the sleep-wake cycle remain limited. Synaptic vesicle glycoprotein 2A (SV2A), a transmembrane protein found in all synaptic terminals regardless of neurotransmitter type, serves as a marker of synaptic density. In this study, we explored changes in synaptic density in sleep-deprived mice using SV2A PET imaging with the tracer [^{18}F] SynVest-1.

Methods: Animals (C57BL/6J, 2-5 months, n=40, 24 male and 16 female) had a light-dark cycle of 12/12 hours. PET images were acquired (Inveon, Siemens) for 60mins following intravenous bolus injection of [18F] SynVesT-1. Scans were performed at the end of the light-on phase (following sleep, S, ZT0), after which the mice were sleep-deprived through gentle handling until the subsequent scan (following sleep deprivation, SD, ZT24). Time activity curves (TACs) were collected from regions including striatum, cortex, hippocampus, thalamus, and cerebellum. Standard uptake values (SUV) were calculated using PMOD (version 3.408, Zurich, Switzerland), normalized to body weight and injected dose, and time-weighted averages from 30 to 60 minutes were used to derive adjusted SUV (aSUV). A noninvasive image-derive input function (IDIF) was used to estimate the volume of distribution (V_T) using a two-tissue compartment model (2TCM). A 10 mm cubic volume of interest (VOI) was placed over the heart in the early frames, and an automatic algorithm in PMOD identified voxels with activity exceeding 50% of the total activity to determine whole blood activity. To obtain the plasma activity curve, a population-based curve of intact tracer in plasma, the plasma-to-whole-blood ratio, and a scaling factor (α= 1.77) were applied (He and Wedekind et al., 2020; Bertoglio and Zajicek et al., 2022).

Results: After sleep deprivation, both SUV and V_T showed a significant decrease in several brain regions, including the striatum, cortex, hippocampus, and thalamus. While there was no difference in SUV in the cerebellum, the V_T values indicated a reduction in SV2A expression in the cerebellum following sleep deprivation.

Discussion: We observed a significant decrease in synaptic density in mice after sleep deprivation. Notably, previous research has shown that long-term potentiation (LTP) is linked to the functional vesicle pool and that sleep deprivation leads to significant long-term depression (Kuhn M et al., 2016;Rey S et al., 2020). The observed decrease in SV2A expression in synaptic vesicles is consistent with the long-term depression effects following sleep deprivation.

Characterization of Somatostatin-Expressing Neurons in the Anterior Olfactory Nucleus: Morphological Diversity and Functional Implications

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Somatostatin-expressing GABAergic neurons constitute a major class of inhibitory neurons in the mammalian brain. Somatostatin (SST)-positive neurons have been shown in all olfactory areas, e.g. in the olfactory bulb (OB) where they modulate physiological gamma rhythms and olfactory discrimination responses, or the piriform cortex, where they control dendritic integration of afferent and recurrent inputs to the network. SST function in the anterior olfactory nucleus (AON), which receives the majority of olfactory input from the OB, remains unknown. As the strongest decline of SST-positive neurons in neurodegenerative diseases is detectable in the AON, we feel that the nature and function of these neurons could be of high interest to understanding the olfactory sensory decline in these pathologies. Our study aims to investigate the nature of SST interneurons in the AON and their function in the modulation of AON local activity.

We analyzed coronal and sagittal sections of SST-Cre tdTomato mice to characterize neurons in the AON morphologically. Cell density varied considerably between layer I and layer II, with layer II having more than five times the cell density of layer I. SST neurons in the AON constitute a heterogenous population: small multipolar neurons, previously assumed to be the predominant form of SST cells in AON, were located primarily in layer I, while most of the SST positive neurons in layer II seemed to consist of horizontal and vertical bipolar cells and large multipolar shaped cells. Differences between layer I and II SST-positive neurons could also be detected in average soma size: while cells in layer I measured 61.3±12.4 µm2, cells in layer II were much larger measuring 139.5±26.8 µm2.

The heterogeneous morphology of SST-positive neurons suggests that these cells might have different functions in the AON circuit. Further research is required to characterize these different SST-positive cells according to their physiological properties.

Behavioral algorithms of ontogenetic switching in larval and juvenile zebrafish phototaxis

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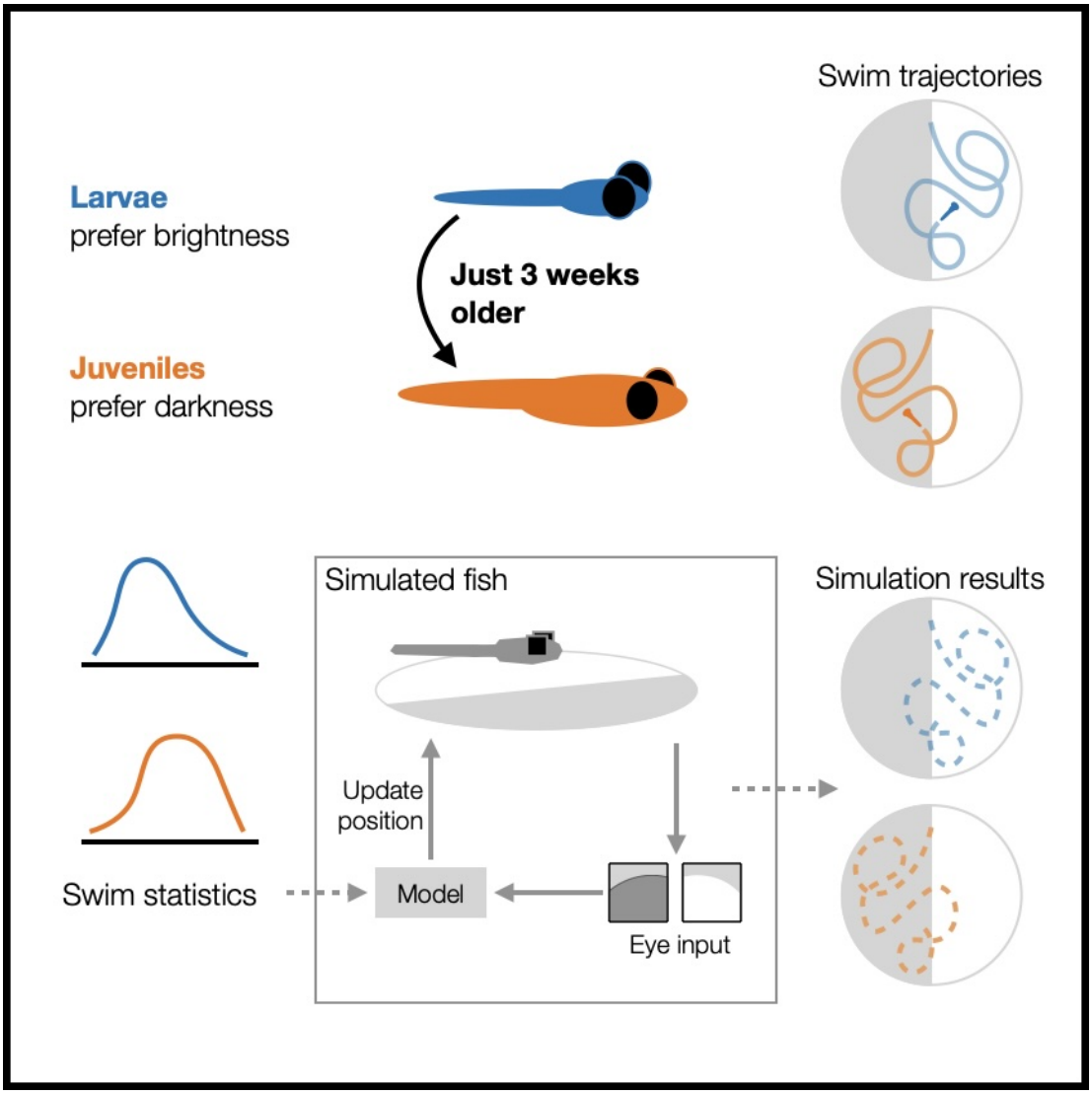
Animals show major behavioral changes throughout their ontogenetic development. However, the cognitive computations and neural mechanisms controlling this process remain elusive. Here, we use a combination of multiple complementary phototaxis assays and high-throughput behavioral tracking to explore how young zebrafish adjust their brightness preferences while growing from larval to juvenile stage. We observed that larvae are attracted to luminance but repelled by changes in luminance, whereas juveniles are becoming attracted to darkness but remain repelled by luminance changes. Using the observed swim event statistics, we build a library of generative agent-based models, with unique parameter sets for each fish. We validate these models by their predictive power of animal behavior in more complex visual environments. The behavior of both larvae and juveniles can be captured best by a superposition of two competing elements: one element senses the current global luminance level, while the other processes information regarding eye-specific luminance change. We think that the implementation of phototaxis through a competitive arrangement of these two processing streams allows animals to flexibly adapt their behavior in dynamic visual environments, based on their internal state, and their changing behavioral goals during development. Our rigorous model-based dissection approach is a novel way to identify the algorithmic and cognitive changes during ontogeny. To explore the mechanistic implementations of the adjustments in the brain, we will now leverage the rich molecular genetic toolkit and whole-brain single-cell-resolution imaging techniques available for zebrafish, at both stages of development.


Swim trajectories
Larvae
prefer brightness
Just 3 weeks older
Juveniles
prefer darkness
Simulated fish
Simulation results
Update position
Swim statistics
Model
Eye input

Transcriptomic decoding of the Locus Coeruleus region identifies differential vulnerability in an early stage mouse model of Parkinson's Disease

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One Sentence Summary: Transcriptomic decoding of the Locus Coeruleus has unveiled a differential vulnerability in new noradrenergic neuronal subtypes and potentially offers fresh avenues for Parkinson's disease intervention.

The Locus Coeruleus (LC) is a small nucleus in the brainstem that serves as the primary source of noradrenaline (NA) in the central nervous system. Its functions range from brain-wide effects impacting arousal, or vascular constriction to localized actions such as facilitating synaptic plasticity in the hippocampus, or modulating working memory in the prefrontal cortex. Degeneration of the LC is a hallmark of several neurodegenerative diseases, including Alzheimer's and Parkinson's disease (PD).

Here, we present the first unbiased transcriptomic analysis of the LC and peri-LC regions in healthy mice and in a neuromelanin (NM) mouse model that recapitulates early symptoms of PD. In humans, aging leads to the accumulation of NM - a dark pigment - in catecholaminergic neurons. Since rodents do not naturally produce NM, we developed a mouse model that conditionally expresses human tyrosinase (hTYR), an oxidoreductase that converts catecholaminergic metabolites into reactive quinones, ultimately forming NM.

To investigate how the cellular identity and cellular vulnerability of noradrenergic neurons changes with NM accumulation, we prepared tissue punches from the LC of control and hTYR-expressing animals at 8 weeks and 40 weeks of age (n = 4 per group for each time point), including both males and females. Putative LC neurons were identified by the expression of relevant marker genes (*Dbh, Th, Net*).

Interestingly, we identified several new genetic subtypes of Th-positive neurons, confirming heterogeneity among noradrenergic LC neurons. For peri-LC neurons, we focused on the expression of GABAergic marker genes (*Vgat, Gad1, Gad2*), which exert strong control over noradrenergic neurons. In the young cohort, we observed a decrease in neurons with clear noradrenergic identity and an upregulation of *Th*, indicating a compensatory mechanism in the remaining neurons at this early stage. We did not observe changes in the number of non-noradrenergic neurons. Moreover, we identified an increase in microglia numbers in hTYR animals but not in wild-type controls, suggesting an inflammatory response to NM accumulation.

In the older cohort, we observed a significant decrease in noradrenergic neurons, aligning with previous

reports on the NM accumulation mouse model. Notably, we found differential vulnerability among subpopulations of noradrenergic neurons, potentially opening avenues for targeted interventions to rescue the highly vulnerable NA3 sub-type.

Our entire dataset is publicly available and provides a valuable resource to guide future research on noradrenergic systems. This study enhances our understanding of molecular changes in the LC associated with NM accumulation and offers insights into potential therapeutic targets for neurodegenerative diseases like PD.

Retinal input integration in excitatory and inhibitory neurons in the mouse superior colliculus *in vivo*

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The superior colliculus (SC) is a midbrain structure that receives inputs from retinal ganglion cells (RGCs). The SC contains one of the highest densities of inhibitory neurons in the brain but whether excitatory and inhibitory SC neurons differentially integrate retinal activity *in vivo* is still largely unknown. We recently established a recording approach to measure the activity of RGCs simultaneously with their postsynaptic SC targets in vivo, to study how SC neurons integrate RGC activity (Sibille et al., 2022). Here, we employ this method to investigate the functional properties that govern retinocollicular signaling in a cell type-specific manner by identifying GABAergic SC neurons using optotagging in VGAT-ChR2 mice. In total, I recorded 350 RGC-SC pairs, identified via cross-correlation analysis, out of which around one third of retinal afferents connect onto SC neurons that express GABA. To characterize the RGC-SC connections we estimated the connection efficacy and connection contribution (Usrey et al., 1999). Our results demonstrate that both excitatory and inhibitory SC neurons receive comparably strong RGC inputs and similar wiring rules apply for RGCs innervation of both SC cell types, unlike the cell type-specific connectivity in the thalamocortical system. Moreover, retinal activity contributed more to the spiking activity of postsynaptic excitatory compared to inhibitory SC neurons, measured by the connection contribution, a measurement for how strong SC neurons are coupled to the activity of individual RGC inputs. This suggests that excitatory SC neurons are are strongly coupled to their RGC afferent inputs, while GABAergic SC neurons likely also integrate inputs from other sources. This study deepens our understanding of cell type-specific retinocollicular functional connectivity and emphasizes the importance to uncover neural diversity in the SC.

Spatiotemporal Deep Learning Pipeline for Decoding Stimulus-Driven Whole-Brain Calcium Imaging

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Whole-brain calcium imaging has emerged as a powerful technique for capturing neuronal dynamics with high spatial and temporal resolution, offering valuable insights into brain-wide activity patterns. However, the complexity of these recordings poses substantial challenges for analysis. We present a deep learning-based pipeline that enables end-to-end analysis of 3D whole-brain continuous recordings, directly decoding the spatiotemporal dynamics of raw imaging data to reveal responses to various stimuli. This approach eliminates the need for extensive manual preprocessing and feature engineering by allowing the model to learn relevant features directly from the raw data. We validate this approach using Light Field Microscopy (LFM) data of calcium activity in *Drosophila melanogaster* - comparing well-fed and food-deprived animal groups in response to different odor, taste, or combined stimuli. This pipeline effectively identifies brain-wide neuronal patterns in response to chemosensory stimuli as influenced by the animal's metabolic state, advancing our understanding of how global brain activity encodes stimulus processing and providing new insights into the neural mechanisms of chemosensory processing.

This Work is supported by iBehave, receiving funding from the programme "Netzwerke 2021", an initiative of the Ministry of Culture and Science of the State of Northrhine Westphalia.

Extraembryonic source of Serotonin involved in Neurodevelopment

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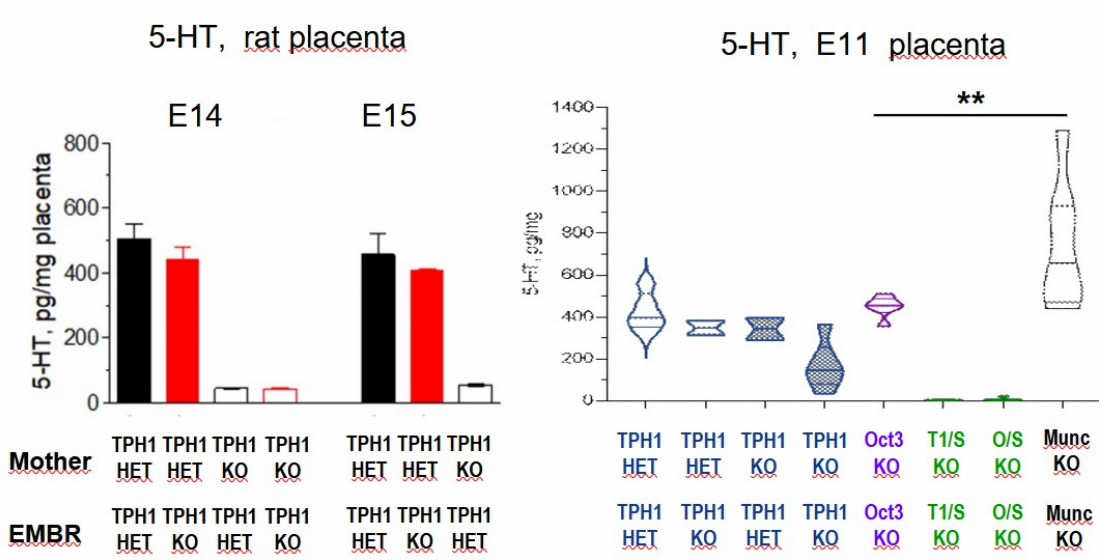
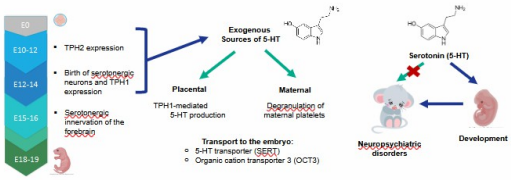
Studies in recent years have suggested that maternal and extraembryonic sources of serotonin, such as placenta, play pivotal roles in embryonic brain development. However, the identity of serotonergic system components and cell types expressing serotonergic genes during development, as well as mechanisms of serotonin transport to the embryo remain controversial (Bonnin et al., Nature. 2011; 472(7343):347-50; Kliman et al., Endocrinology. 2018;159(4):1609-1629).
The aim of this project is to evaluate the contribution of extraembryonic sources of serotonin to PFC development and to dissect the involved cellular and molecular components. Since such an approach is not possible in humans, we use mouse models deficient in genes encoding the serotonin synthesizing enzymes, TPH1 (Walther et al. Science 2003;299:76) and TPH2 (Alenina et al. Proc Natl Acad Sci USA 2009;106:10332-7), and the monoamine transporters, SERT and OCT3, to clarify if these proteins contribute to the supplementation of the fetus with serotonin in the absence of own serotonin production and what is their role in brain development. We investigate the effect of maternal and placental SERT, OCT3, TPH1 and TPH2 depletion on the serotonin levels in placenta and different parts of the embryonic brain before the onset of Tph2 expression at embryonic day (E) 10-11; after the birth of serotonergic neurons (E12-14) and upon serotonergic innervation of the forebrain (E15-16) and on serotonergic-innervation pattern at later stages of embryogenesis.
For this purpose, this project takes advantage of available animal models, including double and triple knockouts for genes involved in serotonin synthesis and transport. We use breeding strategies and embryo transfer technology to create mothers and fetuses with different genotypes. Furthermore, we use tetraploid aggregation (Popova et al. Hum Reprod 2011;26:662-70) to segregate the effects of serotonin production from extraembryonic tissues and the embryo itself. The serotonin content in the embryonic fore- and hind-brain will be measured by HPLC-MS. The PFC maturation in different mutants will be assessed in embryonic development using immunohistochemistry and ECi 3D recostruction. The morpho-functional structure and serotonergic transport mechanisms of the placenta will be assessed using multifluorescent labeling of placental tissues. Additionally, any abnormalities in the placentas of KO animals will also be investigated.
This research is part of “The Serotonin & Beyond project” and has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No. 953327.



Background



Aim

a) Dissect the role of the involved molecular components

b) Identify the mechanisms of serotonin transport in the placenta

c) Detect neurodevelopmental changes at different stages, and the resulting morphological and functional alterations.

Methods:

a) Profiling of serotonergic content in placental and embryonic material

b) Morpho-functional characterization of the placenta

c) Assessment of PFC maturation and serotonergic wiring

Defining the Roles of PV and VIP Neurons in Texture Discrimination of Mice via Chemogenetics

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Rodents are gathering information regarding the surrounding through their whiskers for differentiation of textures, and objects as well as navigation, and each whisker is represented in the barrel cortex (wS1) by a single cortical column in an isomorphic manner. Because of the precisely defined somatotopic map, wS1 is a favorable model for the study of microcircuits and the investigation of the roles of various neuronal subtypes in the processing of sensory information (for a broader review please see Staiger & Petersen, 2021). The microcircuits within each column of the barrel cortex consist of both excitatory and inhibitory neurons. There is also a wide literature on genetically defined GABAergic neurons (parvalbumin [PV], vasoactive intestinal polypeptide [VIP], and somatostatin [SST]) on tactile perception through whisking including either passive simulation of the whiskers (Simons, 1978; Moore & Nelson, 1998) or active touch (O'Connor et al., 2010; Yu et al., 2019). Today, it is very well known that activation of wS1 has an integral role in texture discrimination (i.e. coarse vs. soft textures; Guic-Robles et al., 1989; Carvell & Simons, 1990). However, it remains unknown how PV, VIP, and SST interneurons contribute to the information processing during tactile perception in whisker-based texture discrimination. In the present study, we aim to further investigate the role/contribution of inhibitory neurons, via chemogenetic manipulation, in the processing of whisking information in awake freely behaving mice that were trained for texture discrimination.

For this reason, we initially needed to establish a novel whisker-based texture discrimination task, textured T-maze (TT), which allows mice to explore and move within the maze freely while they are being trained on texture discrimination. It is a 2-choice task in which food-restricted animals were trained to discriminate 2 textures (e.g. 120 vs. 1500 grit sandpaper) to find the correct arm to enter and then reach the food reward. A cre-dependent chemogenetic virus (pAAv-hSyn-DIO-hM3D(Gq)-mCherry – AAV5) was injected in wS1 of PV- (N = 6, Mage = 13.2 weeks) and VIP-cre (N = 11, Mage = 13.9 weeks) mice bilaterally for the expression of DREADDs which allowed us later to depolarize PV cells in PVcre animals and VIP cells in VIPcre animals by intraperitoneal injection of clozapine-N-oxide (CNO).

In total 19 AAV-injected mice started training in TT a week after the stereotactic surgery. Once they passed the criteria to be accepted as advanced in the task, the initial dose (3 mg/kg) of CNO was intraperitoneally injected 75 mins before the test started. Performances of the animals in both groups (5/6 of PVcre and 10/11 of VIPcre animals) dropped to chance level. In other words, chemogenetic activation (i.e. depolarization) of either PV or VIP neurons despaired the ability to discriminate textures. The brains of the animals who did not show a change in discrimination behavior, later went through IHC and FISH procedures to control virus expression area and in both cases it was observed that virus expression was outside of wS1 area. Other than these blind controls, we also confirmed with whole-cell patch-clamp (7 VIP and 5 PV cells) that intrinsic properties of the transfected cells were compatible with the non-transfected samples from the prior recordings in our lab. Depolarization effect of the CNO on transfected cells was also tested via bath application, and recordings shoed that while PV cells (n = 4) were depolarizing on average up to -48 mV, VIP cells (n = 5) were depolarizing on average up to -25.8 mV which lead a depolarization block.

As a conclusion, although the depolarization effect of the chemogenetic virus was not observed to be comparable for PV and VIP cell, either "activation" of PV or "deactivation" of VIP cells in wS1 despaired perceptual discrimination of freely behaving mice in a texture discrimination task.

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Symposium

S14: Circuits for behavior: cross-species strategies for adaptation and plasticity

S14-1 Neural Circuits of Context-Dependent Behavior in Flies
Ilona C. Grunwald Kadow

S14-2 Integration of Information in the Absence of Action in Drosophila
Johanna Aurelia Schweizer

S14-3 Towards the neural basis of the magnetic sense in subterranean mole-rats: behavior and recordings
Erich Pascal Malkemper

S14-4 Simple pleasures: regulation of social and feeding behaviors by lateral hypothalamic neuronal populations
Tatiana Korotkova, Anne Petzold, Rebecca Figge-Schlensok, Hanna Elin van den Munkhof

S14-5 Experience-dependent modulation of cortical circuits for perception and behavior
Simon Musall

Neural Circuits of Context-Dependent Behavior in Flies

Ilona C. Grunwald Kadow[1]

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The dynamic interplay between brain and body ultimately guides behavior and decision-making. How does the brain detect and influence the state of the body, its physiological or behavioral state? How do changes in body state influence the state of the brain and how it processes sensory information and creates memories?
To answer these questions, we use the genetic model fly Drosophila melanogaster in combination with state-of-the-art methods of behavioral analysis, computational methods, (opto)genetics and in vivo functional imaging and microscopy. At the meeting, I will present our recent work on the interaction of brain and body and its underlying mechanisms.

Integration of Information in the Absence of Action in Drosophila

Johanna Aurelia Schweizer

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In dynamic environments learned information must be continuously revised to predict the most feasible outcome of a situation and initiate appropriate behavioral responses. Associations that turn out to be unreliable must be updated by new learning. Thus, learning from repeated non-reinforced re-exposure to cues that elicit inadequate fear or favor drug-related relapse provides an opportunity to alleviate the consequences of maladaptive memories. Extinction based therapy has the potential to weaken associations between cues and reinforcement thereby helping to prevent relapse and promoting new learning. However, whether such extinction learning requires the expression of learned responses is not known. Here we provide evidence that extinction of reward memories does not require conditioned food seeking behavior. Satiated flies (*Drosophila melanogaster*) do not express learned approach behavior to a sugar predicting odor. However, despite the absence of food seeking behavior, satiated flies learn about the omission of the predicted sugar and adapt their behavior accordingly. This learning in the absence of seeking behavior depends on peripheral sensing of the sweet taste of the food. However, interference of novel stimuli can perturb extinction learning in satiated but not hungry flies. Thus, although extinction learning can take place in the absence of behavioral expression of the memory, it seems to be more sensitive to contextual information. Understanding how extinction learning does or does not depend on behavioral responses will be essential to improve its application in therapeutic approaches. Furthermore, the decoupling of computation of information from action might allow to understand cognitive operations.

Towards the neural basis of the magnetic sense in subterranean mole-rats: behavior and recordings

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The ability to sense the Earth's magnetic field and use it for orientation and navigation is widespread in the animal kingdom. However, the cellular and neuronal mechanisms behind this sensory ability are still poorly understood. African mole-rats of the genus *Fukomys* are subterranean rodents suggested to use magnetic cues for orientation in their dark tunnels. We use mole-rats as a model system to investigate how a mammal employs a magnetic compass, which brain regions are involved, and how magnetic cues are neuronally encoded.

In the first step, we developed behavioral assays to demonstrate their ability to perceive magnetic fields, including a virtual magnetic anomaly test and maze navigation. Next, we conducted a whole-brain neuronal activity screen to identify neurons that process magnetosensory input. Finally, we hypothesized that the mole-rat brain contains spatial neurons, such as place cells and head direction cells. However, unlike in surface-dwelling rodents, we expected that inputs from the somatosensory—and possibly the magnetosensory—systems would dominate over visual cues. We tested this hypothesis by performing single-unit recordings in the hippocampus of freely moving mole-rats exploring different environments under controlled magnetic conditions.

Simple pleasures: regulation of social and feeding behaviors by lateral hypothalamic neuronal populations

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Feeding is largely regulated by neuronal populations in the lateral hypothalamus, an evolutionarily conserved brain region. Animals must coordinate and prioritize multiple, sometimes competing, basic needs to ensure survival and reproduction. The fulfilment of nutritional needs has to be balanced and weighed against competing needs such as mating. Using optogenetics, chemogenetics, deep-brain calcium imaging, and electrophysiology in freely behaving mice, we investigated the neuronal mechanisms enabling state-dependent representation and orchestration of multiple innate drives. We identified neuronal populations in the lateral hypothalamus, including neurotensin- and leptin receptor-expressing neurons that act in a complementary manner and in a state- and sex-dependent way to enable the flexible fulfilment of multiple essential needs.

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Experience-dependent modulation of cortical circuits for perception and behavior

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Understanding how cortical circuits integrate multisensory information to adapt to behavioral challenges requires context-specific investigation of cortex-wide activity patterns. To address this, we used widefield imaging to measure activity across the cortex in two major excitatory pyramidal neuron types as mice learned a series of decision-making tasks. By recording calcium dynamics in pyramidal-tract (PT) and intratelencephalic (IT) neurons, we uncovered significant, cell-type-specific differences in the complexity and spatial organization of activity patterns, both locally and across the cortex. These findings suggest the presence of specialized subcircuits for distinct functional roles. Notably, cortical activity patterns adapted during learning, with IT neurons displaying faster changes than PT neurons. Over the course of training, sensory representations in the parietal and frontal cortices strengthened, while choice-predictive signals in the frontal cortex emerged earlier in the decision process. Two-photon imaging in the frontal cortex further revealed a marked increase in the number and magnitude of choice-predictive neurons. These changes also persisted when task contingencies were altered. In addition, high-density Neuropixels recordings in the frontal cortex and superior colliculus (SC) of trained animals demonstrated context-dependent choice signals in the frontal cortex, but not in the deep SC, highlighting distinct cortical-subcortical coding strategies. Our findings reveal dynamic, learning-driven changes in excitatory neuron activity, particularly within the parietal and frontal cortices. This work underscores the role of cortical circuits in adaptive behavior and demonstrates how distinct cortical-subcortical interactions support multisensory decision-making.

Symposium

S15: Building blocks of the brain: insights into CNS circuits and ultrastructure

S15-1 GAUSS-EM: Guided accumulation of ultrathin serial sections with a static magnetic field for volume electron microscopy of whole brains
Kevin Briggman, Kara Fulton, Paul Watkins

S15-2 Array tomography: trails to discovery in neuropathology
Martina Schifferer

S15-3 Clustered postsynaptic density dynamics in CA1 hippocampal neurons
Kristina Evguenievna Ponimaskine, Christian Schulze, Thomas G. Oertner

S15-4 Microcircuits in the marmoset prefrontal cortex analyzed with large volume electron microscopy
Yoshiyuki Kubota

S15-5 Organization principles of the neuronal ultrastructure revealed with 3D electron microscopy
Matthias Haberl

GAUSS-EM: Guided accumulation of ultrathin serial sections with a static magnetic field for volume electron microscopy of whole brains

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Serial sectioning electron microscopy (EM) of millimeter-scale 3D anatomical volumes requires the collection of thousands of ultrathin sections. Here we report a high-throughput automated approach, GAUSS-EM, utilizing a static magnetic field to collect and densely pack thousands of sections onto individual silicon wafers. The method is capable of sectioning hundreds of microns of tissue per day at section thicknesses down to 35 nm. Relative to other automated volume electron microscopy approaches, GAUSS-EM democratizes the ability to collect large 3D EM volumes because it is simple and inexpensive to implement. The method is compatible with correlative approaches to combine permeabilization-free immunohistochemical labelling with EM and/or functional recordings with EM. I will present numerous large-scale EM volumes we have collected with this approach with a focus on sensory circuits in a range of vertebrate species including fish, reptiles, birds and mammals.

In addition, we report a computational pipeline to align the resulting petabyte-scale datasets that were imaged with multibeam scanning electron microscopy. The pipeline places an emphasis on aligning the data while minimizing distortions to the raw data. The combination of these approaches have allowed us to accelerate the acquisition and alignment of cubic millimeter EM volumes to timescales on the order of a couple months.

Array tomography: trails to discovery in neuropathology

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Volume Electron Microscopy (vEM) provides high-resolution data of target structures and resolves three-dimensional representations of otherwise ambiguous biological geometries. While EM provides high spatial resolution, search processes in volumes of several hundreds of square microns is tedious. Multimodal methods like correlated light and electron microscopy (CLEM) allow to bridge these scales. Among the available vEM techniques, array tomography methods like automated tape collecting ultramicrotomy (ATUM) have proven particularly powerful for targeting specific or rare biological structures as needed for correlation as they enable repetitive and large field of view imaging.
Here we show how to apply ATUM-CLEM approaches to reveal ultrastructural correlates of neurodegenerative pathologies. While classic correlation techniques annotate ultrastructural data with one or a few molecular targets, we have developed STcEM, a method that links spatially-resolved gene expression of single cells with their ultrastructural morphology. Our results offer a comprehensive view of the spatial, ultrastructural, and transcriptional reorganization of single cells after brain injury. Moreover, we have developed ATUM-Tomo, a hybrid method that, bridges scales from scanning to transmission EM. As a proof-of-principle, we applied correlative ATUM-Tomo to study ultrastructural features of blood brain barrier (BBB) leakiness around microthrombi in a mouse model of traumatic brain injury. Overall, our new ATUM-Tomo approach will substantially advance ultrastructural analysis of biological phenomena that require cell- and tissue-level contextualization of the finest subcellular textures.

Clustered postsynaptic density dynamics in CA1 hippocampal neurons

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As changes in synaptic strength and the formation and elimination of dendritic spines occur on different time scales, possible connections between synaptic strength and synaptic lifetime have been difficult to investigate. Patch-clamp recordings of synaptic currents are limited to about 1 h, while *in vivo* imaging of spine dynamics is performed at 24 or 48 h intervals. To extend the imaging timeline of synaptic dynamics in live tissue, we developed an on-stage microscope incubator for chronic 2-photon imaging of organotypic hippocampal slice cultures. Using this setup, we measured the size of endogenous postsynaptic densities (PSDs) with fluorescently labeled intrabodies in CA1 pyramidal neurons every 20 min. To analyze the dynamics and interactions of all labeled synapses simultaneously, we developed an automated image analysis workflow, to first process and optimize large 3D multi-view image stacks for subsequent automated spine detection and tracking based on deep learning. This large-scale analysis with high temporal and spatial resolution revealed that PSDs located on apical (oblique) dendrites of CA1 cells were more dynamic than PSDs located on basal dendrites. Dynamic PSDs were spatially clustered on individual dendritic branches and smaller in size.

In addition to studying baseline conditions to understand spontaneous synaptic fluctuations, this approach can be easily combined with (opto-)genetic and/or pharmacological manipulations. Silencing or activation of the entire slice culture led to homeostatic plasticity, which underscores the capacity to detect subtle dynamic changes with this analysis. Our objective is to monitor PSD dynamics for three days following the optogenetic induction of LTP.

Microcircuits in the marmoset prefrontal cortex analyzed with large volume electron microscopy

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In recent decades, brain connectomics using large volume electron microscopy (vEM) has been introduced in several neuroscience laboratories. This methodology allows us to elucidate synaptic connections on a large scale, such as cortical columnar wiring. To implement vEM, we used a modified automated tape collecting ultramicrotome (ATUM) to collect large numbers of serial ultrathin sections, and a high-throughput EM imaging system (Blade, Voxa, USA). Firstly, we remodeled the original ATUM (add reference?) to control the timing of cutting to achieve reliable collection of serial ultrathin sections on individual slots of a grid tape. We succeeded in collecting more than 1000 serial ultrathin sections. These sections were placed securely and fairly reliably at similar locations within each grid slot. Secondly, a transmission EM (TEM) equipped with Blade was used for imaging the ultrathin sections on the grid tape. The Blade-TEM system captured high-resolution (3.2 nm/pixel) images of an area 1.1 x 1.6 mm2 in size, and a vEM dataset from the 1000 serial ultrathin sections (50-nm thick sections) in about a month. Processing imaging data of such a huge size can be challenging. However, we developed a new high-throughput EM pipeline, and we have been analyzing the circuit architecture of a marmoset prefrontal cortex (PFC). The PFC has dramatically expanded in primates. However, their connectivity at synaptic levels remains unclear. We injected combined antero- and retrograde viral tracers into the A10 area to visualize reciprocal projections between columnar patches in the A9 area in the marmoset PFC (Watakabe et al., Neuron. 2023; 111(14):2258-2273). To unravel the cortico-cortical circuit between the A10 and A9 areas, we used confocal laser scanning microscopy of PFC sections in which both axons and dendrites were labeled anterogradely and retrogradely, and acquired a large TEM dataset of the A9 PFC area. The results with this correlative light and EM using vEM will be reported.

Organization principles of the neuronal ultrastructure revealed with 3D electron microscopy

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Form and function of neurons are intricately linked. Long axons reach far away targets and dendrites branch wide to integrate signals, allowing neurons to form an extremely dense network to process external stimuli. While we are gaining a better understanding of the rules of circuit wiring, at the intracellular level we still lack an understanding about which features are random, which are controlled organization and which are cell type specific. Reconstructions of the neuronal ultrastructure is a multiscale problem spanning from the few nm-scale of ER diameter to the dimension of cells and neuronal projections. We imaged large-scale 3D EM volumes of the rodent cerebellum and also performed electron tomography on high-pressure frozen tissue. We reconstructed the neuronal ultrastructure of different cell types, focusing on the ER, mitochondria and membrane contact sites, to then characterize the intracellular organization in detail. At the gross level organization, we found that the intracellular composite of organelles is a cell type specific feature, with large differences between cerebellar cell types. At the fine level organization, we found ultrastructural domains of ER and mitochondria hotspots within Purkinje cells. We expect that our cellular maps will facilitate future studies in health, aging and disease to characterize defined features, by developing a framework for quantitative analysis of the neuronal ultrastructure.

Symposium

S16: Big science, big challenges, and the diversity of life sciences - where does neuroscience go?

S16-2 An interdisciplinary and theory driven approach to integrate multiple data sources that inform brain function across biological scales
Petra Ritter

S16-3 Communicating neuroscience in the era of big data
Alison Claire Abbott

S16-4 Neuroscience – past developments and emerging trends
Peter Stern

An interdisciplinary and theory driven approach to integrate multiple data sources that inform brain function across biological scales

Petra Ritter[1]

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We will present an approach of multiscale brain network modeling and simulation that enables the iterative inclusion of empirical observations (across species) in a unifying computational model describing complex interacting neurophysiological processes at different temporal and spatial scales. These computational models can be tested, falsified and subsequently further refined. The resulting digital twins encapsulate our present understanding of brain function and dysfunction. Personalization through inclusion of single subject/patient data allows for individualized simulations – including the simulation of in silico interventions for a patient. Data standards, ontologies and metadata schemas as well as secure privacy protecting digital high performance compute infrastructure facilitate the integration of empirical and computational data from various sources towards digital brain twins. Digital brain twins can further be integrated with environmental and lifetime information as well as data and models of the body to receive an increasing holistic understanding of the complex interplay of brain, body and environment over the lifetime. Mathematical human digital twins enable us to infer principles and rules underlying brain function and dysfunction that can be utilized upon careful validation for clinical applications.

Communicating neuroscience in the era of big data

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- not available -

Neuroscience – past developments and emerging trends

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When I entered the field of neuroscience as an active researcher the dominant organizational structure was a cottage industry like approach. There were plenty of small laboratories often using their own home-made equipment. Many papers had only one or two author names on it. Over the years the number of coauthors on publications has steadily increased. We also see more and more collaborations between research groups located at different institutions, in different countries and even on different continents. In the last years this continuous trend has seen an exponential increase due to the arrival of mega scale science projects. This brings new opportunities promising to address some of the seriously hard and profound questions in modern neuroscience. However, there are also new challenges for example concerning data quality, data responsibility or conceptual incompatibilities. This presentation will discuss these trends in a broader contextual framework.

Symposium

S17: Mechanisms of reperfusion-failure after cerebral ischemia

S17-1 Role of pericytes in incomplete reperfusion after cerebral and retinal ischemia
Turgay Dalkara

S17-2 The impact of capillary function on tissue oxygenation during ischemia and reperfusion
Leif Østergaard

S17-3 Role of pericytes for reperfusion failure after ischemic stroke in vivo
Joshua James Shrouder, Gian Marco Calandra, Severin Filser, Daniel Peter Varga, Simon Besson-Girard, Uta Mamrak, Maximilian Dorok, Buket Bulut-Impraim, Fatma Burcu Seker, Benno Gesierich, Fabio Laredo, Antonia Clarissa Wehn, Igor Khalin, Patrick Bayer, Arthur Liesz, Ozgun Gokce, Nikolaus Plesnila

S17-4 Breathing New Life into Stroke Therapy: The Anti-inflammatory Power of Inhaled Nitric Oxide
Rebecca Isabella Sienel, Burcu Seker, Nikolaus Plesnila

S17-5 Reperfusion failure following recanalization after ischemic stroke
Nikolaus Plesnila

Role of pericytes in incomplete reperfusion after cerebral and retinal ischemia

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Over the past two decades, our understanding of how blood flow is regulated in CNS tissues has undergone a significant transformation. It was previously believed that blood flow control primarily occurred at the arteriole level, but it is now recognized that the regulation of capillary blood flow by contractile pericytes is crucial in both normal physiological conditions and in limiting blood flow during certain pathological states. In this presentation, we explore the role of pericytes in regulating blood flow in the brain and retina, with a particular focus on the pathological events associated with cerebral and retinal ischemia. Despite the differences in the morphology of cerebral and retinal capillary beds, pericyte-mediated capillary constriction plays an important role in limiting blood flow following ischemia. We conclude by proposing potential therapeutic strategies aimed at relaxing pericytes, which could offer long-term benefits in alleviating incomplete reperfusion after ischemia.

The impact of capillary function on tissue oxygenation during ischemia and reperfusion

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Today, acute ischemic stroke-related symptoms and tissue injury (infarction) are ascribed to critically low cellular oxygen access as vascular changes reduce CBF below the ischemic threshold (~20 mL blood/100 mL tissue/minute) and cause (i) neurological symptoms and (ii) permanent tissue injury unless CBF is restored. Such salvageable brain tissue is referred to as the ischemic penumbra, whose survival is thought to depend on the residual CBF and to range from minutes at CBF levels below 50% of the ischemic threshold to more than 24 hours for milder ischemia. This defines the time-window, and urgency, of reperfusion therapy: intravenous thrombolysis or intraarterial thrombolysis/mechanical thrombectomy to restore CBF. Roughly 35% of AIS patients are eligible for these therapies. Meanwhile, half of the AIS patients who undergo thrombectomy have unfavorable clinical outcomes, ranging from moderate disability to death - despite successful revascularization and minor ischemic tissue injury prior to the treatment – so-called futile reperfusion. Neuroprotection denotes interventions that delay penumbral tissue injury until CBF is restored either therapeutically or spontaneously. However, neuroprotective therapies mostly fail the translation from animal models into human stroke thus far17.
The lecture will discuss how per-ischemic capillary occlusions might affect the oxygenation of penumbral tissue - and how residual capillary occlusions after recanalization could contribute to poor oxygenation and futile reperfusion. With this in mind, capillary perfusion will be discussed as a neuroprotective strategy.

Role of pericytes for reperfusion failure after ischemic stroke in vivo

Joshua James Shrouder[1,2], Gian Marco Calandra[1,2], Severin Filser[1,2,3], Daniel Peter Varga[1,2], Simon Besson-Girard[1,2], Uta Mamrak[1], Maximilian Dorok[1], Buket Bulut-Impraim[1,2], Fatma Burcu Seker[1,2], Benno Gesierich[1,2], Fabio Laredo[1], Antonia Clarissa Wehn[1,2,4], Igor Khalin[1,2,5], Patrick Bayer[1], Arthur Liesz[1,2], Ozgun Gokce[1,2], Nikolaus Plesnila[1,2]

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Incomplete reperfusion of the microvasculature (‘no-reflow’) after ischaemic stroke damages salvageable brain tissue. Previous ex vivo studies suggest pericytes are vulnerable to ischaemia and may exacerbate no-reflow, but the viability of pericytes and their association with no-reflow remains under-explored in vivo.

Using longitudinal in vivo two-photon single-cell imaging over 7 days, we showed that 87% of pericytes constrict during cerebral ischaemia and remain constricted post reperfusion, and 50% of the pericyte population are acutely damaged. Moreover, we revealed ischaemic pericytes to be fundamentally implicated in capillary no-reflow by limiting and arresting blood flow within the first 24 h post stroke. Despite sustaining acute membrane damage, we observed

that over half of all cortical pericytes survived ischaemia and responded to vasoactive stimuli, upregulated unique transcriptomic profiles and replicated. Finally, we demonstrated the delayed recovery of capillary diameter by ischaemic pericytes after reperfusion predicted vessel reconstriction in the subacute phase of stroke. Cumulatively, these findings demonstrate that surviving cortical pericytes remain both viable and promising

therapeutic targets to counteract no-reflow after ischaemic stroke.

Breathing New Life into Stroke Therapy: The Anti-inflammatory Power of Inhaled Nitric Oxide

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Background and aims: Inhaled nitric oxide (iNO) is known to selectively increase collateral blood flow after ischemic stroke. Since iNO exhibits also anti-adhesive properties on adhering leukocytes we hypothesized that iNO, in addition to its cerebral blood flow modulation, may protect the brain by inhibiting post-ischemic inflammation.

Methods: Male C57BL/6 mice underwent 60 minutes of middle cerebral artery occlusion, followed by inhalation of 50 ppm NO upon reperfusion. Visualization of leukocyte-endothelial interactions (LEI) was conducted *in vivo* using deep brain 2-photon microscopy. Five hours post-reperfusion, plasma and tissue samples were collected for the analysis of cytokines, adhesion molecules, leukocyte numbers, nitric oxide synthases, and NO metabolites through qPCR, Western blot, ELISA, or chemiluminescence.

Results: iNO application significantly increased NO-related metabolites, nitrite and nitrate, in plasma four-fold ($p < 0.001$). Furthermore, iNO reduced leukocyte rolling and adhesion by 75% ($p < 0.05$) and 98% ($p < 0.001$), respectively. Additionally, iNO downregulated cytokine and ICAM-1 expression by 60% ($p < 0.05$) and 75% ($p < 0.01$), respectively.

Conclusion: In the context of focal cerebral ischemia, iNO demonstrated a reduction in inflammatory signaling within cerebral vessels. This suggests the potential for iNO as a novel therapeutic approach for ischemic stroke, emphasizing the need for clinical evaluation to further validate its efficacy in reducing vascular inflammation.

Reperfusion failure following recanalization after ischemic stroke

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Ischemic stroke causes brain damage not only during the ischemic period but, counterintuitively, also after recanalization of the occluded artery. As more and more stroke patients receive recanalization therapy, prevention of reperfusion injury may have great clinical potential. Unfortunately, the underlying mechanisms are not fully understood.

Traditionally, the so-called "no reflow phenomenon" has been thought to be one of the main processes contributing to reperfusion injury. "No reflow" describes a process in which cerebral microcirculation is obstructed during ischemia and recanalization is unable to restore microvascular flow. A plethora of processes have been discussed as responsible for no reflow after ischemic stroke, mainly based on histopathological analysis at a single postmortem time point. Recently, however, high-resolution microscopy and MR imaging technologies have become available that allow longitudinal imaging of the cerebral microcirculation with low invasiveness in the living brain during and after cerebral ischemia. The current presentation aims to summarize these recent findings and provide evidence that reperfusion failure may be caused by microvascular constriction, may be a delayed phenomenon, and may be closely related to neuroinflammation and cerebral edema formation, processes that are amenable to therapeutic intervention. Therefore, reperfusion failure may represent a valid therapeutic target in ischemic stroke.

Symposium

S18: How the nervous system builds and maintains myelin

S18-1 Non-synaptic glutamate transfer between axons and oligodendrocytes regulates myelination in vivo – insights from zebrafish
Rafael Gois Almeida

S18-2 Adhesion proteins synergistically regulate myelin formation
Minou Djannatian

S18-3 Cell biological mechanisms of myelin tuning and dynamics
Brad Zuchero

S18-4 Oligodendrocyte mechanotransduction channel TMEM63A fine-tunes myelin sheath geometry
Ram Dereddi

S18-5 Myelin turnover, maintenance and disease: Insight from electron microscopy and 3D imaging by FIB-SEM
Wiebke Möbius, Leonie C. Schadt, Aletta M. R. van den Bosch, Anna M. Steyer, Martin Meschkat, Hauke B. Werner, Inge Huitinga, Klaus-Armin Nave

Non-synaptic glutamate transfer between axons and oligodendrocytes regulates myelination in vivo – insights from zebrafish

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Neurotransmitter signalling has been implicated in the regulation of myelination, but the underlying cellular and molecular mechanisms of this mode of axon-myelin communication remain poorly understood in vivo. To address this, we adapted live reporters of neurotransmission for zebrafish, which are well-suited for imaging intact vertebrate circuits. By live-imaging individual glutamatergic spinal neurons, we found synaptic vesicle fusion and glutamate release to be equally frequent at presynaptic terminals and along the axon. This ‘axonal neurotransmission’ emerged with the onset of myelination, and, as myelination progressed, became enriched in hotspots adjacent to myelin sheaths. This suggests direct regulation of myelin growth by glutamate. Indeed, blocking axonal vesicle fusion destabilized nascent myelin sheaths, while chemogenetically stimulating neuronal activity increased axonal vesicle fusion and promoted myelin growth. Furthermore, the extracellular glutamate reporter SFiGLuSnFR revealed oligodendrocytes responses in myelin sheaths in vivo. Additionally, we found that metabotropic glutamate receptors in oligodendrocytes regulated myelin sheath growth. Our study reveals some of the molecular mechanisms that mediate activity-regulated myelination in vivo, and underscores the need to consider both synaptic and non-synaptic modes of neurotransmission in myelinated circuit formation and function.

Adhesion proteins synergistically regulate myelin formation

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Myelination is a developmentally wired process by which oligodendrocytes in the central nervous system (CNS) enwrap axons with a specialized membrane to ensure electrical insulation. This occurs in a highly orchestrated sequence that includes repetitive axon-glia interactions, membrane wrapping and longitudinal elongation, and membrane compaction. Myelin can to some extent be modified once it has formed, and it can be newly generated later in life, in response to sensory input or a demyelination event. The molecules underlying these critical steps in CNS myelination are however only partially understood. Here, I will focus on the role of adhesion proteins in regulating axon-glia interactions and myelin formation. Using a combination of *in vivo* confocal imaging and 3D electron microscopy in mice and zebrafish, we show that axonal recognition, myelin wrapping and longitudinal extension are severely disturbed in the absence of synergistically acting adhesive systems. Together, these data provide a better mechanistic understanding of how the spatial organization of myelin is achieved.

Cell biological mechanisms of myelin tuning and dynamics

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Myelin is essential for rapid nerve signaling and is increasingly found to play important roles in learning and in diverse diseases of the CNS. Morphological parameters of myelin such as sheath length and thickness are thought to precisely tune conduction velocity and circuit function, but the mechanisms controlling myelin sheath morphology are poorly understood. Our work has converged on two cellular mechanisms within oligodendrocytes that are key to myelin sheath morphology: actin dynamics and vesicular exocytosis. Both actin dynamics and exocytosis are regulated by calcium signaling in other cell types, and local calcium signaling occurs in myelin sheaths in response to neuronal activity. We hypothesize that neuronal activity-induced calcium signaling in myelin sheaths directly regulates actin dynamics and the frequency of exocytosis, which together collaborate to tune sheath length and wrapping. We have developed a powerful genetic toolkit to visualize or perturb each of these cell biological mechanisms (calcium signaling, actin, exocytosis) in oligodendrocytes in culture and in myelin sheaths in vivo. I will discuss my lab's recent and ongoing work using these tools to determine how myelin is tuned in development and remodels during learning, with the longer-term goal of understanding the contribution of myelin dynamics to learning, aging, and disease.

Oligodendrocyte mechanotransduction channel TMEM63A fine-tunes myelin sheath geometry

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Mechanotransduction channels (MTCs) play a crucial role in the process of peripheral myelination by Schwann cells. Although it has been proposed that mechanical forces can regulate myelination in the central nervous system (CNS), very little is known about the expression of MTCs and their role in oligodendrocytes (OLs) development and myelination. To systematically identify the expression of all mechanotransduction ion channels in OLs, we performed single-cell Split-Seq RNA sequencing analysis on Magnetic-activated cell-sorted O4+ OLs from the adult cerebral cortex. We found that several MTCs are expressed by OLs and discovered Tmem63a (also called OCaR1) as one of the most abundant MTCs in OLs. To characterize the expression of Tmem63a in the CNS, we used our newly generated knock-in transgenic mouse lines expressing endogenous levels of Tmem63a protein tagged with enhanced yellow fluorescent protein (Tmem63a-eYFP). An extensive immunohistochemical analysis showed that Tmem63a is mainly expressed by myelinating as well as mature OLs in both grey- and white matter but not in oligodendrocyte precursor cells (OPCs). Our electrophysiological and Ca2+ imaging analysis on OL derived from control and Tmem63a knock-out (Tmem63a-/-) mice revealed that mechanical membrane stretches resulted in Tmem63a-dependent influx of positive cations and induced robust Ca2+ signals in the OL. A systematic histological analysis of the brains from control and Tmem63a-/- mice showed a severe developmental hypomyelination at postnatal days (P) 11, which persisted at the juvenile stage (P21) but was resolved while mice reached sexual maturity and adulthood around 5-6 weeks of age (P35). Although the gross developmental myelination was recovered by P35, a detailed electron microscopic analysis of axonal myelination in the motor cortex and corpus callosum revealed aberrant myelination of very small caliber axons (<150nm) and hypomyelination of large caliber axons, indicating that Tmem63a plays a decisive role in tuning myelin sheath thickness on individual axons. In Tmem63a-/- mice and OL-specific conditional knock-out mice (Mogi-Cre; Tmem63a fl/fl), we observed that OLs layered shorter myelin internodes with diffused and elongated nodes of Ranvier. Next, we performed a systematic motor behavior analysis on 7-8 weeks-old Tmem63a -/- mice to test whether such radial and longitudinal ultrastructural deficits in myelin sheath could result in motor dysfunction. While Tmem63a-/- mice had no significant movement dysfunction and ataxias, mutants exhibited deficits in fine motor functions such as motor coordination and gait. To gain mechanistic insights into intracellular signaling regulated by Tmem63a in OL, we performed bulk-RNA sequencing on enriched OLs isolated from the cortex of Tmem63a -/- mice and littermate controls at P11 and P35. Analysis of differentially expressed genes between the P11 and P35 stages indicates that Tmem63a-mediate Ca^{2+}signaling might regulate vesicular transport and endocytotic secretory pathways. In summary, we identified Tmem63a as a key MCT in OLs, which links mechanical forces sensed by OLs during active myelination with the Ca^{2+}dependent transport and secretory pathways required for fine-tuning myelin sheath thickness on axons.

Myelin turnover, maintenance and disease: Insight from electron microscopy and 3D imaging by FIB-SEM

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Myelin in the central nervous system (CNS) is formed by oligodendrocytes. These cells differentiate from oligodendrocyte precursor cells (OPCs) early in development, generate the first myelin sheaths and persist life-long. In addition, new myelin is continuously formed throughout life, while established myelin sheaths appear stable and contain proteins with exceptionally long lifetimes. To address the question, how myelin sheaths are maintained and turned over, we created an inducible MBP knock-out mouse line to abolish MBP biosynthesis after completion of myelination at the age of 8 weeks. Since lack of MBP prevents the compaction of any newly formed myelin membranes, we used this structural defect to visualize the long-term dynamics and turnover of the individual myelin sheath with volume electron microscopy (vEM) using focused ion beam-scanning electron microscopy (FIB-SEM) and nanoscale secondary ion mass spectrometry (NanoSIMS). Loss of MBP caused a slow demyelination by local integration of newly synthetized non-compacted membranes at the inner tongue, paranodal loops and juxtaparanodes. Thinning of myelin sheaths and shortening of internodes resulted in the loss of 50% of myelin within 20 weeks after recombination indicating that continuous MBP biosynthesis is required for myelin maintenance.

CNS myelin is characterized by a specific proteome. To study the functional relevance of the prevalent structural myelin proteins proteolipid protein (PLP) and myelin associated protein (MAG) we applied vEM using FIB-SEM and three-dimensional reconstruction in *Plp*- and *Mag*-deficient mouse mutants. This revealed that both mutants displayed pathological myelin outfoldings extending up to 10 µm along the internode. In addition, we observed an increased axonal diameter and complex axonal abnormalities underneath myelin outfoldings indicating that structural myelin proteins influence axonal properties.

High resolution immunohistochemistry and quantitative transmission electron microscopy were performed to investigate inflammation and ultrastructure in normal-appearing white matter (NAWM) in multiple sclerosis (MS) in human post-mortem tissue. We detected subclinical alterations of the axon-myelin unit in MS NAWM characterized by activated and phagocytic microglia and CD3+ T cells and changes in paranodes and juxtaparanodes. The myelin structure appeared less compact, showed a higher g-ratio and a higher frequency of axonal mitochondria. These observed subtle changes in MS NAWM may contribute to disease progression.

Symposium

S19: Visual processing in social behaviors

Social distancing: Group behavior and the underlying neural circuits in Drosophila melanogaster larvae

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Decision-making is complex, as animals not only need to consider information based on their own perception, internal state and experience, but also social cues. Living with conspecifics can be beneficial to gain more information regarding the environment and to access better resources. However, it also increases competition for mates and food. How animals sense their conspecifics and then use this information to modulate their behaviour in a social context is not very well understood. To answer this question, we are investigating group behaviour in larvae of the model organism Drosophila melanogaster. In fly larvae we can make use of state-of-the-art behavioural tracking methods and a rich array of genetic tools to understand behaviour and the underlying neural circuits in detail. Behavioural experiments show that Drosophila larvae avoid their conspecifics in an open arena without any food and disperse more than when they are alone. Being in a group also affects decision-making in different sensory contexts and internal states. Furthermore, social distancing is dependent on social experience during development. Preliminary genetic manipulation experiments suggest that larvae sense each other via multiple sensory systems. To better understand the social dynamics, we make use of an agent-based model which can reproduce behavioural effects. Our results will help to better understand the behavioural algorithms and neural processing mechanisms that underlie social interactions between conspecifics.

Sex differences in modulation of defensive behaviours by social motion

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A major benefit of being in a group is the possibility of integrating social information with directly-perceived information about the environment to guide behaviour. Across the animal kingdom, social information acquired via specific signals or cues is used for decisions in reproduction, foraging and protection against predation. Acute fitness benefits of the usage of social information are flagrant in the context of a response to a potential threat: failure to detect a predator can lead to an animal's immediate demise, whereas needless engagement in metabolically costly defence responses can, unnecessarily, impact fitness. We previously showed that female flies in groups decrease their active immobility responses to an external visual threat, i.e., freezing, aimed at becoming inconspicuous. This 'safety in numbers' effect scales with group size and is underlain by the level of motion cues from the social surroundings. Crucially, exposing wild-type female flies to a constant number of surrounding flies whose movement we controlled producing graded social cues of safety led to graded freezing responses. Interestingly, we identified sex dimorphism at the behavioural and neuronal level. Males show a strepper decrease of freezing responses in groups than females, whether surrounded by one or both sexes. Moreover, while in female flies social motion cues are mediated by LC11 visual projection neurons, the group effect on freezing in males is LC11-independent. Importantly these differences in freezing responses seem evident even when flies of both sexes are exposed to similar social motion cues. With tight control over the surrounding social environment by controlling the movement of others, and resorting to a newly developed drift diffusion model, we are now uncovering the mechanisms that underlie this dimorphism. These mechanisms could potentially be linked with other sex dimorphic behaviours and circuits and understanding them will contribute to our understanding of sex differences in social behaviours.

Neural circuits for social affiliation in zebrafish

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Many species live in groups and affiliate with conspecifics upon sensory detection and processing of social information. However, investigating sensory processing during social behavior is inherently difficult because in most cases, the mutual interactions between individuals and the resulting sensory experience are beyond experimental control.
We investigate affiliation pathways in juvenile zebrafish in the context of shoaling, the innate and perpetual drive to swim in groups with continuously moving conspecifics. Using virtual reality psychophysics, we recently identified self-like biological motion as one visual trigger of shoaling. We traced biological motion into the brain and discovered a specifically tuned tecto-thalamic visual pathway that detects this social signal and drives shoaling. We now use the tools available in zebrafish for whole-brain activity mapping and cell type discovery to generate a more complete picture of the neuronal implementation of shoaling. Using candidate screening and artificial selection for extreme social behavior, we have identified a set of socially diverging zebrafish lines to investigate how genetic polymorphisms alter the neuronal processing of social cues. Thus, we can now investigate how individuals coordinate social affiliation at the interface of behavioral algorithms, neuronal circuits, and genetic factors.

From Face Perception to Social Cognition

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Humans and other primates have evolved specialized circuitry to process visual information emanating from the face. Faces reveal a multitude of information, through both structure and dynamics, and for them to serve in social communication, face-recognition mechanisms must exist to extract them and relay them for further processing. Much of the research into the neural mechanisms of face-recognition over the last twenty years has focused on a network of face areas within inferotemporal cortex implementing an information-processing hierarchy to extract facial identify. Other properties, especially changeable or changing ones like expressions and gaze, have received less attention. In this talk I will present recent work from my laboratory that started with the discovery of an additional face-selective area in the brain of the macaque monkey, located closely to where face-cells were initially discovered. Cells in this region encode a wide range of facial properties, including, but not limited to, changeable ones, and also encode facial dynamics, including expression and gaze. I will discuss the detailed properties of this area and how it relays information to other parts of the social brain. What emerges is a circuit that links face perception to social cognition and to facial movements in turn.

Sex, time and the social brain

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By bringing most recent findings on visual social cognition (body language reading, facial assessment of social counterparts, face pareidolia, reading in the eyes and covered faces) along with advanced neuroimaging strategies, I intend to conceive perspectives, open questions, and limitations in our understanding of the social brain. The social brain has many facets playing decisive role in majority of neuropsychiatric conditions such as autistic spectrum disorders, schizophrenia, and depression. I will focus on the following issues: (i) Neuroimaging of the social brain: With the advent of sophisticated techniques over the past decades, brain imaging has energized the rapidly developing field of Social Neuroscience, and has sparked a wide range of research in neuropsychiatry. Yet brain imaging faces with a set of issues that must be addressed. One of them is time, which is a key to understanding the organization of functional networks, since brain topography alone does not allow us to understand neural communication as well as pathological changes in brain activation; (ii) Behavior and brain activity: This relationship is far from simple even in individuals, free from the rich complexities of psychopathology; and (iii) Sex specificity of the social brain: Many neuropsychiatric disorders characterized by aberrant social cognition display a skewed sex ratio: females and males are affected differently in terms of clinical picture, prevalence, and severity. Currently, we are only beginning to understand the origins of gender/sex specificity.

Symposium

S20: Investigating memory using human single-neuron recordings

S20-1 Hippocampal Single-Neuron Dynamics in Working Memory Maintenance and Long-Term Memory Formation
Jonathan Daume, Jan Kaminski, Yousef Salimpour, Andrea Gomez Palacio Schjetnan, Chrystal M. Reed, William S. Anderson, Adam N. Mamelak, Ueli Rutishauser

S20-2 The role of concept cells in memory formation
Sina Mackay

S20-3 Attentional modulation of single-unit activity in the human medial temporal lobe
Ilona Vieten, Jennifer Faber, Valeri Borger, Rainer Surges, Florian Mormann

S20-4 The role of gamma oscillations in stimulus encoding and memory maintenance during a sequential memory task in the human Medial Temporal Lobe
Muthu Jeyanthi Prakash

S20-5 Decoding movie content from neuronal population activity in the human medial temporal lobe
Alana Irene Darcher, Franziska Gerken, Pedro J. Gonçalves, Rachel Rapp, Ismail Elezi, Johannes Niediek, Marcel S. Kehl, Thomas P. Reber, Stefanie Liebe, Jakob H. Macke, Florian Mormann, Laura Leal-Taixé

Hippocampal Single-Neuron Dynamics in Working Memory Maintenance and Long-Term Memory Formation

Jonathan Daume[1,2,3], Jan Kaminski[4], Yousef Salimpour[5], Andrea Gomez Palacio Schjetnan[6], Chrystal M. Reed[2], William S. Anderson[6], Adam N. Mamelak[1], Ueli Rutishauser[1,2,3,7]

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Memory is a complex cognitive process that involves both the retention of information in working memory (WM) and the formation of long-term memories (LTM). In this presentation, we offer new insights into how the brain orchestrates these memory functions at the level of single neurons in humans. First, we uncover the critical role of cognitive control in maintaining WM information in a Sternberg WM task. Using single-neuron recordings from the medial temporal and frontal lobes of patients, we demonstrate that theta–gamma phase–amplitude coupling (TG-PAC) in the hippocampus is crucial for preserving memoranda-specific persistent activity. We identified hippocampal neurons that selectively spike in response to nonlinear interactions of theta phase and gamma amplitude, with their timing coordinated by frontal theta activity during high cognitive control demand. This dynamic, mediated by noise correlations in the hippocampus, shaped population coding geometry, leading to more accurate representations of WM content and enhanced behavioral performance. These findings suggest that TG-PAC serves as a mechanism for integrating cognitive control and working memory storage, highlighting the hippocampus' role in top-down modulation of sensory-driven storage processes. We then show that persistent activity in WM-selective hippocampal neurons predicted the later successful encoding of items into LTM. Notably, the level of persistent activity, rather than visually evoked responses, determined whether an item was recognized with high confidence or forgotten. During memory retrieval, memory-selective neurons demonstrated stronger responses to familiar stimuli when they were previously maintained with strong WM maintenance activity. This indicates that hippocampal persistent activity not only supports WM maintenance but also plays a pivotal role in LTM encoding, suggesting a shared single-neuron mechanism underlying both memory systems. Together, these results provide novel insights into the multicomponent architecture of memory, revealing how cognitive control and hippocampal dynamics contribute to both WM retention and LTM formation.

The role of concept cells in memory formation

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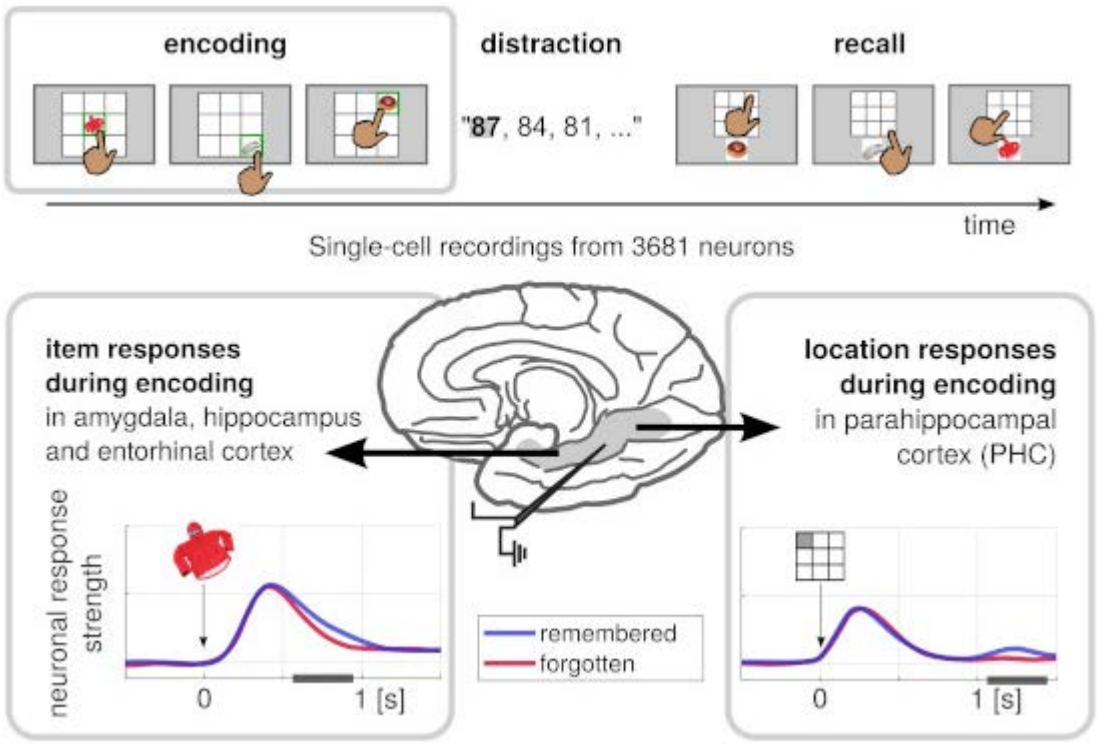
Our brains encode new memories by capturing the 'who/what,' 'where,' and 'when' of everyday experiences. Concept neurons, located in the human medial temporal lobe (MTL), represent semantic concepts and are thought to serve as the building blocks of memory. However, the specific neuronal mechanisms that enable episodic memory encoding remain poorly understood. In the first study, we examined single-neuron activity in the human MTL during the encoding of item-location associations. Two distinct populations of neurons were identified: concept cells in the hippocampus, amygdala, and entorhinal cortex, and location-selective neurons in the parahippocampal cortex. Both groups exhibited significantly higher firing rates during trials that were successfully encoded.
In a second study, we investigated the role of concept neurons in conscious human participants performing an item-location association memory task during a pharmacological intervention: A low dose of propofol was administered, which altered neuronal responses and selectively inhibited memory formation. Attenuated or delayed responses in hippocampal concept neurons disrupted associative memory, which involves linking items to their spatial locations, while object recognition and perception remained unaffected. These results demonstrate that concept neurons are required for episodic memory formation, providing novel insights into how specific neuronal populations contribute to encoding the building blocks of memory.



Stronger responses during successful encoding in the associative memory paradigm

Attentional modulation of single-unit activity in the human medial temporal lobe

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The human medial temporal lobe (MTL) contains neurons characterized by remarkable stimulus selectivity and semantic invariance, called concept cells. These cells play an important role in conscious perception and both working and long-term memory. Based on their response characteristics, they are hypothesized to constitute the semantic building blocks of episodic memory in humans. Attention denotes the ability of the brain to organize and guide perception towards relevant stimuli. Extensive work in non-human primates as well as functional imaging in humans has shown attentional modulation of regional and single-unit responses to preferred stimuli, mostly in the visual system. However, the interplay between attention and visual processing on a single-unit level has not yet been addressed in the human MTL. In our study, 20 epilepsy patients with microwire electrodes embedded in four MTL regions performed 36 sessions of a task designed to isolate the effect of different attentional demands on single-unit activity. Subjects viewed a rapid, pseudo-randomized stream of eight images previously found to specifically activate certain recorded neurons. They were tasked with counting the occurrence of a particular image in the stream while each image was shown around 30 times. This was repeated 8 times using the same images, but with the task focused on a different one during each run. This allowed us to analyze responses to both unattended and attended trials for all images. We recorded from up to now 167 selectively responsive units in the parahippocampal cortex (PHC, 76 units), the entorhinal cortex (EC, 6 units), the hippocampus (H, 30 units), and the amygdala (A, 55 units). We analyzed response magnitude, latency, and duration for each unit in both the target and the non-target condition. We found that the magnitude of stimulus-related activity was attention-modulated in about a third of all responsive cells in each region. However, overall response magnitude was not different between regions. We found that response latency was not attention-modulated, but that PHC units had earlier latencies than those in other regions. In terms of response duration, we found that it differed between conditions in PHC and H, but not between regions. Our results suggest a functional division among stimulus-selective neurons in the human MTL, with a large minority being sensitive to attentional modulation. These subpopulations could play different roles in the transfer of experiences into long-term memory.

The role of gamma oscillations in stimulus encoding and memory maintenance during a sequential memory task in the human Medial Temporal Lobe

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The encoding and maintenance of sequential information is a fundamental component of episodic memory, though the underlying mechanisms are still open to investigation. A prominent theory proposes that maintaining sequential information in memory is reflected in ordered firing of neurons at different phases of theta oscillations (1). One study has recently challenged this view using single-unit and LFP recordings from epilepsy patients in the human medial temporal lobe (MTL, 2). In addition to predicting a specific temporal relationship between spiking and theta oscillations related to temporal order, the theory also assumes that 1. gamma-frequency activity encodes stimulus-relevant information that is maintained within memory and temporally aligned to spiking of individual neurons and 2. proposes a temporal relationship between gamma activity and theta oscillations during memory. Both aspects were not directly explored in the previous study. To address these questions, we utilized the same dataset as in (2) consisting of local field potentials (917 channels) and SUA (1411) from the MTL of epilepsy patients performing a working memory task for temporal order. When assessing whether gamma power (60 - 100 Hz) encoded stimulus information, 31% of the channels exhibited increased activity during visual presentation of stimuli as compared to baseline (test $p<0.001$, Binomial test). Stimulus identity could both be successfully decoded from firing rates of single units and gamma power ($p < 0.05$, Mann Whitney U test). Interestingly, stimulus-encoding channels also showed increased gamma power and higher theta-gamma phase amplitude coupling than non-responsive channels during memory maintenance ($p < 0.001$, Mann Whitney U test). Further, we identified a subset of LFP-neuron pairs (N = 70) with similar stimulus preferences as the corresponding LFP-gamma power. These pairs exhibited increased gamma-spike-field coherence during the encoding and maintenance period as compared to pairs with non-overlapping stimulus preferences ($p < 0.05$, Mann Whitney U test). Taken together, our preliminary analyses show that stimulus information is encoded in 1. gamma power, 2. increased temporal alignment between spiking and gamma during encoding and delay and 3. Increased phase-amplitude coupling between gamma and theta during the delay, as predicted by the theory. We plan to further explore how these findings relate to the temporal order of stimuli, and whether the model holds true in its major prediction about the relationship between stimulus- and theta phase order.

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Decoding movie content from neuronal population activity in the human medial temporal lobe

Alana Irene Darcher[1], Franziska Gerken[2], Pedro J. Gonçalves[3,4,5], Rachel Rapp[3], Ismail Elezi[4], Johannes Niediek[2,8], Marcel S. Kehl[2,9], Thomas P. Reber[2,10], Stefanie Liebe[3,6], Jakob H. Macke[3,7], Florian Mormann[2], Laura Leal-Taixé[1]

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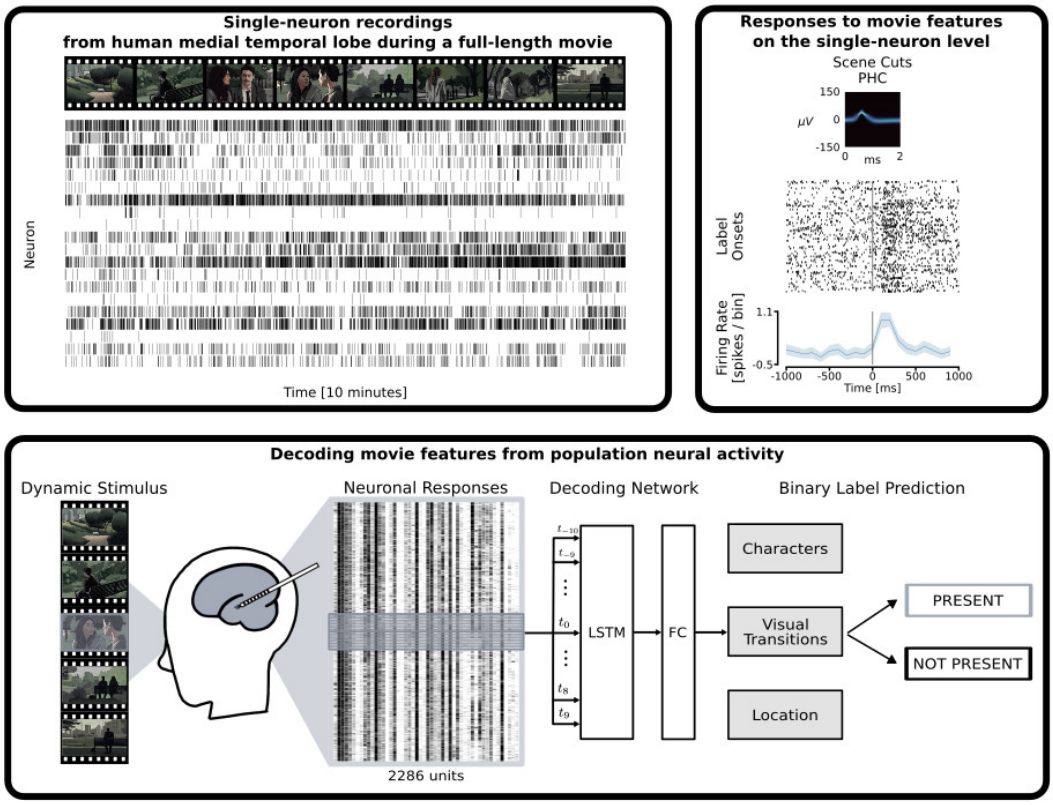
While the characteristics of single neurons in the human medial temporal lobe have been studied extensively via the presentation of static, isolated stimuli, it remains unknown how such characteristics scale to dynamic, continuous stimuli. In this work, we investigated the role played by single neurons in processing semantic information embedded in a more naturalistic setting, namely that of a commercial movie. Using a dataset of sessions collected from 29 intracranially-implanted patients during the presentation of a full-length movie, we studied 2286 neurons recorded from the amygdala, hippocampus, entorhinal-cortex, and parahippocampal cortex. Although few individual neurons exhibited preferential responses to semantic features of the movie, we could nonetheless reliably decode the presence of the movie's main characters, location, and visual transitions on a framewise basis from the population activity of neurons using a machine learning pipeline. By using various model architectures, we further untangled the role of temporal dynamics in each stimulus category, and found that character and location representations do not use timing information, while visual transitions do. We additionally analyzed the role of individual neurons for the decoding by examining the features of the trained models, and found that the information necessary to decode character presence is distributed across the neuronal population, while the information needed to decode visual transitions is concentrated in a subset of highly-responsive single neurons. Our results demonstrate that features of naturalistic stimuli are represented in the neuronal populations of the human medial temporal lobe in a category-dependent manner, and that these features can be decoded on a frame-wise basis.



Decoding features of a naturalistic, dynamic stimulus from populations of human single neurons. Top left panel: selection of spiking activity from a subset of the total 2286 neurons recorded, from 10 minutes of movie presentation. Frames given as an example scene sequence from within the film (images were generated via Stable Diffusion, actual frames not shown due to copyright issues). Top right panel: Example parahippocampal cortex (PHC) unit response to the onset of scene transitions. Upper plot depicts the spike density for all detected spikes, middle plot shows rasters for scene onsets, bottom plot shows the binned spike count (firing rate) for all scene onsets. Bottom panel: Decoding pipeline for prediction movie features from population activity. Features were predicted for each frame from a 1600 ms window of population spiking activity (purple highlights).

Symposium

S21: Social immunity as defense against diseases: from sensory biology to collective animal behavior

S21-1 Social implications of viruses on gustatory, olfactory and visual perception and dietary choices in honey bees
Michael Simone-Finstrom

S21-2 Understanding and selectively breeding for social immunity behaviours in honey bees using proteomics & genomics
Leonard Foster

S21-3 Contributions of chemical ecology to a better understanding of social immunity in the honey bee
Fanny Mondet, Amelie Noel, Charlène Dumas, Alison Mercer, Yves Le Conte

S21-4 Evolution of Social Wound Care Behaviours in Ants
Erik Thomas Frank

Social implications of viruses on gustatory, olfactory and visual perception and dietary choices in honey bees

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Honey bees contend with a suite of parasites and pathogens that contribute to high annual losses in managed colonies. While huge advances have been made over the last decade in our discovery of pathogens and the multifaceted impacts they have on bee health and colony productivity, it has become clear that many effects can be context-dependent and influenced by various biotic and abiotic factors. For example, recent research has shown that viral infection can influence foraging and diet choices, but varies both by the type or strain of virus and the interaction with bee genetic stock. Inherent in this is the potential that host physiology drives perception differences and dietary choices based on infection status, or alternatively that the pathogen alters host behavior in a way that increases its ability to spread within and among hosts. Research on how viral infection alters visual cue perception will be presented with commentary on how this may influence foraging choices in the field. Additionally, a series of experiments will be discussed regarding whether honey bees can detect common honey bee viruses (deformed wing virus, black queen cell virus, and chronic bee paralysis virus) in their environment to provide a more complete picture of how viruses can spread and influence bee behavior.

Understanding and selectively breeding for social immunity behaviours in honey bees using proteomics & genomics

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Social immunity in eusocial insect societies enables unique mechanisms by which they can defend against pests and pathogens. In *Apis mellifera*, the Western honey bee, several related behaviours enable defence against a range of disease-causing organisms, from bacteria to parasitic mites. These behaviours are heritable and so beekeepers are keen to selectively breed for them to enrich these traits in their stock. The conventional behavioural tests for selection are laborious and sensitive to environmental factors. We have shown that we can use gene expression, particularly as measured by proteomics, to select for some of these behaviours. I will discuss how this is done, both at the discovery phase and when applied in industrial-scale beekeeping. As well, I will discuss some of the hypotheses around the molecular mechanisms that lead to these behaviours.

Contributions of chemical ecology to a better understanding of social immunity in the honey bee

Fanny Mondet[1], Amelie Noel[1], Charlène Dumas[1], Alison Mercer[2], Yves Le Conte[1]

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Despite their high susceptibility to invasions and disease spread, many social animals, such as honey bees, have evolved mechanisms that allow effective defence at the group level. These collective strategies play a role at society level that parallels that of an immune system, and the social immunity they confer provide the basis for colony resistance to pathogens and parasites, such as varroa and other diseases. How compromised individuals are accurately diagnosed, and how the collective actions of bees undertaking the immune strategies are regulated at colony level pose many unsolved challenges. It is however known that chemical communication, which plays a central role in homeostasis maintenance within colonies, also plays a key role in host-parasite interactions and in defence against pathogens in general. We will present the current knowledge on the contribution of chemical ecology to social immunity, as well as our latest findings on the mechanisms of hygiene against varroa parasitised brood. This presentation will highlight the input of an in-depth understanding of host defence mechanisms to provide essential tools not only to predict, but also to mitigate against consequences of invasive species events.

Evolution of Social Wound Care Behaviours in Ants

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Animals developed different behavioural adaptations to help injured individuals. In ants permanently injured individuals that lost an extremity are carried back to safety to allow them to recover. In case of an infection, different behavioural strategies have evolved to combat the pathogens. Ants often use the metapleural gland, but some genera lost this gland in their evolutionary history. Here we compare two different behaviours to combat an infected wound, one with the metapleural gland and one without. The ant *Megaponera analis* treats infected wounds with antimicrobial compounds secreted from the metapleural gland, thereby reducing mortality of infected ants by 90%. Further analyses of the metapleural gland secretions of *M. analis* revealed over 121 chemical compounds and 41 proteins, almost half of which have an antimicrobial effect. However, ants from the genus *Camponotus* do not have this gland at their disposal. Remarkably, we observed that workers amputated the infected leg by biting it off at its base. This behaviour halted the infection and guaranteed the survival of the injured ant. The large phylogenetic distance between *Megaponera* and *Camponotus* and their strikingly different natural history (*Megaponera* a group-hunting predator, *Camponotus* a solitary foraging generalist) also suggest that wound care behaviour could be much more widespread in social insects than previously thought. Overall, we reveal a multifaceted care system, which not only allows to differentiate between sterile and infected wounds but also to treat them either with antimicrobial compounds or amputation of the infected leg. Thereby allowing M. analis and Camponotus to combat opportunistic pathogenic pressures present on their frequently inflicted wounds with two very different strategies.

Symposium

S22: The listening brain: frontiers in auditory cognition and health

S22-1 Relationship between hearing loss, cognitive abilities and depressive symptoms in CI users with uni- and bilateral hearing loss
Angelika Illg, Lisa Reuter, Belinda Pletzer, Lennart Weitgasser, Maria Huber

S22-2 States and traits of the listening brain
Jonas Obleser

S22-3 Cerebellar activity predicts vocalization in fruit bats
Shivani Hariharan

S22-4 The link between hearing and cognition - what can we learn from an autism mouse model?
Marlies Knipper, Morgan Hess, Philine Marchetta, Dila Calis, Wibke Singer, Stefan Fink, Steffen Hage, Kerstin Schwabe, Robert Lukowski, Rüdiger Land, Lukas Rüttiger

S22-5 Exploring auditory cognition in non-human primates with automatic, home cage based systems
Marcus Jeschke, Jorge Cabrera-Moreno, Antonino Calapai, Jonas Grunenberg, Tobias Moser

Relationship between hearing loss, cognitive abilities and depressive symptoms in CI users with uni- and bilateral hearing loss

Angelika Illg[1], Lisa Reuter[1], Belinda Pletzer[2], Lennart Weitgasser[3], Maria Huber[3]

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In two study groups, we investigated whether cochlear implantation (CI) has a long-term positive effect on cognitive performance in CI candidates with unilateral and bilateral hearing loss before and after CI, whether depressive problems play a role and whether there are age differences between younger and older hearing-impaired patients

The first cohort study (n = 61) with bilateral profound hearing loss patients was conducted at the MHH and the University of Salzburg. The participants were aged 25 to 75 years, and recruited during outpatient and inpatient care. Twelve months after the CI, the study population had shrunk to 41 participants and devided into two age groups (younger group, 25-59 years old, n=20; older group, 60-75 years old, n=21). Hearing was assessed using speech recognition tests and the Abbreviated Profile of Hearing Aid Benefit (subjective hearing ability). Clinical and subclinical secondary depressive symptoms were assessed using the Beck Depression Inventory (BDI II). Cognitive status was determined using a neurocognitive test battery. Assessments were conducted immediately before surgery, three months, and 12 months after CI.

The second study additionally examined an adult cohort (n = 21; mean age: 55 years) of patients with single-sided deafness (SSD) before and 12 months after CI using the same test battery. Additional the patient rated the severity of their tinnitus. Fifteen patients were still participating in the study after 12 months.

In bilateral hearing loss patients speech recognition and subjective hearing had significantly improved three and 12 months after CI ($p < 0.001$) and the severity of secondary depressive symptoms had decreased ($p = 0.01$). Results of cognitive tests showed only a significant improvement in semantic fluency in the older group ($p = 0.007$). The improvement in hearing was not associated with an improvement in cognitive ability, nor with a reduction in depression. Furthermore, the decrease in depression did not predict the improvement in cognitive performance. It was no difference between the younger and the older group in hearing, depressive status and cognitive status.

The results of the patients with SSD show significant improvement in monosyllables ($p = 0.001$), a decreasing severity of tinnitus ($p = 0.025$), and a not significant decrease of secondary depressive symptoms. Cognitive abilities increases significantly only in the verbal learning and memory test ($p = 0,013$). Age effects can be demonstrated in non-verbal learning tests, where younger patients achieved better results, and in verbal fluency tests, where older patients performed better. Tinnitus and depressive symptoms correlate significantly 12 months after CI. Speech comprehension is also associated with verbal learning and memorization skills.

Secondary depressive symptoms did not mediate between hearing and cognition in younger and older CI patients with bilateral hearing loss. These results justify the hypothesis that cognitive decline may also occur in young and middle-aged adults with hearing loss, which we also found in patients with SSD. Since deficits in semantic language fluency are considered a marker of cognitive impairment and dementia, the improvement in semantic abilities of older CI patients may be interpreted as an indication that the CI can prevent cognitive impairment in bilaterally hearing-impaired patients. New models are needed to illustrate the relationship between hearing loss, secondary depressive symptoms, and cognitive performance.

States and traits of the listening brain

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Amidst a flurry of methodological advances in neuroimaging and data analysis, we have made somewhat limited progress in explaining individual (i.e., trait-like) and momentary (i.e., state-like) differences in a listener's sensations and perceptions, that is, in their behaviour in a given communication situation. Here, I will present results from a series of studies on a large, ERC-funded and longitudinally studied cohort of middle-aged and older listers. We investigated how the neural tracking of speech in auditory cortex (using EEG) as well as the dynamics of large-scale brain networks (primarily using the brain's hemodynamic responses in fMRI) support adaptive and stable listening across varying auditory challenges. These findings underscore the important differentiation of dynamic, flexible state changes versus interindividual trait differences in auditory cognition.

Cerebellar activity predicts vocalization in fruit bats

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Echolocating bats exhibit remarkable auditory behaviors, enabled by adaptations within and outside their auditory system. Yet, research in echolocating bats has focused mostly on brain areas that belong to the classic ascending auditory pathway. This study provides direct evidence linking the cerebellum, an evolutionarily ancient and non-classic auditory structure, to vocalization and hearing. We report that in the fruit-eating bat Carollia perspicillata, external sounds can evoke cerebellar responses with latencies below 20 ms. Such fast responses are indicative of early inputs to the bat cerebellum. After establishing fruit-eating bats as a good model to study cerebellar auditory responses, we searched for a neural correlate of vocal production within the cerebellum. We investigated spike trains and field potentials occurring before and after vocalization and found that the type of sound produced (echolocation pulses or communication calls) can be decoded from pre-vocal and post-vocal neural signals, with prediction accuracies that reach above 85%. The latter provides a direct correlate of vocalization in an ancient motor-coordination structure that lies outside of the classic ascending auditory pathway. Taken together, our findings provide evidence of specializations for vocalization and hearing in the cerebellum of an auditory specialist.

The link between hearing and cognition - what can we learn from an autism mouse model?

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Autism spectrum disorder is discussed in the context of altered neural oscillations and imbalanced cortical excitation–inhibition of cortical origin. We discuss here observations that document that developmental changes in peripheral auditory processing, while preserving basic hearing function, lead to altered cortical oscillations. Local field potentials (LFPs) were recorded from auditory, visual, and prefrontal cortices and the hippocampus of BdnfPax2 KO mice. These mice develop an autism-like behavioral phenotype through deletion of BDNF in Pax2+ interneuron precursors, affecting lower brainstem functions, but not frontal brain regions directly. Evoked LFP responses to behaviorally relevant auditory stimuli were weaker in the auditory cortex of BdnfPax2 KOs, connected to maturation deficits of high-spontaneous rate auditory nerve fibers. This was correlated with enhanced spontaneous and induced LFP power, excitation–inhibition imbalance, and dendritic spine immaturity, mirroring autistic phenotypes. Thus, impairments in peripheral high-spontaneous rate fibers has the potential to alter spike synchrony and subsequently cortical processing relevant for normal communication and behavior. The finding is discussed in the context of defined sensory input during the critical developmental period to set the threshold and operation point for corticofugal plasticity responses in adulthood.

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Exploring auditory cognition in non-human primates with automatic, home cage based systems

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Understanding auditory cognition in non-human primates (NHPs) is essential for advancing both basic neuroscience and clinical applications, such as developing and comparing novel approaches for hearing rehabilitation. However, developing cognitive testing in the auditory domain, presents unique challenges in NHPs which are generally considered to have a visual bias. One further significant hindrance is the bottleneck of human resources required to train a sufficient number of animals in traditional setups, which typically involve labor-intensive environments like sound-proof booths and primate chairs.

Our work addresses these challenges by employing an automatic, home-cage-based system —MXBI— to explore auditory cognition in the highly vocal common marmoset. This automated approach allows for flexible cognitive testing without food or water restriction, dietary limitations, or social separation, facilitating a more naturalistic testing environment. We now leveraged the automatic testing by utilizing a brute-force approach to assess the perception of species-specific vocalizations, testing several thousand different, manipulated vocalizations. This analysis revealed a key role of harmonics and specific combinations of vocalization parameters in auditory perception.

Additionally, we compared auditory cognition across traditional chair-based psychophysical testing and automated, cage-based systems. Our results demonstrate that behavioral audiograms in home-cage environments exhibit higher thresholds compared to controlled lab environments. However, when accounting for auditory background noise, the thresholds in both conditions converged, opening new possibilities for studying attention, informational masking, and related cognitive processes in more naturalistic settings.

We conclude by providing an outlook on the potential for automatic testing systems in exploring novel auditory interventions, such as electrical cochlear implants. These developments hold promise for improving our understanding of auditory cognition and advancing translational applications in hearing rehabilitation.

Symposium

S23: Extracellular matrix alterations in aging and neurological diseases

Interactions Between the Extracellular Matrix, Glia, and Synapses in Stroke Recovery

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Reorganization of brain tissue and neurological recovery after stroke require coordinated rearrangements across neuronal, astrocytic, and microglial networks. While ongoing research continues to reveal new aspects of neuron-glia communication, the role of the extracellular matrix (ECM) in mediating these cellular interactions remains poorly investigated. Emerging evidence shows that neuronal activity can be regulated by balanced synthesis of ECM by astrocytes and its degradation by microglia (1).

Around fast-spiking neurons, the ECM consolidates into perineuronal nets (PNNs), which enwrap neuronal somata and synapses, stabilizing synaptic connections and maintaining neuronal excitability. In our previous studies, using superresolution 3D stimulated emission depletion (STED) and structured illumination microscopy (SIM), we demonstrated that PNNs undergo transient structural changes following stroke (2,3). During the late post-acute phase, we observed an increase in the size of PNN facets, which form synaptic pockets. This expansion allowed for the formation of new synapses, facilitating the reorganization of GABAergic input to motor cortical layer 5 interneurons. The remodeling of PNNs and their associated inhibitory synapses, preceded motor recovery and correlated with the severity of the ischemic injury. These changes in PNN nanostructure were likely driven by activated microglia, which were attracted to PNN-coated neurons and established direct contacts with them after stroke.

One potential mediator of microglia-PNN interactions is hyaluronic acid, a polysaccharide that anchors proteoglycan components of PNNs to neuronal surfaces. After stroke, hyaluronic acid fragments can act as damage signals, attracting microglia. However, in our recent in vitro experiments, we found that physiological concentrations of both long-chain hyaluronan and its fragments did not significantly influence microglial migration or phagocytic activity. Conversely, calcium imaging of GCamp6f-expressing astrocytes revealed that hyaluronic acid-based matrix is crucial for regulating calcium responses in astrocytes following mechanical stimulation.

Our findings highlight the critical role of the ECM in regulating glial function, neuroplasticity, and homeostasis in the brain. However, the molecular mediators of ECM-glia interactions and their role in neuroplasticity regulation remain to be elucidated. Targeting ECM remodeling may offer promising strategies for enhancing stroke recovery, but a deeper understanding of the complex interplay between the ECM, astrocytes, microglia, and neurons in both healthy and injured brains is essential for developing effective therapies.

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Microglia dynamics are affected by hyaluronan structure and distribution in health and disease

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Beyond neurons and glia, the Central Nervous System (CNS) holds a plastic scaffold known as the extracellular matrix, which in the brain consists mostly on long chains of the glycan polymer hyaluronan. The neural matrix serves a dual function of structural framework and signalling hub, communicating via both mechanical and diffusible cues that can elicit different cell responses. We have shown recently that microglia, the never-resting immune cells of the CNS, sense matrix disruption as a damage-associated molecular pattern (DAMP), while having also a role in matrix phagocytosis and turnover. However, it is unknown whether changes in the structural matrix affect microglial dynamics, i.e., motility, and migration. Here we report on the interaction between microglia and hyaluronan, using time-lapse imaging in ex vivo paradigms to characterise microglia dynamics in response to matrix modification. 2-photon time-lapse imaging in acute slices of Cx3Cr1+/eGFP mice showed that microglial motility, ramification and territory surveyed are reduced upon hyaluronan fragmentation, with no changes when other matrix components are degraded. Viral-mediated expression of Neurocan-GFP, a hyaluronan fluorescent reporter, showed that IB4-labelled microglia move and migrate differently through diverse matrix densities in organotypic slices. This observation was confirmed in 3D-cultures, using artificial matrix scaffolds of varying stiffness. These results imply a clear effect of the extracellular matrix upon microglia physiology, and shed light on the dual role of hyaluronan as scaffolding polymer and pro-inflammatory signal in the CNS.

Interplay Between Neural Extracellular Matrix, Microglia and Synapses in Adult and Aged Mice

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ECM proteoglycans of the lectican family, hyaluronic acid, tenascins and link proteins form an extracellular scaffold in the brain, which modulates cellular communication, axonal guidance, and synaptic plasticity[1]. Activity-driven formation of the dense lattice-like perineuronal nets (PNNs)[2] around parvalbumin-expressing interneurons in the cortex and hippocampus signifies the end of the critical plasticity period during brain development. Formation of PNNs restrains developmental forms of plasticity and leads to maturation of perisomatic GABAergic inhibition, less redundant memory engrams, and improved precision of memories[3]. On the other hand, 6-sulfated chondroitin sulfate ECM proteoglycans are secreted by glial cells in an activity-dependent manner, reach perisynaptic sites of excitatory synapses, and promote induction and consolidation of functional and structural synaptic plasticity[4]. These ECM-dependent mechanisms appear to involve signaling through L-type Ca^{2+} channels, SK channels, integrins, mechanosensitive Piezo1 channels, p38, Arc and GluN2B-containing NMDA receptors. Experiments with the depletion of microglia in young and aged mice reveal their importance for the organization of both perineuronal nets and perisynaptic ECM of excitatory synapses[5]. Moreover, recent data indicate that the attenuation of neural ECM modulates microglial state and prevents tagging of excitatory synapses by complement proteins, resulting in a short duration of microglia-synapse contacts and reduced rate of spine elimination, as directly demonstrated by vital two-photon microscopy of microglia-spine interactions[6]. This is opposite to alterations found under inflammatory conditions induced by the administration of lipopolysaccharide or inoculation of tau-rich brain fraction from Alzheimer's patients[7]. These new data further support the concept of tetra-partite synapses, in which glia and neurons shape ECM, which regulates multiple glial and synaptic functions.

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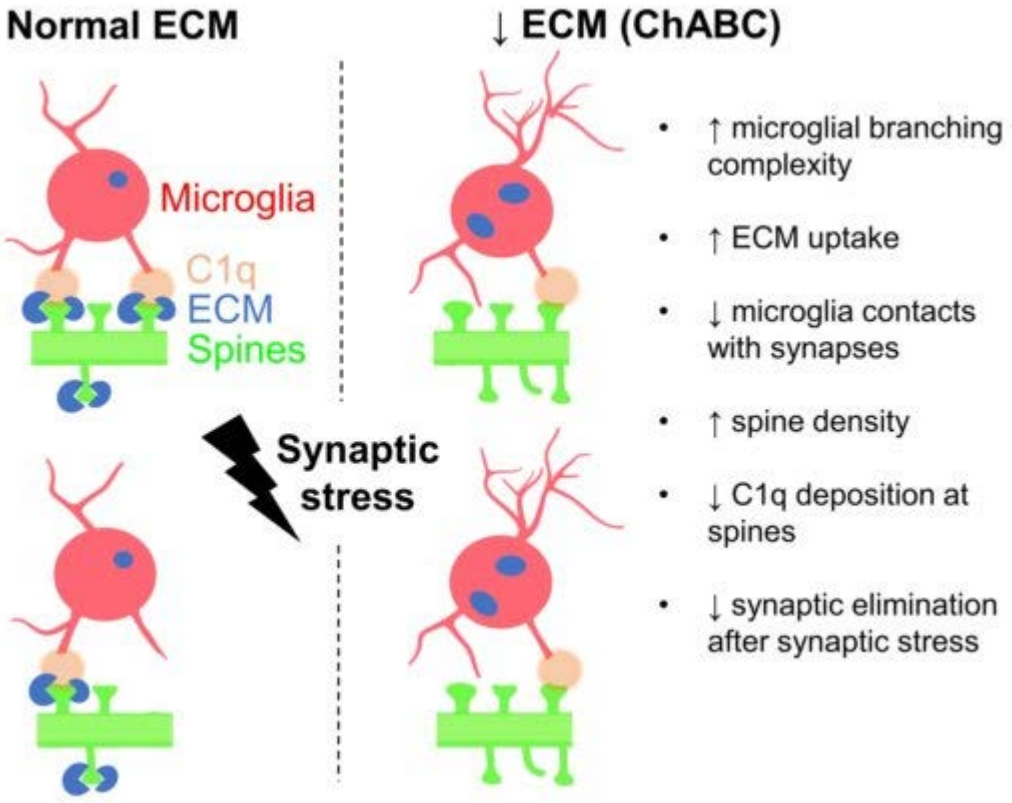
Normal ECM
↓ ECM (ChABC)
Microglia
C1q
ECM
Spines
Synaptic stress
• ↑ microglial branching complexity
• ↑ ECM uptake
• ↓ microglia contacts with synapses
• ↑ spine density
• ↓ C1q deposition at spines
• ↓ synaptic elimination after synaptic stress

Dysregulation of Hyaluronan-based ECM in Epilepsy, Alzheimer's Disease, and ALS

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The brain's extracellular matrix (ECM) is uniquely composed of proteoglycans and polymeric carbohydrates like hyaluronan, and it controls and maintains neuronal excitability, synaptic functions and blood-brain barrier (BBB) functionality. The proteomic and glycomic composition of the neural ECM is affected by neural activity states, development, aging and disease. Pathophysiological remodeling of the ECM and its structural specialization, the perineuronal nets (PNN), occurs at the level of biosynthesis, glycosylation, secretion and proteolytic cleavage. Increasing evidence points to a role for ECM components in the pathogenesis of neurological disorders.
In murine models of epilepsy levels of core components of the hyaluronan-based ECM like Brevican, Neurocan, Aggrecan and link proteins Hapln1 and Hapln4 levels reliably predicted seizure properties across models, suggesting a link between ECM state and epileptic phenotype.
The neural sheddome and ECM secretome are detectable in human plasma and cerebrospinal fluid (CSF), opening a window into their composition under pathophysiological conditions and allowing the detection of candidate molecules. In the CSF of Alzheimer's patients levels of brevican and neurocan positively correlated with age, total tau, p-Tau, neurofilament-L and A beta 1-40, and in ALS patients' CSF samples higher levels of a cleaved Neurocan fragment were detected, indicating disorder-specific neural ECM rearrangements.

Role for amyloid beta as an antimicrobial peptide that enhances autophagy in response to HSV1 infection in a 3D-neuronal cell culture model

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Herpes Simplex Virus 1 (HSV1) infection has been reported to lead to a 2.5-fold increased risk for Alzheimer's Disease (AD). We have previously shown that amyloid beta protein (β) exhibits antimicrobial activities against HSV1 via binding to viral glycoproteins and entrapment of virus, thereby preventing infection. These observations led to the development of the "Antimicrobial Protection Hypothesis of AD", which postulates cerebral β-amyloid deposition is an innate immune host-defense response to an actual or perceived infection. The role of intracellular Aβ in this process has remained unclear. Colocalization of Aβ with autophagic vacuoles during HSV1 infection suggests activation of selective autophagy - a crucial cellular defense mechanism against invading pathogens.

Here, we challenged RenVM neuronal cells with HSV1 in 3D cell culture models to investigate the ability of intracellular Aβ to afford host defense via selective macroautophagy. For this purpose, 24 hours post-infection, we assessed the expression of the selective autophagy receptors (SAR), p62, NDP52 and OPTN, and ATG8 family members LC3B and LC3C via Western Blot analysis. We also measured levels of Beclin1, a component of the Autophagy-Initiation complex, which is actively suppressed by the neurovirulent protein ICP34.5 of HSV1, to evade autophagy. To further investigate the involvement of Aβ in the autophagic response, we conducted immunofluorescence analysis to evaluate the colocalization of Aβ with autophagy markers. Additionally, we performed bulk RNA sequencing analysis to explore potential pathways involved in the autophagic response driven by Aβ.

Our results showed that under basal conditions, autophagic activity was significantly influenced by APP expression, with APP-overexpressing cell lines exhibiting higher LC3B levels compared to naive cells. Immunofluorescence analysis confirmed increased colocalization of LC3B and SARs with Aβ in the APP-overexpressing cells compared to naïve cell line. Following 24-hour HSV1 infection, we observed the expected suppression of autophagy, evidenced by reduced levels of Beclin1 and SARs. This suppression was more pronounced in APPKO cells but was effectively counteracted in APP-overexpressing cells, suggesting that Aβ mitigates HSV1-mediated autophagy suppression.
Treatment with Bafilomycin A1 to block autophagic degradation further confirmed increased colocalization of LC3B and p62 with Aβ in punctate structures under both uninfected and infected conditions measured by immunofluorescence analysis. Bulk RNA sequencing revealed significant activation of the CGAS-STING pathway in APP-overexpressing cells compared to naive and APPKO cells. These findings indicate that the CGAS-STING pathway, a known autophagy activator, may play a pivotal role in Aβ-mediated enhancement of autophagic activity in response to HSV1 infection.

In summary, we provide evidence for increased autophagy 24 hours post-HSV1 infection in Aβ overexpressing cell line versus naïve and APP knockout cells. These findings suggest a potential antimicrobial role for Aβ in enhancing autophagy as a potent cellular defense mechanism against HSV1. Our findings also suggest novel pathways for potential therapeutic strategies that target the underlying microbial triggers of Aβ accumulation.

Symposium

S24: Evolution of behavior: from genes to circuits

S24-1 Mechanisms of behavioral evolution: Lessons from poison frogs
Eva Kristin Fischer

S24-2 Cellular and molecular mechanisms underlying the evolution of central neural circuits and behaviour
Christoph Giez, Ruairi J. V. Roberts, Hui Gong, Lucia L. Prieto-Godino

S24-3 Predatory aggression in nematodes evolved through adaptations to noradrenergic circuits
Monika Scholz, Guniz Eren, Leonard Boeger, James Lightfoot

S24-4 Crowded and hungry locusts: Finding food in smelly swarms
Yannick Günzel, Inga Petelski, Sercan Sayin, Susanne Kraus, Einat Couzin-Fuchs

S24-5 Untangling the web of behaviors used in spider orb-weaving
Andrew Gordus

Mechanisms of behavioral evolution: Lessons from poison frogs

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The emergence of traits initially exhibited only in one sex in the opposite sex has been proposed as a major, though underappreciated, force in the evolution of behavior, including novelty in parental care, courtship communication, and alternative reproductive tactics. Yet, the mechanisms that facilitate this activation and the integration of new behavior into opposite sex physiological, neural, and molecular systems under natural conditions are largely unknown. We leverage diversity in parental care strategies in closely related Neotropical poison frog to probe mechanisms of naturally occurring variation in parental care across levels of organization – from behavior to physiology to neural mechanisms. We report consistent individual differences in care behavior, as well as flexibility in which parent provides care.We link differences at the behavioral level to hormone and brain gene expression patterns. Our findings suggest that sex roles are not fixed or even as biased as previously suggested, with important implications for our understanding of how sex-biased behavior is generated, maintained, and occasionally reversed at immediate and evolutionary timescales.

Cellular and molecular mechanisms underlying the evolution of central neural circuits and behaviour

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The immense variety of sensory-driven behaviours displayed by animals today arose through the evolution of neuronal circuits. Sensory systems face strong selection pressure as they are crucial for navigating the environment. While it is known that selection pressures cause genetic changes in peripheral systems, affecting information input, the ways in which central neuronal circuits change and their contributions to species-specific behaviours remain underexplored. Here, we aim to understand how central neuronal circuits evolve and contribute to new sensory-driven behaviours. We propose to address this question by using the larval olfactory system of closely related *Drosophila* species. We used a comparative connectomics, single-cell sequencing, and live imaging approach. We found that among many differences, the number of synapses between olfactory neurons and their projection neurons varies across species resulting in a potential change of information flow. Furthermore, we found that local neuron population exhibit species-specific changes in their connectivity suggesting changes in olfactory computations. We aim to further explore these differences by combining live imaging and modelling to explore how central neuronal circuits change over time and give rise to species-specific differences.

Predatory aggression in nematodes evolved through adaptations to noradrenergic circuits

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Behaviors are adaptive traits evolving through natural selection. Crucially, the genetic, molecular, and neural modifications that shape behavioral innovations are poorly understood. Here, we identify specialized adaptations linked to the evolution of invertebrate aggression. Using the predatory nematode *Pristionchus pacificus*, we developed a machine-learning model from behavioral tracking data, and identified robust behavioral states associated with aggressive episodes. Strikingly, aggression coincides with a rewiring of key circuits across nematode evolution. We find modifications to the invertebrate noradrenergic pathway, with octopamine promoting predatory bouts while tyramine antagonistically induces non-predatory states. Furthermore, additional octopaminergic neurons are present and possess morphological adaptations including neurites extending to teeth-like structures for attacking prey. Additionally, the octopamine receptors *Ppa-ser-3* and *Ppa-ser-6*, and the tyramine receptor *Ppa-lgc-55* gate predation through a specific group of head sensory neurons. Thus, evolutionary adaptations in noradrenergic circuits facilitated the emergence of aggressive behavioral states associated with complex predatory traits.

Crowded and hungry locusts: Finding food in smelly swarms

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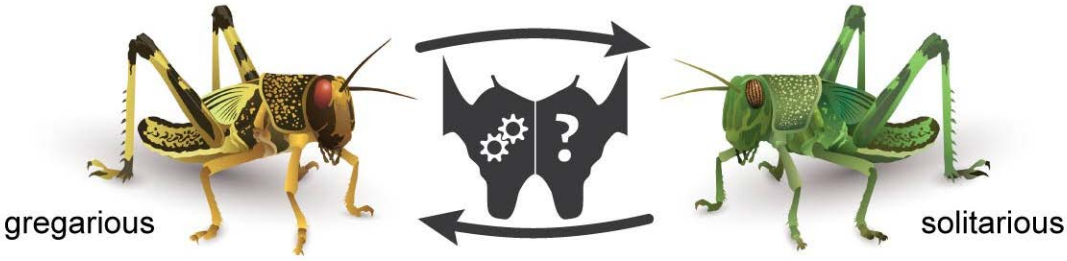
Swarms of the migratory desert locust can extend over several hundred square kilometers, and starvation compels this ancient pest to devour everything on its path. However, despite the plague's enormous socio-economic impact, estimated to affect ten percent of humanity, little is known about their collective decision-making processes. Central to their swarm formation is crowding-induced plasticity, with social phenotypes changing from cryptic (solitarious) to swarming (gregarious), and theory suggests that gregarious behavior benefits foraging efficiency. Here, we elucidate the implications of the transition between the phenotypes on foraging decisions and corresponding neural circuits.

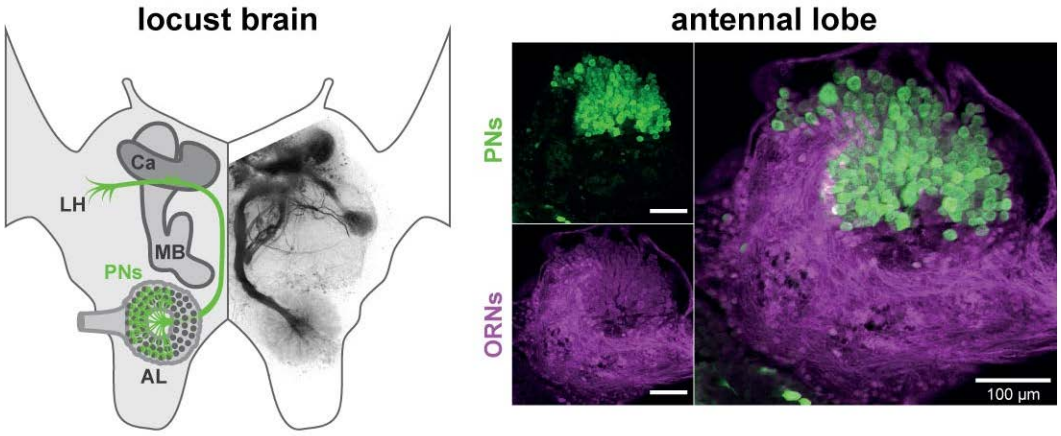
First, we used behavioral experiments and Bayesian modeling to disentangle the relative contribution of different information classes on foraging decisions. Collecting high-resolution trajectories in a group-foraging arena and carefully maintaining each animal's identity over time, we traced how personally acquired experience and socially derived information inform foraging decisions. Our findings suggest that locusts effectively integrate the two classes of information in a way that balances incongruent evidence but reinforces congruent ones, which optimizes decisions.
Next, we aimed to decompose the multi-modal facets of foraging. For this, we employed a simple patch-selection assay that allowed modulating the type of information (olfactory, visual, or both) available to a focal animal. Offering four options – food, social context, food+social, and an empty control – revealed that olfactory social cues play a critical role.

To this end, we investigated how corresponding odors are encoded in the locust olfactory system, allowing appropriate and context-dependent decisions. We established an in-vivo functional calcium imaging protocol to monitor the activity of antennal lobe projection neurons. Further, to characterize the minute spatiotemporal details of odor-induced responses, we developed a data-driven and reproducible approach for unsupervised activity-based functional imaging data segmentation that intends to overcome the challenges of traditional methods. We discovered crowding-dependent synergistic interactions between the neural responses to food and social odors. The observed synergy was specific to the gregarious phase and manifested in distinct odor response motifs distributed across stable combinatorial response maps. Consequently, this allowed us to use the antennal lobe network dynamics to predict reliably whether a locust was gregarious or solitarious. Our results suggest a crowding-induced modulation of the locust olfactory system that enhances food detection in swarms.

We demonstrate how linking sensory adaptations to behaviorally relevant tasks can improve our understanding of social modulation in non-model organisms. Beyond locusts, our findings and methodologies have broader implications, especially for studying olfactory-guided social behaviors in other species, such as rodents. At the same time, our analytical approach can be extended to shed light on the neuronal processes that distinguish different phenotypes of other insect species (e.g., the forest and domestic forms of the yellow fever mosquito, a major vector of viruses).

Synergistic olfactory processing for social plasticity in desert locusts





Rapid density-dependent adaptations pose interesting questions about the neural processing in changing social environments that allow animals to adjust their decision-making appropriately in a context-dependent manner. Adapted from Lehmann et al. (2024) and Petelski et al. (2024).

Untangling the web of behaviors used in spider orb-weaving

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Many innate behaviors are the result of several coordinated sensorimotor programs to produce higher-order behaviors. Knowing the underlying cognitive states that encode how these programs are coordinated is often difficult since we simply can't ask the animal their objective. However, extended phenotypes such as architecture provide us with a window into the mind because the structure itself is a physical record of behavioral intent. A particularly elegant and easily quantifiable structure is the spider orb-web. We have developed a novel assay enabling high-resolution behavioral quantification of web-building by the hackled orb-weaver Uloborus diversus. With a brain the size of a fly's, the spider U. diversus offers a tractable organism for the study of complex behaviors. Using machine vision algorithms for limb and web tracking, and unsupervised behavioral clustering methods, we have developed an atlas of stereotyped movements and sensorimotor transformations used in orb-web construction. The rules for how these movements are coordinated change during different phases of web construction, and we find that we can predict web-building stages based on these rules alone. Thus, the physical structures of the web explicitly represent distinct phases of behavior. To uncover how this sophisticated algorithm is encoded in the brain, we have assembled a genome, a brain atlas, and biological assays to understand which neurons and genes are critical to encoding web-building behavior.

Symposium

S25: Multilevel human brain mapping and atlas as a tool connecting micro- and macro-structures

S25-1 Brain architecture from cells to organ
Katrin Amunts

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S25-3 Multi-scale neuroimaging with synchrotron radiation: volume data for the brain atlas, and also for future connectomics?
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S25-5 High resolution 3d mapping within areas 44 and 45 – new cytoarchitectonic subdivisions in broca's region
Nataliia Fedorchenko, Sabine Ruland, Hartmut Mohlberg, Sebastian Bludau, Christian Schiffer, Katrin Amunts

Brain architecture from cells to organ

Katrin Amunts[1]

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Based on Brodmann's idea of structural-functional relationships at the cortical area level, studies of the human brain benefit from detailed anatomical atlases. However, brain mapping is a dynamically developing field, and Brodmann's 1909 cytoarchitectonic map, which is a schematic view of a single hemisphere, does not meet the requirements of a modern microstructural atlas. At the same time, our concepts of language and other cognitive functions have changed considerably since then and suggest a much more detailed segregation of the brain than shown in Brodmann's map.

This was our motivation for developing Julich-Brain – a multimodal 3D atlas of cortical and subcortical areas. It is based on an analysis of histological sections of 10 post-mortem brains to capture intersubject variability, which differs between brain regions. Reproducible mapping of areas and subcortical nuclei was achieved by using image analysis and statistical tools (Amunts, 2020). Cytoarchitectonic maps serve as a reference to integrate data from different spatial scales, and various aspects of brain organization (e.g., connectivity, molecular and genetic maps) into a coherent system.

However, when it comes to correlating cognitive functions with the cellular level, the size of the human brain, with its billions of nerve cells forming complex networks, plays an increasingly important role. Capturing the cellular level requires the analysis of large amounts of data, and high-performance computing becomes mandatory. Two of the atlas brains are the so-called BigBrains, which represent 3D-reconstructured histological data sets at a spatial resolution of 20 micrometres (Amunts, 2013). This high resolution allows to spatially align data from other optical techniques, e.g. 3D Polarized Light Imaging for studying the fibres architecture of the brain (Axer and Amunts et al., 2022) and ultra-hight resolution X-ray phase contrast imaging, Light sheet imaging or two-photon imaging down to the nanometer scale.

Julich-Brain is openly available via the EBRAINS research infrastructure, to link atlas information to a broad range of other data, tools and services using the siibra toolkit. Such digital platform allows to better distinguish the different components of cognitive function, to improve our understanding of the language network, to contribute to brain medicine and to gain a deeper understanding of the relationship between brain structure and function in general.

Bridging different levels of brain organization using the siibra toolsuite

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Comprehensive empirical data is required for understanding how cognitive networks are related to human brain organization. This data spans across an extensive range of scales and modalities, and typically originates from a plethora of resources. To make multimodal and multidimensional measures of brain organization accessible for different neuroscientific workflows, they need to be integrated into a common reference framework, and exposed via suitable software tools. This talk will present our work on building a highly detailed multilevel atlas of the human brain and making it accessible through the siibra tool suite. It provides streamlined access to brain reference templates at different spatial scales, complementary parcellation maps, and a wide range of multimodal data features. It links macroanatomical concepts and their inter-subject variability with measurements of the microstructural composition and intrinsic variance of brain regions, building on cytoarchitectonic maps as a reference, and integrating the BigBrain model as microscopic reference template. The tool suite includes a web-based 3D viewer (siibra-explorer) and a Python library (siibra-python) to support a broad range of use cases. It makes use of EBRAINS as a data sharing platform and cloud infrastructure, and implements interfaces to other neuroscience resources.

Multi-scale neuroimaging with synchrotron radiation: volume data for the brain atlas, and also for future connectomics?

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In order to unravel physiological and pathological mechanisms of the nervous system, at the cellular level, structure and processes have to be visualized on a wide range of scales. Imaging at cellular and sub-cellular resolution is the realm of histology. For this purpose, the tissue obtained by surgical intervention or from a post mortem autopsy is cut into thin sections, stained and observed in an optical microscope. In conventional histology, images are obtained only of two-dimensional sections but not of the entire three-dimensional (3D) volume. In order to visualise and to quantify the cytoarchitecture in 3D, even deep in the tissue or organ, we use phase-contrast X-ray computerized tomography, as a tool for quantitative and fully digital 3D virtual histology [1]. We have implemented the method using optimized phase retrieval [2], both at highly coherent synchrotron and at inhouse micro-focus sources. In a multi-scale approach, we combine parallel and cone beam illumination to cover a wide range of scales. Since the workflow is non-destructive and fully compatible with standard clinical pathology, we can perform correlative histology studies.

In this talk we discuss image formation and advanced phase retrieval of propagation and inline holography data, the respective resolution limits, object constraints, scaling properties, as well as morphometric image analysis. We show how solutions and algorithms of mathematics of inverse problems and machine learning help us to meet the challenges of phase retrieval, tomographic reconstruction, segmentation, and more generally exploitation of bulky image data. All to the benefit of ambitious imaging projects such as mapping the human brain, and fighting neurodegenerative diseases [3,4].

Since more recently, X-ray tomography is also discussed as a potential non-destructive, robust and scalable alternative to volume EM for mapping neuronal tissue at ultrastructural resolution, in order to unravel neuronal circuits, in particular in the mammalian cortex [5]. In the last part of this talk I will discuss the requirements and challenges to achieve synaptic resolution (<20 nm) in mm sized volumes of neuronal tissue with hard X-rays.
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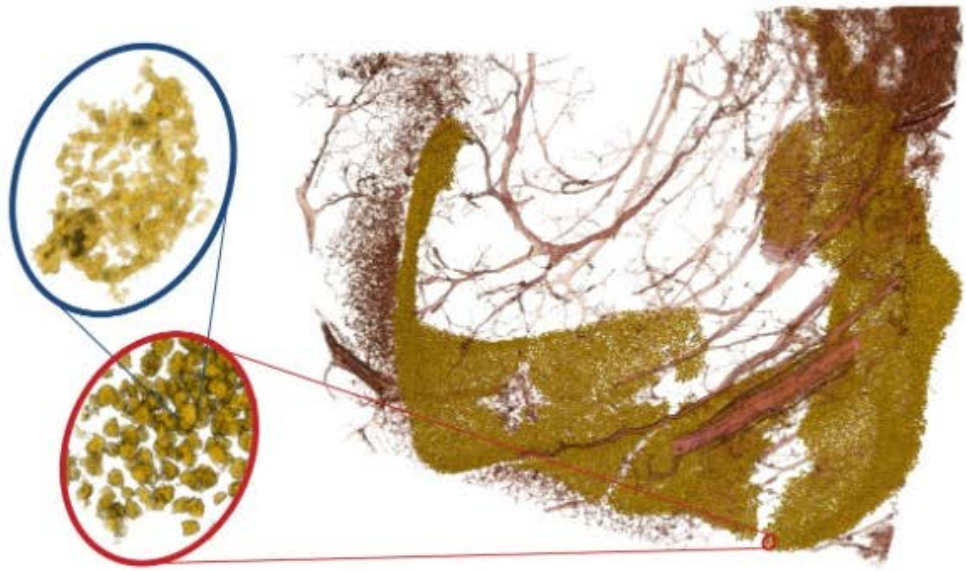

Figure 1 *3D visualisation of human neuronal tissue reconstructed by multi-scale X-ray phase contrast tomography. Neuronal cell nuclei are shown in yellow for the granule neurons in the dentate gyrus region of the hippocampus. Blood vessels are shown in red. By changing the X-ray optical magnification in the multi-scale recordings, one can zoom into regions-of-interest (red ovals). In these scans the resolution is high enough to resolve sub-structures of the nucleus, associated with different DNA packing regimes. Adapted from [3]*

High resolution 3D mapping of the human hypothalamus and its subdivisions

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The hypothalamus is crucial for maintaining homeostasis, regulating sleep-wake cycles, appetite, circadian rhythm, and thermal regulation (Nieuwenhuys et al., 2008). Despite its importance, its structural organization, precise boundaries, and functional differentiation of nuclei remain incompletely understood. Existing anatomical maps of the hypothalamus do not reflect interindividual variability in 3D space; they often lack the spatial resolution and morphological detail to provide a comprehensive understanding of this complex region and to inform neuroimaging studies about the brain microstructure. Therefore, we aimed to develop probabilistic cytoarchitectonic maps to address intersubject variability and provide a high-resolution 3D reference map of the hypothalamus for informing studies in the living human brain.

We delineated the hypothalamus and its nuclei on every 15th cell-body stained brain section in 10 brains (5 female) including BigBrain (Amunts et al., 2013). To create the high-resolution BigBrain model, we used a deep-learning based tool (Schiffer et al., 2021) that delineated the remaining sections. Other brains were used to create probability maps that capture intersubject variability in space and location of areas. To do this, brains were 3D reconstructed and superimposed in standard reference space (Amunts et. al., 2020). Quantitative tools, including texture analysis (Devakuruparan, 2023) and contour proposal network (Upschulte et al., 2021), were used for detailed subdivision analysis.

We generated a high-resolution 3D map of 23 nuclei of the human hypothalamus, that show their shapes and neighbourhood relationships with high precision. These nuclei were categorized into three rostro-caudal zones:

Preoptic Zone: Includes the anterior periventricular and median preoptic nuclei lining the third ventricle, with the uncinate and intermediate nuclei forming a cluster around the medial preoptic nucleus. Additionally, it contains the paraventricular nucleus with dark magnocellular neurons ventrolaterally and less intense parvocellular neurons medially, the supraoptic nucleus with densely packed magnocellular neurons, the supra- and retrochiasmatic and anterior periventricular nuclei.

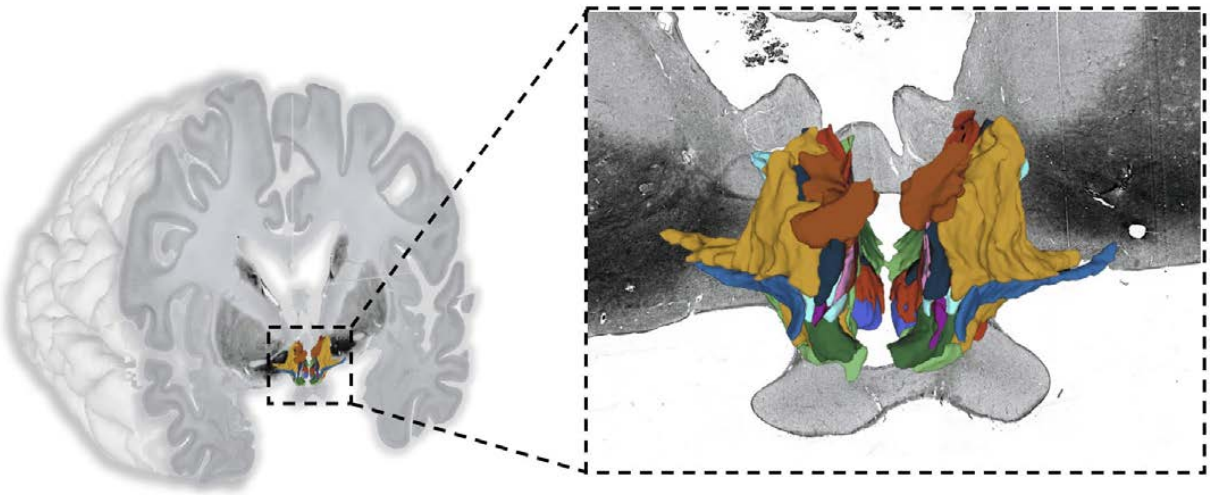
Tuberal Zone: Features the ventromedial nucleus with high peripheral cell density, the smaller posteromedial nucleus between the ventromedial nucleus and mammillary body, the dorsomedial nucleus with densely packed small neurons at its centre, and the arcuate nucleus within the tuber cinereum.

Mammillary Zone: Contains the medial and lateral mammillary nuclei. The tuberomammillary and supramammillary nuclei contain large dark magnocellular neurons, and the lateral tuberal nucleus housing medium-sized neurons in the basolateral mammillary zone.

The mean hypothalamic volume was 1492 mm^3. The Lateral (514 mm^3) and Posterior hypothalamic areas (262 mm^3) showed the highest volumes, while the uncinate and lateral mammillary nuclei exhibited the lowest values (0.845 mm^3; 1.8 mm^3). Permutation tests found no significant effects of hemisphere, sex, or their interaction on the shrinkage-corrected volumes for each nucleus. Intersubject variability was reflected in the probabilistic maps that will be part of the Julich-Brain Atlas (Amunts, 2020) and available via EBRAINS and other platforms.

In sum, we provide a detailed microstructural map of the hypothalamus that provides an anatomical basis for interpreting and comparing neuroimaging data, helping to refine the functional organization of the hypothalamus.

3D reconstruction of the hypothalamus in the BigBrain



Based on 414 annotated sections with 1 µm in-plane resolution

High resolution 3d mapping within areas 44 and 45 – new cytoarchitectonic subdivisions in broca's region

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Neuroimaging shows the participation of Broca's region in key aspects of language processing, in action understanding, imitation, music processing, complex hand movements, and associative sensorimotor learning. However, such functional diversity is not reflected in existing anatomical maps of areas 44 and 45 as part of this region. Previous work of our lab on the receptorarchitecture has suggested a more detailed parcellation, but these areas have not been systematically analysed and mapped in cytoarchitectonic sections (Amunts et al., 2010).

Therefore, we mapped the cytoarchitecture of areas 44 and 45 in histological sections of ten post-mortem human brains, stained for cell bodies, using image analysis and an observer-independent approach for defining borders (Amunts et al., 1999). One of the brains was the so-called BigBrain model, which represents a 3D-reconstructed brain with a spatial resolution of 20 microns isotropic. We supplemented borders of areas in-between the analysed sections using deep learning (Schiffer et al., 2021).

We identified two subdivisions of area 44 (44a and 44p) and two of area 45 (45a and 45p) arranged in anterior posterior orientation, which differ in their cytoarchitecture. Area 44p was identified by its dominant layer III, with magnopyramidal cells spread throughout its length, very thin, invaded by the cells from neighboring layers, layer IV. Area 44a differed by the high concentration of magnopyramidal cells mainly in the lower part of layer III, and broader layer IV. Area 45p appeared with magnopyramidal cells present in middle and lower parts of layer III, and well-developed layer IV. Area 45a, in contrast, had less cells in layer III, with magnopyramidal cells located mainly in the lower part of it, layer IV was wide and well-developed. 3D maps of all four subdivisions were computed in the single subject template MNI Colin 27, and the asymmetric ICBM 2009c. In addition, high-resolution 3D reconstructions were computed in the BigBrain to reveal fine details of anatomy.

The maps will be openly available as FAIR data in the Julich-Brain Atlas (Amunts et al., 2020) as part of the EBRAINS infrastructure. They facilitate neuroimaging studies on structure-function relationships, inform modelling, simulation and brain stimulation, and serve for education purposes in brain anatomy.

Symposium

S26: Neural circuits for flexible social behavior

Neural mechanisms of vocal learning and production in songbirds

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Learning and execution of complex motor skills are often modulated by sensory feedback and contextual cues arriving across multiple sensory modalities. Vocal motor behaviors are primarily influenced by auditory inputs, both during learning and mature vocal production. The importance of auditory input in shaping vocal output has been investigated in several songbird species that acquire their adult song based on auditory exposure to a tutor during development.

We explored song imitation in juvenile zebra finches raised either in the presence or absence of females providing vocal feedback. We found that male zebra finches raised with a female copied the spectral and temporal features of the tutor song more accurately than compared to birds, that were raised socially isolated. We found that females emitted more calls as young birds improved their song performance, indicating that females can provide practice-specific feedback. To decipher whether female vocal feedback has an impact on the neural activity within the song learning pathway, we performed intracellular recordings in HVC, a premotor area involved in song learning and production, in singing and listening zebra finches. In juvenile zebra finches, we found that female vocalizations can modulate neural activity in HVC during passively listening and singing. These results highlight the contribution of female vocal feedback to developmental song learning and how vocal input other than the tutor song can influence the neural circuit involved in song learning and production.

Once the bird reaches adulthood this song remains stable. We discovered that inhibition within the premotor area HVC plays a major role in closing this critical period by suppressing the influence of the tutor once song proficiency has been achieved. We then developed a cell-type specific viral strategy to target inhibitory neurons in adult zebra finches and were able to re-open the critical period by teaching an adult zebra finch novel song elements. This finding might have important implications to understand and expand motor skill learning capabilities or improve sensory and motor recovery after injury.

Sensory circuits underlying social context-dependent decision-making in *Drosophila* larvae

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All animals must make appropriate, but also flexible, foraging decisions, especially when facing starvation. *Drosophila* larvae need to eat throughout their life to pupate, eventually become a fly, and mate. It has been shown that they can even feed and survive on a conspecifics diet when no other food is present. We investigated how fly larvae sense each other and which sensory systems are involved in cannibalistic behavior. We find that alive and dead larvae provide overlapping, but also different multisensory cues, for example, chemosensory and mechanosensory. We investigated under which circumstances fly larvae turn to cannibalism and how internal state and social context influence these foraging decisions. A group of fed larvae shows weak attraction towards dead conspecifics, however, this preference can be enhanced by starvation. A single alive larva shows an enhanced attraction towards dead conspecifics even when fed, thus social group context prevents cannibalistic behavior. We hypothesize that a cannibalistic context provides the presence of a potential food source, but also the danger of being eaten. We are investigating how larvae integrate social multisensory cues with internal state and how this modulates feeding on conspecifics. Flexibility in foraging behavior enables fly larvae to optimally weigh food availability vs. threat in a social foraging situation and to expand their feeding choices to overcome starvation.

Neural Circuits Regulating Avoidance and Tracking

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Animals survive and reproduce by responding appropriately to environmental stimuli, relying on rapid and accurate information processing for tasks such as predation, evasion, and courtship. Vision is a key sensory modality in Drosophila, constituting more than half of the inputs to the central brain [1-3]. Vision is also essential in social interactions [4-8], with competition for resources, aggression and courtship occurring frequently on food substrates crowded with Drosophila vinegar flies. The central unit of the anterior optic tubercle (AOTu) is the largest retinorecipient area in the central brain that receives input from different neuron types, primarily from the LC10-group. LC10a neurons mediate female tracking in courtship behavior [7], whereas LC10d neurons mediate avoidance of visual objects deprived of a chemosensory signature [9]. Despite sharing functional characteristics and downstream neurons, LC10a and LC10d neurons mediate opposing behaviors. How AOTu-output neural circuits orchestrate these distinct behaviors remains unknown. In this study, we explored the connectivity of LC10a and LC10d neurons with downstream targets in the AOTu to identify neuron types that might be involved in avoidance or tracking. Utilizing genetic access to single AOTu-output neuron types [10] to drive expression of a neuronal silencer, we employed single-pair behavior assays to search for neurons involved in avoidance or tracking. We found that specific AOTu-output neuron types were required for avoidance or distinct steps of tracking. The major phenotypes observed upon silencing of AOTu-output neurons can be grouped in those that result in elimination of avoidance, early courtship initiation, an increase in the latency to initiate courtship, and a modest decrease in female tracking. Our findings suggest the presence of intricate relationships in AOTu-output circuits that result in high robustness in the ability of the male to maintain close proximity to the female when courting on one hand, and more straightforward roles in the transition from avoidance to tracking on the other. The central unit of the AOTu thus appears to function as a hub processing visual cues subserving different behaviors in social interactions.

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Comparing human and monkey neural circuits for processing social scenes

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Recognizing agents, their actions, and their interactions is essential for understanding the world around us. Using functional Magnetic Resonance Imaging, we discovered in the macaque monkey brain a network of areas centered on the medial and ventrolateral prefrontal cortex that is selectively engaged in social interaction analysis. Its extent and location suggest that this function is an evolutionary forerunner of human mind-reading capabilities. A comparative fMRI investigation in humans additionally revealed which neural strategies adapted to the needs of each species, and emphasized human interest in understanding actions of our peers directed towards objects. Together these studies show how our primate brains continuously decode the complex visual scenes unwinding in front of us: both the nature of material entities, such as individuals and objects, and their immaterial interactions.

Effects of social experience on neural function in *Drosophila*

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Learning from social feedback is crucial for success in a social world. Conversely, lacking such social flexibility can lead to social isolation and strong impairments to an individual's quality of life. Therefore, understanding the neural basis for social flexibility is key to understanding how circuit malfunctions lead to social disorders. Gaining such understanding is challenging, since it requires high-resolution measurements of social interactions and characterization of neural function, the combination of which is not available in most systems.

We therefore focus on the social flexibility of the vinegar fly, *Drosophila melanogaster*, which exhibits highly quantifiable social behavior and allows for optogenetic neural interrogation in unrestrained and freely behaving animals [1]. Prior work has established that past social experience influences the behavioral strategies of male flies. Specifically, experiencing ongoing sexual rejection from female flies leads to subsequent suppression of male courtship even towards receptive females. How this and other types of social experience influence the function of individual neurons to facilitate social flexibility remains elusive.

To test how different types of past social experience shape the function of individual neurons along the neural circuitry controlling social behavior in male *Drosophila*, we combine real-time pose estimation, closed-loop optogenetic behavioral manipulation and neural interrogation, and unbiased behavioral quantification. We first let individual males experience one of several possible 'alternate social realities', in which females provide different types of social feedback to the male. We then quantify the function of targeted neurons in the male, using a combination of optogenetic neural characterization with stochastic stimuli and unbiased behavioral classification [2]. We then investigate how this behavioral readout of neural function differs between males that experienced different social realities in the past, with a female that was either a) receptive, b) rejecting, or c) walking backwards every time the male extends a wing to produce courtship song. To facilitate c), we use optogenetic activation of Moonwalker Descending Neurons [3] in the female, triggered on male wing extension in closed loop. To ensure specific stimulation of the female and to prevent unintended optogenetic stimulation of the male during this experience phase, we track location and identity of the male and female in real time [4] and use this information to guide a laser onto the female, generalizing a previous approach for rapid neural manipulation in freely behaving *Drosophila*[5] to socially interacting animals.

Together, this novel approach can reveal how social experience shapes the function of individual neurons to enable learning from social feedback.

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Symposium

S27: Brain organoids for modelling immune-neural interactions in epilepsy

S27-1 Studying immune-neural interactions in a model of T-cell driven hippocampal sclerosis: pitfalls and translational value
Nico Melzer

S27-2 In vitro modelling of maternal immune activation (mia) in cerebral organoids
Denise Haslinger, Nico Melzer, Andrea Rossi, Andreas G. Chiocchetti, Julia Ladewig

S27-3 Genome-editing to model selective somatic mutations associated with focal epileptogenic lesions
Andrea Rossi

S27-4 Omics insights into LIS1-patient-derived cerebral organoids unravel novel molecular pathways underlying disease severity and suggest therapeutic strategies
Julia Ladewig, Lea Zillich, Andrea Carlo Rossetti, Olivia Fechtner, Matteo Gasparotto, Camille Maillard, Anne Hoffrichter, Eric Zillich, Ammar Jabali, Fabio Marsoner, Ruven Wilkens, Christina B. Schroeter, Andreas Hentschel, Sven G. Meuth, Tobias Ruck, Philipp Koch, Andreas Roos, Nadia Bahi-Buisson, Fiona Francis

Studying immune-neural interactions in a model of T-cell driven hippocampal sclerosis: pitfalls and translational value

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Epilepsy is a central nervous system (CNS) disorder hallmarked by seizures and abnormal brain activity [8]. Current anti-seizure drugs block seizures in only ~70% of patients, do not address the underlying pathology and do not impact the progression of the disorder [5]. Hippocampal sclerosis (HS) as well as malformations of cortical development (MCD) such as focal cortical dysplasia (FCD) and lissencephaly as well as low-grade epilepsy-associated tumors (LEAT) such as ganglioglioma are among the most frequent causes for pharmacoresistant focal epilepsy [2, 3]. All these types of brain lesions harbor innate and adaptive immune cell infiltrations which likely contribute to and modulate their epileptogenicity [4, 6, 10, 11, 12, 13].

Understanding the specific mechanisms involved in the interaction of immune cells and cells of the brain parenchyma for the generation and progression of seizures and epilepsy in these disorders will allow the development of novel drugs that modify the process of epileptic neural network transformation itself.

Human induced pluripotent stem cells (hiPSCs) reprogrammed from patient somatic cells have proven as powerful tool to model human diseases including epilepsies [1, 7, 9]. Platforms in which neurons, astrocytes, oligodendrocytes and microglia derived from healthy or diseased subjects mature in a single system represent a robust method to model human brain disorders.

In this symposium we will discuss the potentials and pitfalls of using hiPSC-derived glioneuronal cell cultures (2D) and brain organoids (3D) to address role the development and mechanisms of epileptic neural network aberration and the role of immune-neural interactions therein.

At the beginning, PD Dr. Nico Melzer will give an overview on immune-neural interactions in a model of T-cell driven hippocampal sclerosis especially with regards to its translational value. Dr. Denise Haslinger will give then provide an overview of an in vitro model of maternal immune activation (MIA), i.e., inflammation induced via pro-inflammatory cytokines in cerebral organoids. She will discuss preliminary data on how this model affects distinct cell types and electrophysiological activity in developing cerebral organoids. Dr. Andrea Rossi will give an overview of the generation of induced pluripotent stem cells from human somatic cells, their genetic manipulation, and their use in creating mini brains to model focal epileptogenic brain lesions. Finally, Dr. Julia Ladewig will elucidate how multi-OMICS analyses of patient-derived forebrain-type organoids can shed light into the molecular mechanisms underlying different severity grades of LIS1-lissencephaly, a malformation of cortical development resulting in clinical phenotypes including epilepsy.

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In vitro modelling of maternal immune activation (mia) in cerebral organoids

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Epilepsy is a central nervous system (CNS) disorder hallmarked by seizures and abnormal brain activity. Current anti-seizure drugs block seizures in only ~70% of patients, do not address the underlying pathology and do not impact the progression of the disorder. Hippocampal sclerosis (HS) as well as malformations of cortical development (MCD) such as focal cortical dysplasia (FCD) and lissencephaly as well as low-grade epilepsy-associated tumors (LEAT) such as ganglioglioma are among the most frequent causes for pharmacoresistant focal epilepsy. All these types of brain lesions harbor innate and adaptive immune cell infiltrations which likely contribute to and modulate their epileptogenicity.
Understanding the specific mechanisms involved in the interaction of immune cells and cells of the brain parenchyma for the generation and progression of seizures and epilepsy in these disorders will allow the development of novel drugs that modify the process of epileptic neural network transformation itself.
Human induced pluripotent stem cells (hiPSCs) reprogrammed from patient somatic cells have proven as powerful tool to model human diseases including epilepsies. Platforms in which neurons, astrocytes, oligodendrocytes and microglia derived from healthy or diseased subjects mature in a single system represent a robust method to model human brain disorders.
In this symposium we will discuss the potentials and pitfalls of using hiPSC-derived glioneuronal cell cultures (2D) and brain organoids (3D) to address role the development and mechanisms of epileptic neural network aberration and the role of immune-neural interactions therein.
At the beginning, PD Dr. Nico Melzer will give an overview on immune-neural interactions in a model of T-cell driven hippocampal sclerosis especially with regards to its translational value. Dr. Denise Haslinger will give then provide an overview of an in vitro model of maternal immune activation (MIA), i.e., inflammation induced via pro-inflammatory cytokines in cerebral organoids. She will discuss preliminary data on how this model affects distinct cell types and electrophysiological activity in developing cerebral organoids. Dr. Andrea Rossi will give an overview of the generation of induced pluripotent stem cells from human somatic cells, their genetic manipulation, and their use in creating mini brains to model focal epileptogenic brain lesions. Finally, Dr. Julia Ladewig will elucidate how multi-OMICS analyses of patient-derived forebrain-type organoids can shed light into the molecular mechanisms underlying different severity grades of LIS1-lissencephaly, a malformation of cortical development resulting in clinical phenotypes including epilepsy.

Genome-editing to model selective somatic mutations associated with focal epileptogenic lesions

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Epilepsy is a central nervous system (CNS) disorder hallmarked by seizures and abnormal brain activity [8]. Current anti-seizure drugs block seizures in only ~70% of patients, do not address the underlying pathology and do not impact the progression of the disorder [5]. Hippocampal sclerosis (HS) as well as malformations of cortical development (MCD) such as focal cortical dysplasia (FCD) and lissencephaly as well as low-grade epilepsy-associated tumors (LEAT) such as ganglioglioma are among the most frequent causes for pharmacoresistant focal epilepsy [2, 3]. All these types of brain lesions harbor innate and adaptive immune cell infiltrations which likely contribute to and modulate their epileptogenicity [4, 6, 10, 11, 12, 13].

Understanding the specific mechanisms involved in the interaction of immune cells and cells of the brain parenchyma for the generation and progression of seizures and epilepsy in these disorders will allow the development of novel drugs that modify the process of epileptic neural network transformation itself.

Human induced pluripotent stem cells (hiPSCs) reprogrammed from patient somatic cells have proven as powerful tool to model human diseases including epilepsies [1, 7, 9]. Platforms in which neurons, astrocytes, oligodendrocytes and microglia derived from healthy or diseased subjects mature in a single system represent a robust method to model human brain disorders.

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Omics insights into LIS1-patient-derived cerebral organoids unravel novel molecular pathways underlying disease severity and suggest therapeutic strategies

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Lissencephaly is a developmental cortical malformation characterized by reduced to absent gyri anda disorganized cortex, often leading to severe impairments in affected individuals and a reduced life expectancy. Heterozygous mutations in the LIS1gene, encodingaregulator of the microtubulemotor dynein, cause lissencephaly with differentclinicalseverities.While the clinical disease spectrum correlates with the degree of lissencephaly, location and type of mutation may not. Weleveraged forebrain-type organoids from LIS1-lissencephalypatients,diagnosedwith mild, moderate or severelissencephalyto investigate, in a cytoarchitecture and multi-omics approach, how the severity degreein patientsmight relate to specific mutations in the LIS1gene. We questionedwhichprocesses during cortical development might bedifferentially affected by severity grade, and whether they could be pharmacologically targeted.We found alterations in neurodevelopment often with a severity-dependent gradient. Specifically, we identifiedalterations of the cytoarchitecture, progenitor cell homeostasisand neurogenesis.Particularly important disease-linked molecular mechanisms were microtubule destabilization, WNT-signaling,and perturbed cadherin-and unfolded protein-binding.Some mechanismsexhibiteda severity-dependent gradient,or were specific to asevere grade. We present strategies to reverse phenotypic changes in LIS1-patient organoids,and an insilicoapproach with therapeutic potential. Thus, we show that different LIS1-severity grades can be recapitulatedin vitro,that there is a direct link between the phenotype and genotype, that organoid-based disease modeling can identifymolecular underpinnings of malformations of cortical development and that organoidsprovide a valid platform to develop and test therapeutic strategies.

Symposium

S28: Early dysfunction of the locus coeruleus noradrenergic system in neurodegenerative diseases

S28-1 Cytoarchitecture and cellular tau pathology of the human locus coeruleus – pericoerulear complex revealed by 3D imaging
Csaba Adori

S28-2 *In vivo* imaging of mitochondrial transport across neuronal cell types reveals tau-mediated dysfunction in the locus coeruleus
Theresa Niedermeier

S28-3 Early locus coeruleus system degeneration underlies olfactory dysfunction in Alzheimer's disease
Lars Paeger, Carolin Meyer, Theresa Niedermeier, Paul Feyen, Felix L. Strübing, Boris Rauchmann, Johanna Gentz, Yannik Tillmann, Katharina Ochs, Karin Wind-Mark, Gloria Biechele, Jessica Wagner, Selim Guersel, Carolin Kurz, Meike Schweiger, Richard Banati, Guo-Jun Liu, Ryan J. Middleton, Gerda Mitteregger-Kretzschmar, Robert Perneczky, Jonas J. Neher, Sabina Tahirovic, Matthias Brendel, Jochen Herms

S28-4 Probing the noradrenergic system to investigate early stages of Alzheimer's Disease
Dorothea Hämmerer

S28-5 Locus coeruleus and central noradrenaline targeting to counteract cortical hyperexcitability in amyotrophic lateral sclerosis
Caroline Rouaux

Cytoarchitecture and cellular tau pathology of the human locus coeruleus – pericoerulear complex revealed by 3D imaging

Csaba Adori[1]

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Early tau pathology of the brainstem nucleus locus coeruleus (LC) is a hallmark of several age-related neurodegenerative disorders, including Alzheimer's disease. However, comprehensive neuropathological examination of the LC is difficult due to its small size and rod-like shape.

To investigate the LC cytoarchitecture and tau cytoskeletal pathology in relation to possible propagation patterns of disease-associated tau in an unprecedented large-scale three-dimensional view, we applied volume immunostaining and whole-mount optical clearing technology, combined with light sheet fluorescence microscopy. We examined pathological ($AT8^+$) tau in the LC/pericoerulear region of 20 brains from Braak neurofibrillary tangle (NFT) stage 0 to 6.

We demonstrate a high morphological complexity of $AT8^+$ cellular structures in the LC, representing various intracellular stages of NFT maturation and their diverse transition forms. We show that gradual dendritic atrophy is one of the the first morphological signs of the degeneration of tangle-bearing neurons. Irrespective of the Braak NFT stage, tau pathology is more advanced in the dorsal LC that preferentially projects to vulnerable forebrain regions in Alzheimer's disease, like the hippocampus or neocortical areas. Moreover, already in the precortical Braak 0 stage, 3D analysis reveals clustering tendency and dendro-dendritic close appositions of $AT8^+$ LC neurons, $AT8^+$ long axons of NFT-bearing cells that join the ascending dorsal noradrenergic bundle after leaving the LC, as well as $AT8^+$ processes of NFT-bearing LC neurons that target the 4th ventricle wall.

Our study suggests that the unique cytoarchitecture, comprised of a densely packed and dendritically extensively interconnected neural network with long projections, makes the human LC to be an ideal anatomical template for early accumulation and trans-neuronal spreading of hyperphosphorylated tau.

In vivo imaging of mitochondrial transport across neuronal cell types reveals tau-mediated dysfunction in the locus coeruleus

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The Locus Coeruleus (LC) is a brainstem nucleus of special interest in the context of neurodegenerative diseases such as Alzheimer's disease (AD), where it is the first region to show hyperphosphorylated 'pre-tangle' tau. Due to the bioenergetic needs of the tonically active LC neurons with their extensive unmyelinated axonal projections throughout the entire forebrain, tau-dependent impairment of mitochondria has been suggested to underlie early LC axon loss.
The study of mitochondria is often restricted to immunohisto- or cytochemical analysis, limiting conclusions about dynamics and progression over time. Reports on the fraction of motile mitochondria show discrepancies between *in vitro* and *in vivo* models. To expand on available *in vivo* studies we utilized mice expressing GFP in the outer mitochondrial membrane in a Cre-dependent manner. We applied *in vivo* acousto-optic two-photon imaging to visualize mitochondrial transport cell type specifically.
The presence of a significant number of moving mitochondria in adult mammals has been a matter of controversy. We reveal for the first time *in vivo* in LC, Parvalbumin (PV) and CamKIIα neurons alike a high fraction (15-20%) of motile mitochondria. Intriguingly, long unmyelinated LC axons showed drastically increased velocities as compared to PV and CamKIIα neurons.
Custom build AAVs were utilized to express human tau (P301S) Cre-dependently in all three cell types. Dbh-Cre animals revealed a significant reduction in mitochondrial velocity in LC axons *in vivo*, which progressively increased over the course of three months. Critically, decreased mitochondrial velocity correlated with a progressive loss of axonal projections in the cortex. The effect on mitochondrial motility was exclusive to LC mitochondria across the studied neuronal types, highlighting the cell type specific vulnerability of the LC during tauopathy.
To further explore this vulnerability under disease conditions, Dbh-Cre animals were transfected with a custom Cre dependent AAV to model α-synucleinopathy (A53T). Mitochondrial velocity was unaltered, showcasing the differences between neurodegenerative diseases. Extending the imaging paradigm to control mice aged over 12 months revealed significantly slower mitochondria in LC axons, which, however, did not reach the same extent as during tauopathy.
Collectively, we show not only an abundance of mitochondrial axonal transport past neonatal stages across cell types, but also the importance of further investigations of mitochondria in tauopathies and other diseases *in vivo*. For the first time we correlate mitochondrial dysfunction with the "dying-back" hypothesis in the LC *in vivo*.

Early locus coeruleus system degeneration underlies olfactory dysfunction in Alzheimer's disease

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Alzheimer's disease (AD) is often accompanied by early non-cognitive symptoms, including olfactory deficits, such as hyposmia and anosmia. These have emerged as predictors of cognitive decline, but the underlying mechanisms of hyposmia in early AD remain elusive. Pathologically, one of the brain regions affected earliest is the brainstem locus coeruleus (LC), the main source of the neurotransmitter noradrenalin (NA) and, a well-known neuromodulator of olfactory information processing. Classically, the degeneration of the LC noradrenergic system is related to the microtubule associated protein tau (MAPT). The contribution of β-amyloid to noradrenergic system dysfunction has been studied less extensively within the past. Using an β-amyloid mouse model of AD, we here show that early (at the age of two months) and distinct loss of LC derived noradrenergic input to the olfactory bulb (OB) coincides with impaired olfaction, even before pronounced appearance of extracellular amyloid plaques. Mechanistically, OB microglia detect externalized phosphatidylserine and MFG-E8 on hyperactive LC axons and subsequently initiate their clearance. Translocator protein 18 kDa (TSPO) knockout reduces phagocytosis, preserving LC axons and olfaction. Importantly, patients with prodromal AD display elevated TSPO-PET signals in the OB, similarly to APP^{NL-G-F} mice. As the disease progresses, patients develop olfactory deficits, supporting recent studies describing olfactory deficits in humans as predictor of cognitive decline in AD. We further confirm early LC axon degeneration in post-mortem OBs in patients with early AD. Collectively, we uncover an underlying mechanism linking early LC system damage and hyposmia in AD. Our work may help to improve early diagnosis of AD by olfactory testing and neurocircuit analysis and consequently enable early intervention.

Probing the noradrenergic system to investigate early stages of Alzheimer's Disease

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Cognitive and brain physiological aging is a multifactorial process whose protective and risk factors we are increasingly understanding. Due to the increased life expectancy, we have been confronted with an increase in dementia-related diseases in recent decades, for which there is currently no cure. A striking aspect of dementia-related diseases is that neuromodulatory systems are at the center of affected brain structures. It is currently still unclear why neuromodulatory systems are particularly vulnerable, and to what extent early impairments of our neurochemical supply in the brain can accelerate the progression of dementia. In my talk, I will present our current research on the noradrenergic system in ageing which uses cognitive neuroscientific approaches to better understand the extent and consequences of an affected noradrenergic locus coeruleus in ageing. The focus hereby is on the non-invasive imaging of the structure and function of cell nuclei using magnetic resonance imaging as well as drug and brain stimulation interventions that are intended to investigate the consequences of a change in noradrenergic activity.

Locus coeruleus and central noradrenaline targeting to counteract cortical hyperexcitability in amyotrophic lateral sclerosis

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The fatal neurodegenerative disease amyotrophic lateral sclerosis (ALS) is characterized by the death of upper (UMN) and lower motor neurons (LMN) in the motor cortex, brainstem and spinal cord, that triggers progressive paralysis and death within only two to five years. Despite decades of research, ALS remains incurable, challenging to diagnose, and of extremely rapid progression.
A unifying feature of ALS is cortical hyperexcitability, a typical dysfunction of the motor cortex neuronal network which has been demonstrated to precede motor symptom onset and to negatively correlate with survival [1]. We recently demonstrated that cortical hyperexcitability can be unraveled, both in patients and mouse models of the disease using novel electroencephalography and electrocorticography approaches. In addition, we convincingly identified central noradrenergic deficit as a driver of cortical hyperexcitability. Using mass spectrometry analyses of central nervous system neuropeptides, we identified a presymptomatic reduction of noradrenaline (NA) in the motor cortex of ALS mouse models, further validated by in vivo two-photon imaging, that revealed pronounced reduction of locomotion-associated NA release. NA deficits were also detected in postmortem tissues from patients with ALS, along with transcriptomic alterations of the noradrenergic signaling pathway. Pharmacological ablation of noradrenergic neurons with DSP-4 triggered cortical hyperexcitability in wild-type mice and administration of L-DOPS, a synthetic precursor of NA could significantly moderate cortical hyperexcitability in ALS mice [2]. Building on this recently published work, we hypothesize that noradrenergic impairment is a very early feature of ALS that strongly contributes to cortical hyperexcitability and disease onset and progression. Work is in progress to determine whether the locus coeruleus, along with the broader central noradrenergic system, may represent a new therapeutic target, paving the way to the development of new treatment options in ALS.

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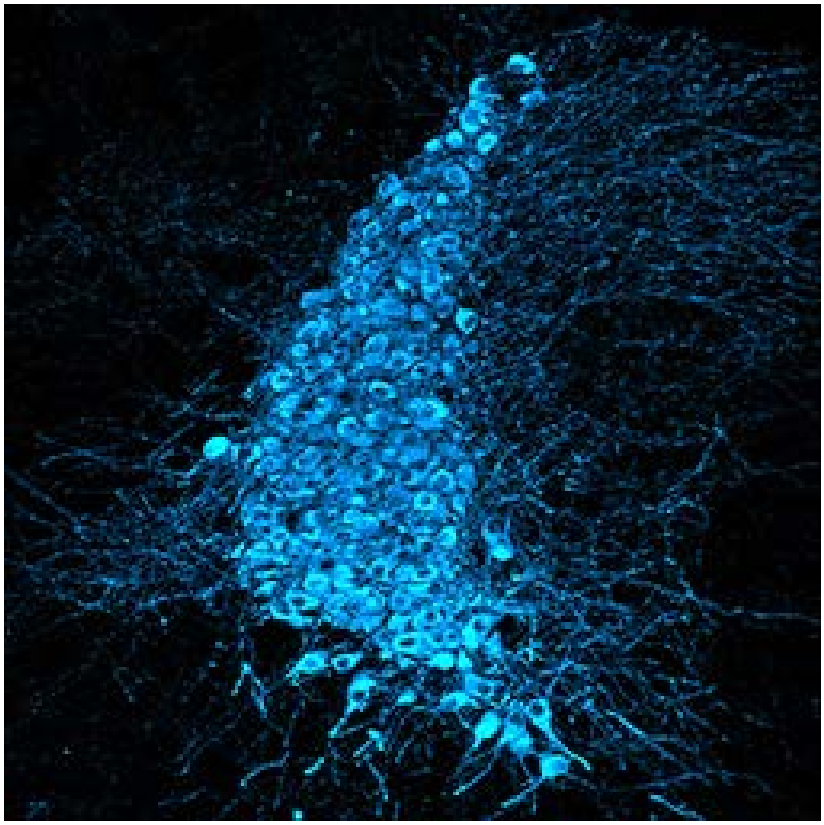

Noradrenergic neurons of the left locus coeruleus revealed by thyrosine hydroxylase immunofluorescence on a mouse brain coronal section.

Symposium

S29: Neural circuits and decision strategies for behavioral trade-offs

From Stimulus to Action: How the Brain Balances Reproductive and Survival Needs

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Romantic engagement can bias sensory perception. This 'love blindness' reflects a common behavioural principle across organisms: favouring pursuit of a coveted reward over potential risks1. In the case of animal courtship, such sensory biases may support reproductive success but can also expose individuals to danger, such as predation2,3. However, how neural networks balance the trade-off between risk and reward is unknown. Here we discover a dopamine-governed filter mechanism in male Drosophila that reduces threat perception as courtship progresses. We show that during early courtship stages, threat-activated visual neurons inhibit central courtship nodes via specific serotonergic neurons. This serotonergic inhibition prompts flies to abort courtship when they see imminent danger. However, as flies advance in the courtship process, the dopaminergic filter system reduces visual threat responses, shifting the balance from survival to mating. By recording neural activity from males as they approach mating, we demonstrate that progress in courtship is registered as dopaminergic activity levels ramping up. This dopamine signalling inhibits the visual threat detection pathway via Dop2R receptors, allowing male flies to focus on courtship when they are close to copulation. Thus, dopamine signalling biases sensory perception based on perceived goal proximity, to prioritize between competing behaviours.

Embodied neuroAI: decision making with Drosophila Larva

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Animals are not free-floating neural networks that perform classification tasks. Our body, the temporal continuity of our actions, and our sensory word shape the property and the computing of biological neural networks. Advances in Drosophila genetic manipulation, neural connectomics, and amortised inference (acceleration of the sampling procedure using neural networks) have allowed us to study small neural circuits' organisation, structure, and function. We still have a limited understanding of the forces organising small neural circuits.
In this presentation, I will address finite element simulations of the Drosophila larva body coupled to neural dynamics simulation of selected circuits, phenotyping larva as a statistical test in a learned latent space and neuromodulation at the single neuron scale.

Neural basis of visual information integration and decision-making in larval zebrafish

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Decision-making is a long-studied topic in neuroscience. We have an increasingly good mechanistic understanding of the neural circuits that allow animals to temporarily integrate specific decision variables. However, it remains unclear how these circuits combine, often conflicting, information from multiple sensory channels to form a single decision. Recently, we have described how the larval zebrafish anterior hindbrain integrates visual motion to decide about swimming direction. Other studies, focusing on different sensory stimuli, have identified the same brain area as a central processing structure for sensory-motor control. This raises the hypothesis that the anterior hindbrain forms a general integration hub for decision-making. Here, we employ a combination of behavioral experiments, computer simulations, and two-photon functional imaging to algorithmically and mechanistically describe how larval zebrafish integrate motion and luminance cues. Our behavior experiments and computational simulations argue for a parallel arrangement, in which separate modules temporally integrate information from distinct visual processing streams. Our imaging experiments support these findings, revealing distinct activation patterns with slow temporal dynamics that match the model predictions. These results allow us to build precise neural networks whose connections we test using newly established circuit dissection tools. Together, this means we can describe in mechanistic detail how brains combine and evaluate information extracted from multiple visual features.

A subcortical switchboard for controlling exploratory, perseverative and disengaged states

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To survive in evolving environments with uncertain resources, animals need to dynamically adapt their behaviour and exhibit flexibility in choosing appropriate behavioural strategies, for example, to persevere a familiar choice, to explore alternative options and acquire novel information, or to disengage altogether. Previous studies have mainly investigated how forebrain regions represent choice costs and values as well as optimal strategies during such decisions. However, the neural mechanisms by which the brain implements alternative behavioural strategies such as persevering, exploring or disengaging from the environment, remains poorly understood. Here we identify a neural hub critical for flexible switching between behavioural strategies, the median raphe nucleus (MRN). Using cell-type specific optogenetic manipulations, calcium fibre photometry and circuit tracing in mice performing diverse instinctive and learnt behavioural tasks, we found that the MRN's main cell types, GABAergic, glutamatergic (VGluT2-positive), and serotonergic neurons, have complementary functions and drive perseverance, exploration and disengagement, respectively. Suppression of MRN GABAergic neurons, for instance through inhibitory input from lateral hypothalamus which conveys strong positive valence to the MRN, leads to perseverance in current actions and goals. In contrast, activation of MRN VGluT2+ neurons drives exploratory behaviour. Activity of serotonergic MRN neurons is necessary for general task engagement. Input from the lateral habenula conveying negative valence suppresses serotonergic MRN neurons, leading to disengagement. These findings establish the MRN as a central behavioural switchboard, uniquely positioned to flexibly control behavioural strategies. These circuits thus may also play an important role in the aetiology and possible treatment of major mental pathologies such as depressive or obsessive-compulsive disorders.

Theoretical models of social foraging

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Foraging is a widespread behavior, and being part of a group may bring several benefits compared to solitary foraging, such as collective pooling of information and reducing environmental uncertainty. Often theoretical models of collective behavior use coarse-grained representations, or are too complex for analytical treatment, and generally do not take into account the noisy decision making process implemented by individual agents. This calls for the development of a mechanistic, analytically tractable, and stochastic framework to study the underlying processes of social foraging, tying the microscopic to the macroscopic levels. Based on an evidence accumulation framework, we developed a model of patch-leaving decisions in egalitarian and hierarchical groups. Across a variety of environmental statistics and information sharing mechanisms, we were able to analytically derive optimal agent strategies. The environmental statistics considered are either two non-depleting or several successive depleting patches. The social information sharing mechanisms are either through observation of others' food rewards or through belief sharing, with continuous sharing, pulsatile observation of others' departures or arrivals, or through counting the number of individuals in a patch. Throughout all these conditions, we quantified how cohesive a group is over time, how much time agents spend on average in a patch and what are their group equilibrium dynamics. We found that social coupling strongly modulates these features across a variety of environmental statistics. This general modeling framework is crucial to both designing social foraging experiments and generating hypotheses that can be tested.

Symposium

S30: Glia-neuron interactions sculpting functional circuit architecture; insights from genetic animal models

S30-1 Glial cells, integrators of neural circuit architecture throughout life: insights from *C. elegans*
Georgia Rapti, Francesca Caroti, Jolita Cibulskaite, Carlo Bevilacqua

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Glial cells, integrators of neural circuit architecture throughout life: insights from *C. elegans*

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Specialized functions of the nervous system rely on the proper assembly of its elaborate circuit architecture. This remarkably complex architecture is built early in development and is sensitive to age and environmental challenges throughout life. Circuit architecture influences animal behavior and cognition, and its improper formation or age-dependent aberrations of neural cells manifest as circuit malformations, mental conditions, or cognitive decline.

Investigating the mechanisms that shape circuit architecture is inherently challenging, requiring an integrated approach across molecular, cellular, tissue scales. Large cell numbers and intricate interactions complicate the dissection of molecular contributions underlying these processes. Neurons receive much attention, but glial cells remain understudied despite composing the non-neuronal half of our nervous system. Glial cells and their interactions with neurons have recently been implicated in patterning neural circuit architecture, contributing to proper circuit connectivity and function. Failure to form or preserve glial cell integrity results in compromised circuit function. Thus, dissecting the mechanisms underlying glial cell architecture is crucial to understanding circuit architecture and maintaining functional circuit integrity. Yet, despite extensive nervous system studies, the mechanisms underlying glial cell architecture remain understudied.

We investigate mechanisms shaping and maintaining glial cell architecture, leveraging the advantages of the model organism *C. elegans*. *C. elegans* offers a powerful setting, with stereotyped and mapped lineage, connectivity, and nervous system anatomy at single-cell resolution, and glia implicated in circuit development and function but largely dispensable for neuronal viability, facilitating their manipulation. *C. elegans* astroglial cells have architecture, functions, and molecular content analogous to vertebrate astroglia, as we and others have shown. We study *C. elegans* astroglia and identify factors that shape their formation and their interactions with neurons that shape circuit architecture or ensure its age-progressive integrity.

To identify genes regulating astroglial architecture, we perform candidate-gene or unbiased screens and generate toolkits labeling astroglial (sub)cellular architecture in relation to interacting cells. We isolated mutants harboring astroglia with abnormal gene expression or defective cellular architecture, resulting in the consequent disruption of neuronal circuit features. To dissect the underlying molecular mechanisms and glial functions, we study these mutant glial cells by combining advanced genetics, quantitative imaging of subcellular features and the extracellular matrix (ECM), genetic manipulation of glial cells or environmental properties, and biophysical measurements. Our work uncovers conserved factors driving astroglial gene expression, patterning their development, or safeguarding their age-progressive architecture. We will report on our findings in these directions, the molecular and cellular analogies between *C. elegans* and vertebrate astroglia, the mechanisms of glial development, and their functions in circuit assembly and maintenance. Overall, our studies highlight glial assembly as an integrator of circuit functional architecture throughout life and may provide insights into astroglial biology beyond *C. elegans*.

Exploring the relationship between glial morphologies and transcriptomes

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Morphology is a defining feature of neuronal identity. Like neurons, glia display diverse morphologies, both across and within glial classes, but are also known to be morphologically plastic. Here, we explored the relationship between glial morphology and transcriptional signature using the Drosophila central nervous system (CNS), where glia are categorised into 5 main classes (outer and inner surface glia, cortex glia, ensheathing glia, and astrocytes), which show within-class morphological diversity. We analysed and validated single-cell RNA sequencing data of Drosophila glia in 2 well-characterised tissues from distinct developmental stages, containing distinct circuit types: the embryonic ventral nerve cord (VNC) (motor) and the adult optic lobes (sensory). Our analysis identified a new morphologically and transcriptionally distinct surface glial population in the VNC. However, many glial morphological categories could not be distinguished transcriptionally, and indeed, embryonic and adult astrocytes were transcriptionally analogous despite differences in developmental stage and circuit type. While we did detect extensive within-class transcriptomic diversity for optic lobe glia, this could be explained entirely by glial residence in the most superficial neuropil (lamina) and an associated enrichment for immune-related gene expression. In summary, we generated a single-cell transcriptomic atlas of glia in Drosophila, and our extensive in vivo validation revealed that glia exhibit more diversity at the morphological level than was detectable at the transcriptional level. This atlas will serve as a resource for the community to probe glial diversity and function.

Influence of glial cells in positioning voltage-gated ion channels along Drosophila axons

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Neuronal information conductance often involves the transmission of action potentials. The spreading of action potentials along the axonal process of a neuron is based on three physical parameters: the axial resistance of the axon, the axonal insulation by glial membranes, and the positioning of voltage-gated ion channels. In vertebrates, myelin and channel clustering allow fast saltatory conductance. Similarly, voltage-gated sodium and potassium channels, Para and Shal, co-localize and cluster in an area resembling the axon initial segment, both in sensory and motor neurons. In larvae, relatively low levels of Para channels are needed to allow proper signal transduction and axons are simply wrapped by glial cells. In adults, the concentration of Para increases and is prominently found at the axon initial segment of motor neurons. Concomitantly, these axon domains are covered by a mesh of glial processes forming a lacunar structure that possibly serves as an ion reservoir. Directly flanking this domain glial processes forming the lacunar area appear to collapse and closely apposed stacks of glial cell processes can be detected, resembling a myelin-like insulation.
Surprisingly, we found that the presence of wrapping glial cells regulates both the levels of Para expression as well as its positioning within the axonal membrane. To address how glial cells modulate expression of this neuronal ion channel we have identified the interactome of Para using mass spec analysis and follow genetic strategies to understand how glial cells are able to modulate neuronal protein expression.

Pharmacological targeting of Smoothened receptor as a promising approach to enhance oligodendrocyte differentiation

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Remyelination is a natural repair process of the central nervous system (CNS), that restores axonal insulation, promoting neuroprotection and functional recovery after myelin damage. Several demyelination pathologies could benefit from improved CNS remyelination, especially during ageing. Despite this, to date, no remyelination agent arrived at the clinic. There is an urgent need for innovative pharmacological strategies to enhance remyelination and improve the effectiveness of current therapeutic molecules. Recent phenotypic screening studies have highlighted the promyelinating properties of some glucocorticoids (GCs) in multiple sclerosis animal models. This specific class of GCs interacts, not only with the Glucocorticoid Receptor (GR), but also with the Smoothened (Smo) receptor of the Hedgehog pathway. However, how their binding to Smo influences oligodendrocyte precursor cells (OPCs) remains unclear (Al Jaf et al., 2024). Additionally, the individual contributions of each receptor to the observed promyelinating effects are yet to be fully understood. Gaining deeper insights into how these ligands modulate Smo receptor activity could provide critical information for structure-based drug design, paving the way for more precise and effective remyelination therapies.
Building on this knowledge, we focused on studying two molecules that bind Smo: the GSA-10, a synthetic molecule derived from the pharmacophore of Smoothened agonist SAG, and the Budesonide, a GC that binds to the cysteine-rich domain (CRD) of Smo. Both these drugs prevent Smo activation in fibroblasts. Our latest study employed a combination of cellular, biochemical, and molecular dynamics approaches to demonstrate that budesonide treatment promotes myelination in oligodendroglia cells by facilitating synthetic axon ensheathment. Moreover, Budesonide reduces the conformational flexibility of the Smo CRD, thereby inhibiting canonical Smo-mediated signaling. At the same time, Budesonide activates the Liver Kinase B1 (LKB1)/AMP-activated protein kinase (AMPK) pathway, which leads to the upregulation of Myelin Basic Protein (MBP) expression (Recchia et al., 2024). These data reinforce previous evidence that Smo plays a key role in remyelination via activation of the LKB/AMPK pathway. How activation of this pathway can regulate MBP gene transcription represents the next crucial step in understanding a basic mechanism of OPCs differentiation in myelinating oligodendrocytes. Together, these findings lay a solid foundation for pharmacologically targeting the Smo as one of the strategies to enhance OPC differentiation and promote remyelination, opening up new avenues for therapeutic intervention in demyelinating diseases.

Al Jaf, A.I.A., Peria, S., Fabiano, T., and Ragnini-Wilson, A. (2024). Remyelinating Drugs at a Crossroad: How to Improve Clinical Efficacy and Drug Screenings. Cells 13, 1326. https://doi.org/10.3390/cells13161326.
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Radial astrocyte synchronization modulates the visual system during behavioral-state transitions

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Glial cells were thought to support the function of neurons. Recent evidence show that astrocytes are involved in brain computations. To explore whether and how their excitable nature affect brain computations and motor behaviors, we used two-photon Ca2+ imaging of zebrafish larvae expressing GCaMP in both neurons and Radial Astrocytes (RAs). We found that in the optic tectum, RAs synchronize their Ca2+ transients immediately after the end of an escape behavior. Using optogenetics and ablations, we found that RA synchronous Ca2+ events are mediated by the locus-coeruleus-norepinephrine system and the medulla oblangata. The latter functions as an integrator of the internal state of the animal.

RAs synchronization modulated the direction selectivity of tectal neurons and their long-distance functional correlations. This mechanism may support freezing behavior following a switch to an alerted state and improve visual detection. These results show that LC-mediated neuro-glia interactions modulate the visual system during transitions to arousal states.

Symposium

S31: From olfaction to emotions

Mice navigate the odour landscape using plume temporal dynamics

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Olfactory signals convey a plethora of crucial information about our surroundings. Unlike in other senses, olfactory information is directly conveyed to the brain's limbic system, involved in emotion, memory, and motivation. This underpins the ability of odours to elicit immediate and potent behavioural responses during vital scenarios such as predator scent detection which can trigger an instant fear response. Similarly, the detection of conspecific odour cues such as pheromones plays a crucial role in social and reproductive behaviours, and can decrease responses to aversive stimuli within social contexts.
Natural odours, shaped by turbulent airflow, are transported as temporally complex odour plumes. The spatiotemporal dynamics of natural odours provide valuable cues about the sources' nature and location. Our experiments showed that correlated odour intensity fluctuations arise when odours originate from the same source, while a separation of sources results in uncorrelated odour profiles. Olfactory cues are initially processed in the olfactory bulb and we showed that within the local olfactory bulb network, the temporal structure of odours can be extracted to guide behavioural output.
We further investigated whether mice use spatial information carried by odour plumes for distance discrimination. Using a wind tunnel and innovative odour delivery devices, we generated and recorded odour plumes, replicating them in an "olfactory virtual reality" system. We propose that odour features at frequencies higher than the respiratory cycle are more significant for distance discrimination than slower timescales such as average concentration. Presenting odour plumes recorded at various distances in the virtual reality environment, we found that a subset of olfactory bulb projections neurons exhibited differential responses corresponding to different distances. Notably, these responses were linked to the temporal features of odour plumes and correlated with sub-sniff temporal patterns.
We are now extending these investigations to freely moving animals to determine which features of complex odour plumes mice use for odour source localisation. To this end, we combine the acquisition of navigation behaviour, active sampling strategies, dynamic odour profiles, and neural activity to reveal how naturalistic olfactory information informs behaviour.
Taken together, our findings highlight the capability of mammals to extract and utilize temporally complex odour information to navigate the odour landscape. At the cellular level, we shed light on the mechanisms underlying odour source localization in olfactory bulb neurons. This paves the way to directly connect the temporal dynamics of naturalistic odours with higher order processing mechanisms and emotional functions.

Potential integration of main and accessory olfactory system information in the mouse amygdala

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Olfactory stimuli are processed via at least two central pathways in the rodent brain: the main and accessory olfactory pathways. These systems are specialized to detect and process non-exclusive olfactory stimulus spaces. Yet, olfactory-guided behavior relies on integration along both pathways. Here, we set out to identify and investigate central structures anatomically suited to integrate main and accessory olfactory information. To this end, we used a viral transduction approach to trace and compare axonal projections of main and accessory olfactory bulb principal neurons, respectively. We identified axonal convergence in the bed nucleus of the accessory olfactory tract (BAOT) and anteroventral medial amygdala (MeAav). Next, we used single-cell patch-clamp recordings in acute brain slices to investigate the electrophysiological and morphological properties of cells in these adjacent nuclei. We identified unique cell types in both nuclei. Our morphological and electrophysiological results suggest the integrative capabilities of BAOT and MeAav cells. Next, we investigated synaptic connectivity using pharmacological intervention, providing insights into the excitatory and inhibitory connectivity of BAOT and MeAav cells. Altogether, we describe convergent axonal projections from the main and accessory olfactory bulb as well as the morphological and physiological profiles of neurons in the convergence zones, i.e., BAOT and MeAav.

The nucleus of the lateral olfactory tracts is a center for odor-emotion interactions

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The sense of smell is tightly linked to emotions, a link that is thought to rely on the direct synaptic connections between the olfactory bulb and nuclei of the amygdala. A small number of amygdaloid nuclei are the recipients of such direct input from the olfactory bulb and their unique functions are not known. Among them, is the nucleus of the lateral olfactory tract (NLOT) that has been very little studied and consequentially its function is unknown. We recently developed the methods for specific targeting of the NLOT with various viruses and used them for behavioral, anatomical, and physiological studies. We found that intact NLOT activity is critical for odor fear learning but not for odor identification. We analyzed the connectivity of NLOT using pseudo-rabies input tracing as well as adeno-associated viruses to reveal NLOT projection targets. We found that the NLOT is interconnected with several olfactory brain regions and with the basolateral amygdala. Some of these connections were reciprocal, and some showed unique interhemispheric patterns. We tested the excitable properties of NLOT neurons and the properties of each of the major synaptic inputs. We found that the NLOT receives powerful input from olfactory cortical regions, and the basolateral amygdala, but only very weak input from the olfactory bulb. When input crosses threshold, NLOT neurons respond with calcium-dependent bursts of action potentials. These data indicate that NLOT plays a role in behaviors that combine smell and emotion, possibly assigning emotional value to odors.

Functional diversity of inhibitory amygdala microcircuits

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Neural circuits undergo experience-dependent plasticity to form long-lasting memories. Excitatory projection neurons are considered to be the primary neuronal substrate for memory acquisition and storage. Inhibitory interneurons control the activity of projection neurons in a in a spatially and temporally precise manner, yet their contribution to memory acquisition, storage and expression remains poorly understood. Here, we employ a miniature microscope imaging approach to monitor the activity of large amygdala interneuron populations in freely moving mice during aversive learning and extinction at the single cell level. We find that amygdala interneurons display mixed-selectivity and show complex plastic responses at both the ensemble and single neuron level across the acquisition, expression and extinction of aversive memories. Taken together, our study identifies complex neuronal plasticity within amygdala interneuron ensembles that goes beyond a passive processing function, suggesting a critical role of inhibitory microcircuit elements for memory selectivity and stability.

Neuronal types in the mouse amygdala and their transcriptional states in fear memory

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The amygdala is a brain region primarily associated with emotional response. For example, fear learning and memory are known to activate neurons in the amygdala, which induce gene expression to strengthen the formation of stable engrams. Single-cell transcriptomics can provide insights into behavior-associated cell state changes. Here we present a detailed cell- type taxonomy of the adult mouse amygdala during fear learning and memory consolidation. We perform single-cell RNA sequencing on naïve and fear-conditioned mice, identify 130 neuronal cell types and validate their spatial distributions. We found that only a subset of all neuronal types is transcriptionally responsive to fear learning and memory retrieval. Within the responsive populations, activated engram cells upregulate activity-response genes and coordinate the expression of genes associated with neurite outgrowth, synaptic signaling, plasticity and development. We identify known and previously undescribed neuronal populations and candidate genes responsive to fear learning. Our molecular atlas may be used to generate hypotheses to unveil the neuron types, neural circuits and genes regulating the emotional component of learning and memory.

Symposium

S32: Dendritic inhibition - role in network dynamics, memory and behavior

S32-1 Top-down control of threat memory through neocortical layer
Johannes J. Letzkus

S32-2 The contribution of dendritic inhibition to cortical network dynamics
Matthew Evan Larkum

S32-3 Dendrit inhibition shapes encoding of space and context in the dentate gyrus of behaving mice
Marlene Bartos

S32-4 Inhibitory control of circuit dynamics by dendrite-targeting interneurons - insights from computational models
Panayiota Poirazi

Top-down control of threat memory through neocortical layer

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Accurate perception of the environment is a constructive process that requires integration of external bottom-up sensory signals with internally-generated top-down information reflecting past experiences and current aims. Decades of work have elucidated how sensory neocortex processes physical stimulus features. In contrast, examining how memory-related top-down information is encoded and integrated with bottom-up signals has long been challenging. Here, I will discuss our recent work identifying the outermost layer 1 of neocortex as a central hotspot for processing of experience-dependent top-down information during threat perception, one of the most fundamentally important forms of sensory perception.

The contribution of dendritic inhibition to cortical network dynamics

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Dendritic inhibition critically modulates cortical network function by influencing how information is processed and encoded. Inhibitory neurons shape synaptic integration within the dendrites of cortical pyramidal neurons, particularly affecting active processes that lead to dendritic spikes essential for synaptic plasticity and nonlinear integration.

Specific dendrite-targeting inhibitory interneurons, such as somatostatin (SST)-expressing and neuron-derived neurotrophic factor (NDNF)-positive cells, precisely target subcompartments of pyramidal neuron dendrites for fine-tuned regulation of neuronal output. Our recent findings reveal that similar inhibitory mechanisms operate within the dendrites of inhibitory neurons themselves, adding a new layer of complexity to cortical modulation. We hypothesize that dendritic processes in inhibitory neurons modulate their control over cortical network dynamics. Utilizing whole-cell recordings, two-photon calcium imaging, and optogenetic manipulations in vitro and in behaving animals, we demonstrate that dendritic inhibition influences network dynamics and memory consolidation in cortical networks. These results highlight the pivotal role of dendritic inhibition in shaping both individual neuron activity and the collective dynamics of neuronal populations during learning and memory.

Dendrit inhibition shapes encoding of space and context in the dentate gyrus of behaving mice

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To select appropriate behaviour, individuals need to rely on the encoding of relevant features within their environment in the context of current and past experiences. Here, we show that changes in goal-locations in familiarized environments relate to altered activity of hippocampal somatostatin-expressing interneurons (SOMIs). By applying single unit recordings of optogenetically identified SOMIs in the dentate gyrus of head-fixed mice trained on a spatial goal-oriented reward-learning task in a virtual reality, we show that in expert mice characterized by goal anticipatory behaviour, elevated SOMI activity temporally precedes reward-locations. Translocation of learned goals to novel previously unrewarded locations resulted in rapid reduction of anticipatory behaviour, lost predictive SOMI signaling and rapid reconfiguration of SOMI activity to times after reward onset in association with non-anticipatory behaviour. Chemogenetic silencing of SOMIs caused a loss in memorizing that trained goal-sides are no longer available. Thus, our data show a so far unrecognized ability of SOMIs to flexibly encode goal-locations depending on current and past experiences to bias behavioral outcomes.

Inhibitory control of circuit dynamics by dendrite-targeting interneurons - insights from computational models

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In this presentation I will discuss work from our lab in which we investigate the role of different type of interneurons in controlling the circuit dynamics of simulated neuronal networks. I will also discuss how nonlinearities in the dendrites of specific interneuron subtypes can affect circuit computations in such networks, offering important gains in learning and memory capacity.

Symposium

S33: Non-canonical contribution of oligodendrocyte precursors in brain circuits

Neuro-glia crosstalk shapes brain morphogenesis across species

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In the forebrain, oligodendrocyte precursor cells (vOPCs) originating from ventral regions migrate tangentially towards the cortex alongside cortical interneurons. Most vOPCs do not survive postnatally, raising questions about their function. In this study, we demonstrate that vOPCs play a temporary and non-canonical role in cortical development by supporting the migration of cortical interneurons. Additionally, we present new evidence showing that this transient function is conserved across different species.

How neuronal connectivity is shaped by oligodendrocyte precursor cells

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It is becoming increasingly clear that glia cells play crucial roles in the regulation of circuit connectivity through modulation of synapse formation and remodelling. Recently, we have attributed this function to a particular type of glial cells called the oligodendrocyte precursor cell (OPC). Traditionally, OPCs have established roles in giving rise to oligodendrocytes that myelinate axons. However, unlike other progenitor cells of our body, OPCs do not reside within specialised niches but are instead evenly distributed across the CNS, making up ~5% of all CNS cells lifelong. The roles of these non-myelinating OPCs are largely unknown.

To investigate how OPCs affect the CNS independently of myelin formation, we have identified the optic tectum of zebrafish as a region which contains OPCs but which is devoid of myelin during stages when a functional visual system is present. We show that OPCs are tightly integrated into tectal circuitry, where they form an elaborate process network that tiles throughout the synaptic neuropil and where they dynamically interact with arbours of retinal ganglion cell axons (RGCs) and local interneurons. Genetic depletion as well as ablation of OPCs led to erroneous RGC axon branching and enlarged arbour sizes of individual RGC axons. Functionally, OPC ablation impaired prey capture, and degraded visual acuity in response to optic flow, indicating that neuronal connectivity must be perturbed. To investigate how OPCs exert these functions, we carried out longitudinal in vivo imaging of single neurons over time. Our experiments revealed enhanced remodelling dynamics of RGC terminals in the absence of OPCs, reduced stability of neuronal pre-synapses in the short term, and loss pre-synapses in the longer term. Thus, our work demonstrates a non-canonical role of OPCs in regulating neural circuit connectivity.

Rxrg regulates brain oligodendrogenesis during key events of life

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Overcoming remyelination failure in multiple sclerosis (MS) is a key challenge to generate a therapy. To remyelinate, oligodendrocyte precursor cells (OPCs) need to engage in differentiation, but this process is impeded in MS. RXRg is a nuclear receptor known to stimulate OPC differentiation, and its downregulation in MS correlates with remyelination failure. However, the mechanism of such Rxrg function in remyelination process, and its relevance for developmental oligodendrogenesis remains unknown.

Using a new in vivo reporter system, we found Rxrg-expressing OPCs contribute to the generation of mature oligodendrocytes at key time point throughout mouse life. Additionally, we found that RXRg expression can also be induced in young adult mouse brain following myelin lesion. Such RXRg-positive OPCs generate mature OLs and contribute to regeneration of myelin scar.

To better characterize the differentiation mechanism controlled by Rxrg, we studied primary OPC cultures. mRNAseq analyses revealed a significant increase of gene expression in a key signaling pathway, as well as other genes associated with this pathway in Rxrg-/- OPCs. Pharmacological inhibition of this pathway restored normal differentiation of Rxrg-/- OPCs, pointing to the role of hyperactive signaling in the differentiation block observed in these cells. The relevance of this in vitro data for in vivo oligodendrogenesis is supported by an increase of this same pathway in OPCs in Rxrg-/- null mutants. Our data indicate that Rxrg is involved during key moment in the life of the mouse -or during disease-associated oligodendrogenesis. Thus, further deciphering molecular mechanisms of its action may point to new strategies for treatment of multiple sclerosis or others myelin diseases.

Changes in OPC-neuron interactions in the hippocampus upon increased neuronal activity

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NG2 cells, or oligodendrocyte progenitor cells, are a glial cell population that covers the entire parenchyma of the central nervous system. NG2 cells differentiate into mature oligodendrocytes, ensheathing axons with myelin. However, it is still unknown whether all NG2 cells have the same potential to generate oligodendrocytes or whether a subpopulation of them becomes permanent NG2 cells. The mechanisms of neuron-NG2 cell interaction are not fully understood, but this interaction is thought to be regulated by neuronal activity. Using mouse models with increased neuronal activity, we investigate how altered neuronal excitability affects the structural relationship between neurons and NG2 cells in specific layers of the hippocampus. We detected an increase in the number of OPC processes extending towards the soma of activated neurons and will discuss the functional implications of this interaction.

Oligodendrocyte progenitor cells facilitate exocytosis of neuronal lysosomes

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Oligodendrocyte precursor cells (OPCs) shape brain function through complex regulatory mechanisms. Here, we observed that OPCs facilitate neuronal lysosome exocytosis by forming contacts with neuronal somata. Loss of OPCs or reduced OPC-process branching decreased these contacts, leading to lysosome accumulation, altered neuronal metabolism and more senescent neurons with aging. Our findings are relevant for prevention of aging-related pathologies and therapeutic strategies of neurodegenerative diseases.

Symposium

S34: Modelling CNS recovery from autoimmune neurodegeneration

S34-1 Metabolic control of the regenerative potential in autoimmune CNS lesions
Mikael Simons

S34-2 Reorganization of neurons into circuits as a checkpoint of CNS recovery after traumatic and autoimmune lesions
Florence Martine Bareyre

S34-3 Immunophenotyping of the brain after recurrent ischemic stroke in mice
Polina Bugaeva, Laura Kate Ismajli, Sylwia Piatek, Eduart Temaj, Amido Daugardt, Marco Foddis, Markus Winkler, Ronja Marion Dörk, Tingting Wang, Amelie Weber, Susanne Mueller, Stefan Paul Koch, Philipp Boehm-Sturm, Nikolaus Wenger, Christian Hoffmann, Linda Hammerich, Christian Oeing, Christoph Harms

S34-4 The role of the meninges in autoimmune CNS inflammation
Arianna Merlini, Alexander Flügel, Francesca Odoardi

S34-5 Adjuvant-free experimental autoimmune encephalomyelitis as a model to study brain inflammation and neurodegeneration
Djordje Miljkovic, Suzana Stanisavljevic, Bojan Jevtc, Neda Nikolovski, Miljana Momcilovic, Goran Stegnjaic, Mirjana Dimitrijevic

Metabolic control of the regenerative potential in autoimmune CNS lesions

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We study the question of how myelin drives a chronic inflammatory response, and how this inflammation is linked to the pathogenesis of diseases. Another goal is to understand the role of microglia in lesion recovery after demyelinating injury. Acute CNS damage is followed by a multicellular response that encompasses different cell types and spans different scales. Currently, we do not understand which factors determines lesion recovery. Failure of inflammation to resolve is a key underlying reason of poor regeneration, and one focus is therefore on the biology of microglia during de- and remyelination, and their cross talk to other cells, in particular oligodendrocytes and the progenitor cells. In addition, we are exploring the link between lipid metabolism and inflammation, and its role in the regulation of regeneration.

Reorganization of neurons into circuits as a checkpoint of CNS recovery after traumatic and autoimmune lesions

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In this talk we will detail the cellular basis of axonal reorganization of neurons into circuits as a checkpoint of CNS recovery after traumatic and autoimmune lesions. We will give a major emphasis to traumatic injuries of the spinal cord and inflammatory lesions. Aim is to detail therapeutic strategies aiming at enhancing axonal reorganization to promote recovery following traumatic and autoimmune lesions.

Immunophenotyping of the brain after recurrent ischemic stroke in mice

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Introduction: Recurrent stroke occurs in 19% of patients within a year after the first-time ischemic stroke. Notably, recurrent strokes result in higher mortality than first-time strokes. Stroke elicits multiple neuroimmune reactions, some of which may have Janus-faced effects. Though promoting neurorepair, neuroinflammation might also be detrimental: Infiltrating B-cells produce antibodies that supposedly lead to neurodegeneration and subsequent cognitive decline. Activated microglia and infiltrating lymphocytes maintain the chronic inflammation. However, how a second stroke promotes this chronic neuroinflammation and how neuroinflammation affects stroke outcomes after the second stroke is unclear. This study aimed to immunophenotype brain tissue in a mouse model of recurrent stroke.

Methods: Male and female mice of 12-week age underwent 30-minute middle cerebral artery occlusion (MCAo) followed by reperfusion. They were subjected to permanent distal middle cerebral artery occlusion (dMCAo) 14 days after the first surgery to model a recurrent stroke. Sham surgeries were conducted following the procedures for MCAo and dMCAo, but without occlusion of the respective arteries. To explore the effects of each surgery, four groups of animals were investigated: MCAo/dMCAo, MCAo/sham, sham/dMCAo, and sham/sham. T2-weighted MRI and incidence maps assessed the lesion size 24 hours after the interventions. To mark the cells that proliferate after the first or second strokes, mice received nucleotide analogues BrdU or EdU, respectively. The brains were studied by immunofluorescent staining and spectral flow cytometry analysis 35 days after the second stroke.

Results: We successfully established the recurrent stroke model in mice, primarily affecting the striatal area with 30-minute MCAo and limiting the dMCAO-induced lesion to the somatosensory cortex. Histological analysis of brain tissue revealed atrophy of the affected hemisphere and cell proliferation in both lesion areas. Spectral flow cytometry showed an increase in immune cell infiltration in the ischemic hemisphere and detected populations of helper and cytotoxic T cells, B cells, plasma cells, and border-associated macrophages.
The model can be used in further preclinical studies to evaluate the pathophysiology of immune response in recurrent stroke.

The role of the meninges in autoimmune CNS inflammation

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Multiple Sclerosis (MS) is an autoimmune disease marked by recurrent acute inflammatory episodes targeting the central nervous system (CNS), leading to either full recovery or permanent neurological deficits, significantly impacting patients' quality of life. The mechanisms dictating these divergent outcomes remain not well understood. We propose that the meninges—thin membranes encasing the CNS—play a pivotal role in shaping the consequences of these episodes. The meninges consist of the pia mater and arachnoid (termed leptomeninges) and the dura mater. The pia mater is vascularized and separated from the arachnoid by the cerebrospinal fluid-filled subarachnoid space. The avascular arachnoid connects to the outer dura mater, which has a vascular bed, innervation, and lymphatic vessels linked to cervical lymph nodes.

In experimental MS models, meninges serve as the entry port for immune cells into the CNS. Here, autoreactive T cells are activated by local antigen-presenting cells, which are crucial for disease onset. The meninges and subarachnoid space can also act as a microenvironment where inflammatory cells persist, proliferate, and form lymphoid structures, potentially damaging CNS tissue.

Recently, we analyzed the distinct contribution of the different meningeal layers to the immune process in MS. Using mouse and rat models of MS (experimental autoimmune encephalomyelitis, EAE), we discovered that the leptomeninges were massively invaded by immune cells in every phase of the disease, while the dura was largely excluded from the inflammatory process.

This was unexpected, since the dura is immunologically competent due to its dense and fenestrated vascularization, lymphatic network and varied immune cell populations. Two features of the leptomeninges could explain this discrepancy. First, leptomeningeal vessels, unlike those in the dura, express adhesion molecules necessary for T cells to exit circulation and enter tissue. Second, CNS proteins, which trigger inflammation in MS, are largely confined to the CNS parenchyma and leptomeninges, with minimal exposure to the dura, allowing only leptomeningeal antigen-presenting cells to activate autoreactive T cells and initiate inflammation.

Since meningeal infiltrates are observed in progressive MS and correlate with disability progression, we also investigated the role of meningeal layers in chronic autoimmunity by inducing repeated bouts of EAE. Even after a single inflammatory event, the leptomeninges showed incomplete recovery, with tissue thickening and immune infiltration. These changes became more pronounced after multiple episodes, confirming the leptomeninges as a critical site for chronic inflammation. Consistently, RNA sequencing revealed lasting alterations in leptomeningeal tissue months post-inflammation, while the dura remained unaffected. Corroborating these findings, human samples from chronic MS patients displayed significant cellular infiltration in the leptomeninges but not in the dura.

Our findings highlight the leptomeninges as a central checkpoint in MS activation and progression, suggesting therapies should target this region to regulate immune cell access and reactivation. Future work will clarify the cellular and molecular mechanisms governing immune recruitment and persistence in the leptomeninges and their impact on CNS integrity.

Adjuvant-free experimental autoimmune encephalomyelitis as a model to study brain inflammation and neurodegeneration

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Experimental autoimmune encephalomyelitis (EAE) is a valuable animal model for studying central nervous system autoimmunity. Complete Freund's adjuvant (CFA), routinely used for the induction of EAE, is an obstacle in translation of knowledge obtained in this animal model towards understanding multiple sclerosis pathogenesis and therapeutic options. To overcome this, we have recently developed EAE induced in Dark Agouti rats with spinal cord homogenate (SCH) without CFA. As a consequence of SCH immunization heterogeneous clinical outcomes are observed in Dark Agouti rats. According to the different clinical courses of EAE, rats can be grouped into one of four categories: mild, moderate, severe and lethal EAE. The moderate and the severe clinical course are in the focus of our investigation, as they parallel relapsing-remitting and chronic form of multiple sclerosis. So far, we have been able to identify major cellular players, and we are currently working on deciphering molecular mechanisms, involved in the divergence. Further, infiltration of immune cells into various brain compartments, including pons, cerebellum, hippocampus, and cortex demonstrate was observed in SCH-immunized rats. Here, possibilities to use this model to study brain inflammation and neurodegeneration will be discussed.

Symposium

S35: New perspectives on the locus coeruleus - noradrenergic activity during sleep and its role in memory function

S35-1 Unraveling the role of sleep in vocal learning
Artemis Gkinakou

S35-2 The activity dynamics of the Locus Coeruleus noradrenergic neurons during sleep and its role in systems-level memory consolidation
Oxana Eschenko

S35-3 Locus coeruleus Activity Fluctuations in Mouse Non-REM Sleep:
Coordinators of Brain and Peripheral Rhythms, Gatekeepers of the Non-REM-REM Sleep Cycle, Culprits of Sleep Disruptions
Anita Lüthi, Georgios Foustoukos, Alejandro Osorio-Forero, Romain Cardis, Najma Cherrad, Laura Fernandez

S35-4 The role of norepinephrine-driven sleep microstructure on cognitive performance in health and disease
Celia Kjaerby, Mie Andersen, Viviane Compete, Klaudia Tokarska, Yi Qian

S35-5 Of Mice and Men: Autonomic activity that support Memory Consolidation During Sleep
Sofie Smith Jacobsen, Sara Mednick

Unraveling the role of sleep in vocal learning

Artemis Gkinakou

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As a juvenile bird learns to sing, it must undergo a complex memory task that involves the formation of auditory memories, sequences of motor output, and associative higher-order representations of learned vocalizations (Margoliash & Schmidt, 2010). As soon as a juvenile bird is exposed to the song of another male bird (usually its father, the “tutor”), the young bird begins to imitate aspects of those songs in squeaky and noisy subsongs, which are often compared to the babbling of human babies (Aronov et al., 2008; Brainard & Doupe, 2000). Through a process of auditory feedback and motor learning, juvenile subsongs transition from acoustically simple songs to complex and stereotypical adult songs in a process known as crystallization.

How do neural circuits change to incorporate these newly learned events? Numerous studies in mammals have shown that offline periods like sleep might provide a ideal state to facilitate the reactivation and consolidation of recent events in the absence of new sensory input (Maquet, 2001).
Could sleep serve a similar function during vocal learning in songbirds? Indeed, compelling behavioral (Brawn et al., 2013; Derégnaucourt et al., 2005), electrophysiological (Dave & Margoliash, 2000; Elmaleh et al., 2021; Rauske et al., 2010; Shank & Margoliash, 2009), and molecular (Phan et al., 2006) evidence indicates that sleep is crucially involved in vocal learning.

In this work, we investigated brain activity during natural sleep and singing behavior as juvenile birds transitioned from variable subsong to crystallized songs. We used advanced clustering algorithms to track the spectral features of song syllables over the course of learning. We found that song syllables that changed substantially over the course of learning deteriorated overnight during sleep and were subsequently practiced and improved over the next days of singing. In contrast, syllables that did not change substantially over the course of learning were improved and consolidated overnight. We found that this vocal behavior was correlated with changes in the durations of electrophysiological sleep states during the night. Overall, our results provide the first mechanistic insight into the interplay between sleep and learning in songbirds.

The activity dynamics of the Locus Coeruleus noradrenergic neurons during sleep and its role in systems-level memory consolidation

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The cortical slow oscillations, thalamocortical sleep spindles, and hippocampal sharp-wave ripples have been causally implicated in sleep-dependent memory consolidation. Our electrophysiological recordings in the LC during natural sleep in rats revealed a coordinated firing of the LC-NA neurons with these forebrain oscillations. Our results have been supported by the dynamics of NA release, which has been recently characterized in several forebrain regions at a fine temporal scale using genetically encoded sensors. These observations combined with the emerging view on the complexity of the structural and functional organization of the LC-NA system renewed interest in its previously overlooked role during 'offline' states, including sleep. Earlier behavioral pharmacology studies in humans and animals highlighted the importance of noradrenergic transmission during post-encoding periods. While the faciliatory role of the NA for synaptic plasticity is well-established, only recently has LC-NA activity been considered to play a role in systems-level memory consolidation. Our recent work provided further insights into the mechanisms underlying the role of the LC-NA system in facilitating the reactivation of memory-encoding neural representations and their integration into the neocortex for long-term storage.

Locus coeruleus Activity Fluctuations in Mouse Non-REM Sleep:

Coordinators of Brain and Peripheral Rhythms, Gatekeepers of the Non-REM-REM Sleep Cycle, Culprits of Sleep Disruptions

Anita Lüthi[1], Georgios Foustoukos[1], Alejandro Osorio-Forero[2], Romain Cardis[1], Najma Cherrad[1], Laura Fernandez[1]

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Sleep leaves us still, disengaged from our surroundings, focused inward. I will argue that this common view on sleep needs to be revised because neurochemicals that promote wakefulness, such as noradrenaline, are released at high levels in the sleeping brain. In sleeping mouse, noradrenaline (NA) determines how sleep states follow each other, arousability levels, and autonomic output. Furthermore, NA release in sleep is altered by preceding wake experience, which implies it in sleep disruptions caused by the challenges of daily life.

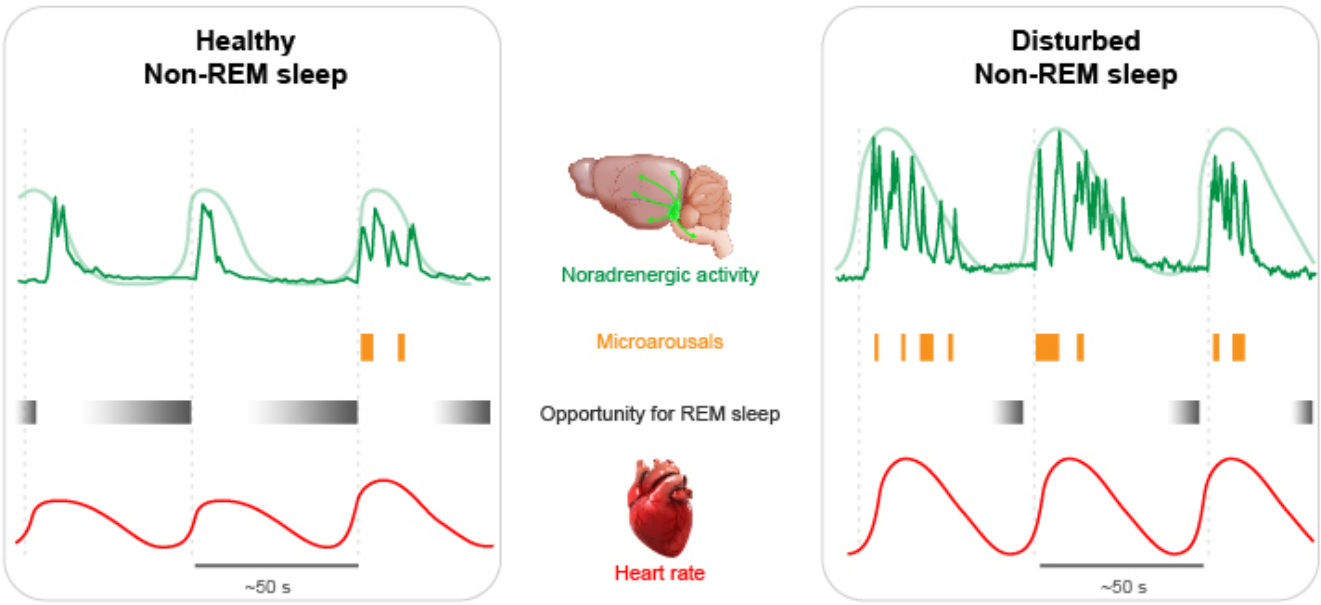
I will start by presenting a subtle but remarkably persistent fluctuating spectral activity pattern of non-rapid-eye-movement (non-REM) sleep that makes the healthy sleeping mouse more susceptible to spontaneous and evoked sensory disruption on an infraslow, 50-second timescale (1). This previously undescribed pattern is qualitatively the same in mice and men and it is correlated with fluctuations in the heart rate and pupil diameter. By in-house developed closed-loop optogenetic interference techniques in combination with sleep monitoring and genetically encoded calcium and neurotransmitter biosensors, and through cellular recordings, we identified neuronal activity of the noradrenergic locus coeruleus (LC), and release of NA from the LC fibers projecting to the sensory thalamus, to underlie these fluctuations (2) (Figure, left).

Aside regulating susceptibility to sensory stimuli, the surges and troughs of LC activity partition non-REM sleep into two functionally different brain states. Depending on their size, LC surges lead to partial arousals involving subcortical brain areas, or brief microarousals involving cortex (3). Conversely, during the troughs, transitions to REM sleep are made possible (3). The infraslow LC activity fluctuations act thus as gatekeepers of the ultradian non-REM-REM sleep cycle. On-going work shows that infraslow LC activity fluctuations in non-REM sleep may be multiply regulated, both by external and internal signals. For example, a brief period of stimulus-enriched wakefulness strengthens LC surges, disrupts sleep through microarousals, and prevents REM sleep (Figure, right). More evidence on regulation by internal signals, notably through vagal sensory afferents, will also be presented.

1. Lecci S, Fernandez LM, Weber FD, Cardis R, Chatton JY, Born J, Lüthi A (2017) Coordinated infraslow neural and cardiac oscillations mark fragility and offline periods in mammalian sleep. Science Adv 3(2):e1602026.

2. Osorio-Forero A, Cardis R, Vantomme G, Guillaume-Gentil A, Katsioudi G, Devenoges C, Fernandez LMJ, Lüthi A (2021) Noradrenergic circuit control of non-REM sleep substates. Curr Biol 31, 5009-5023.e7.

3. Osorio-Forero A, Foustoukos G, Cardis R, Cherrad N, Devenoges C, Fernandez LMJ, Lüthi A (2024) Infraslow noradrenergic locus coeruleus activity are gatekeepers of the NREM-REM sleep cycle. Nature Neuroscience, in press



Infraslow LC activity dynamics and associated sleep signatures in healthy and disturbed sleep

The role of norepinephrine-driven sleep microstructure on cognitive performance in health and disease

Celia Kjaerby[1], Mie Andersen[1], Viviane Compete[1], Klaudia Tokarska[1], Yi Qian[1]

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Sleep is essential for cognitive performance and it is well established that impaired sleep reduces cognition. Sleep is not a homogeneous brain state, but composed of several micro-structures regulated by many regions within the brain. In recent years, short arousals have been recognized as an integral part of normal sleep adding to the complexity of sleep. We recently demonstrated a slow rhythmic activity of the locus coeruleus during NREM sleep that creates subdomains of NREM sleep enriched in spindles. The level of spindle density in these arousal-driven subdomains are determining memory consolidation. We are currently exploring how the locus coeruleus rhythm is affected in aging and how we might use sleep-targeted optogenetic modulation of locus coeruleus to improve cognition.

Of Mice and Men: Autonomic activity that support Memory Consolidation During Sleep

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Sofie Jacobsen will present research from Prof. Sara C. Mednick's human sleep lab that focuses on the coupling of autonomic/central physiological events in human sleep and their impact on cognition. These studies reveal that the autonomic and central nervous systems interact in both coordinated and complementary ways that benefit human memory consolidation. First, we will examine autonomic/central events or 'ACE', where increased slow oscillation (0-1Hz) and spindle (12-15Hz) activity that is grouped by a burst in heart rate is associated with improved long-term and working memory. Additionally, the coupling of autonomic/central physiological events also work in competitive and antagonistic ways that can lead to a performance trade-off with enhancements in one cognitive domain at the expense of another domain. These principles are seen in connection with the Slow Oscillation Switch Model, where slow oscillations coupled with high frequency heart rate variability is linked to improved working memory. However, spindle and slow oscillation coupling links to increases in long term memory but at the cost of working memory. Given the recent findings of locus coeruleus (LC) phasic activity as a gating mechanism for spindles, and that the link between LC-driven changes in spindle activity and memory improvement has been found cross-species, these findings build a mechanistic bridge between animal and human studies by emphasizing the role of LC for sleep-mediated memory consolidation.

Symposium

S36: Neuronal representation of space, directions and goals in insects and vertebrates

S36-1 Neural representation of space: From compass coding to spatial goal coding in insects
M. Jerome Beetz

S36-2 Neural circuit mechanisms for working memory and evidence integration during olfactory navigation
Katherine Nagel

S36-3 How larval zebrafish orient and move in space
Ruben Portugues

S36-4 Evaluation of CA3 place cell remapping in the APP/PS1 model mouse of Alzheimer's Disease
Eva Maria Robles Hernandez

S36-5 Origin of inhibitory tuning in the rodent head-direction cortex
Adrian J. Duszkiewicz

Neural representation of space: From compass coding to spatial goal coding in insects

M. Jerome Beetz[1]

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Each actively moving animal needs a sense of orientation. Whether it is a bat using a biosonar for short-range orientation or monarch butterflies that use a sun compass to keep their direction for thousands of kilometres during their migration to their overwintering site, each individual must know where to go. This information could be a goal direction, as it is the case for the monarch butterfly, or it could be an actual goal location. Unlike compass coding, the neuronal substrates of spatial goal coding are less understood in insects. In this talk, I will present neuronal data which I monitored in tethered flying monarch butterflies that were free to steer with respect to a compass cue. By changing the butterfly's goal direction, I was able to characterize goal direction neurons in the insect brain (Beetz et al. 2023; DOI: 10.1038/s41467-023-41526-w). While monarch butterflies are ideal model organisms to study directional coding, they may be less suited to study the representation of goal locations because olfactory cues at the migratory goal are thought to stop their migration. To study place coding in insects, I recently shift my research focus on bees, which exploit patches of flowers in a fixed order on a daily basis. Mastering such a complex navigational behavior requires a sophisticated memory of the spatial goals. With neural recordings from freely walking honeybees that forage in a circular arena, we started to study place and spatial goal coding in the insect brain.

Neural circuit mechanisms for working memory and evidence integration during olfactory navigation

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To navigate towards an unknown food source, animals must accumulate evidence about the location of a goal and store this information in working memory. Here we identify a population of local neurons in the fan-shaped body of Drosophila that exhibits both evidence integration and working memory dynamics. We developed a closed-loop virtual odor navigation paradigm that allows us to image neural activity during navigation of a simulated turbulent plume. Using this paradigm, we observe that bump activity in a population of fan-shaped body local neurons gradually ramps up with stochastic odor encounters, and can persist for seconds after odor offset. While activity persists, we find that flies maintain the goal trajectory adopted during odor, suggesting that this signal represents a directional working memory. Silencing of this population reduces the persistence of upwind heading in response to odor, arguing for a causal role of these neurons in directional memory. To determine the circuit and cellular mechanisms underlying these dynamics, we are employing connectomics, whole-cell electrophysiology, 2-photon imaging, and computational modeling. Our current results suggest that both recurrent connectivity and slow signaling mediated by GPCRs interact to produce a robust and tuneable working memory signal. Our work reveals the dynamics of neural circuits that compute goals in a stochastic natural environment, and illustrates how the tools of Drosophila can be used to illuminate cellular and circuit mechanisms supporting cognitive computations.

How larval zebrafish orient and move in space

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Animals need to move in their environment in order to survive, and generating an internal representation of this interaction can be highly advantageous. It has been shown that some vertebrate and invertebrate nervous systems can encode the direction the animal is heading in. These heading direction networks are supported by persistent activity, need to be anchored to the external sensory world and can influence behavior.
In this talk I will present efforts from the lab to understand the heading direction network in larval zebrafish that we recently discovered. The tools available in this model organism, coupled with its small size, give us hope of a thorough mechanistic understanding of this cognitive process in this small vertebrate.

Evaluation of CA3 place cell remapping in the APP/PS1 model mouse of Alzheimer's Disease

Eva Maria Robles Hernandez

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Spatial navigation impairments are among the earliest clinical manifestations of Alzheimer's Disease (AD). Pyramidal cells of the hippocampus fire selectively when the animal is in a specific location in the environment, leading to the theory that the hippocampus plays a crucial role in forming a cognitive map of the environment. The phenomenon of "remapping", where specific cells exhibit selective firing in distinct environments, is thought to support the formation of different memories. By performing in-vivo electrophysiological recordings in freely moving mice while they navigate through different environments, we characterized 1) if the remapping of different hippocampal place cells (CA1, CA3, DG) is altered in the APP/PS1 mouse model of AD, a model known for spatial navigation deficits; 2) the potential involvement of CA3 interneurons in the early hyperexcitability of the CA3 network, a feature shared by both AD patients and the APP/PS1 mouse model. While interneuron firing rates and place cell remapping are mostly
maintained in the CA1 cells during the early phases of plaque deposition, we found several alterations that are present in the CA3 cells. By investigating the interplay between CA3 place cell remapping and the role of interneurons, our research contributes to a deeper understanding of the neurophysiological changes associated with spatial navigation impairments in AD. This knowledge may pave the way for novel therapeutic approaches targeting specific alterations in the hippocampal network.

Origin of inhibitory tuning in the rodent head-direction cortex

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Local inhibition is critical for the maintenance of high-fidelity tuning across many cortical areas. Furthermore, excitatory-inhibitory recurrent connectivity is thought to support attractor dynamics in many of the brain's spatial navigation systems. However, while the firing patterns of inhibitory interneurons in these areas are known to be spatially non-uniform, not much is known about the principles governing their spatial tuning. To investigate it, we surveyed the spatial tuning properties of putative fast-spiking interneurons (FS cells) in the cortical head-direction (HD) system – the most fundamental of the brain's allocentric spatial representation systems.

To this end, we used 64-channel linear electrode arrays to conduct population recordings in postsubiculum (PoSub) of freely moving mice (n = 32), and recorded assemblies of up to 174 PoSub cells (n = 2930 total cells). We first established that the firing rate of PoSub-FS cells (n = 427) varied by an average of 50% as a function of animal's HD and that this HD tuning was stable both within and across exploration sessions and followed rotation of a distal visual landmark to the same degree as PoSub-HD cells.

In order to compare the tuning fidelity of PoSub-FS and PoSub-HD cell populations, we utilized Fourier transform to decompose individual tuning curves into their spectral components. Importantly, we found that while tuning curves of PoSub-HD and PoSub-FS cells differed in their shape, the average Fourier spectrum of PoSub-FS cell tuning was virtually indistinguishable from that of PoSub-HD cells but different from that of the upstream thalamic HD cells, suggesting that tuning of PoSub-FS cells has a local and not thalamic origin. Two observations corroborated this hypothesis. First, optogenetic modulation of thalamic gain indicated that PoSub-FS cell tuning was independent of upstream thalamic inputs. Second, PoSub-FS cell tuning was tightly coupled to the local PoSub-HD cell activity even during sleep, when sensory inputs are dampened.

Together, these findings provide evidence that the resolution of neuronal tuning is an intrinsic property of local cortical networks, shared by both excitatory and inhibitory cell populations.

Poster Topics

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 Beneficial effects of voluntary running upon adult neurogenesis depends on the levels of available BDNF in the brain
Monique Klausch, Viola von Bohlen und Halbach, Oliver von Bohlen und Halbach

T1-2A Elucidating SYNGAP1 Isoform Functions in Human Neurodevelopment Using Cerebral Organoids
Ivanna Kupryianchyk-Schultz, Daniel Bauersachs, Ralf Kühn, Manuel Irimia, Sarah Shoichet, Agnieszka Rybak-Wolf

T1-3A High-throughput knockdown screening for modifiers of neuronal morphology in patient-derived neurons
Selene Lickfett, Carmen Menacho, Markus Schülke, Andrea Rossi, Sidney Cambridge, Alessandro Prigione

T1-1B Adult neurogenesis in the mouse vomeronasal organ
Lena Terlau

T1-2B Systematic analysis of the transcriptome and proteome of human iPSCs during differentiation into cortical neurons
Shreejoy Tripathy

T1-3B BAF Complex Modulates MGE-Derived GABAergic neuron Development
Xiaoyi Mao, Eman Abbas, M Sadman Sakib, Pauline Antonie Ulmke, Tonatiuh Pena Centeno, Linh Pham, Joachim Rosenbusch, Jochen F. Staiger, Andre Fischer, Huu Phuc Nguyen, Tran Tuoc

T1-1C Molecular mapping of the neuroectoderm across phyla – conservation and divergence of brain regions between insects and vertebrates
Gregor Bucher, Nico Posnien, Vera Hunnekuhl

T1-2C Olfactory neuron regeneration in adult *Drosophila*
Ismael Fernández-Hernández, Eric Hu, Michael A. Bonaguidi, André Fiala

T1-1D C-terminal binding protein 1 is required for adult hippocampal neurogenesis
Burcu Sucu, Neeraja Suresh, Nader Mounzer, Bartomeu Perelló-Amorós, Renato Frischknecht, Anna Fejtová

T1-2D The role of MAST3 in neurodevelopment and disease
Carolina Rodrigues Duro, David Anthony Keays

T1-3D *In vitro* models to explore mechanisms of hypoxia resistance in the naked mole-rat
Paraskevi Kakouri, Alice Rossi, Oscar Sanchez-Carranza, Severine Kunz, Gary R. Lewin

Beneficial effects of voluntary running upon adult neurogenesis depends on the levels of available BDNF in the brain

Monique Klausch[1], Viola von Bohlen und Halbach[1], Oliver von Bohlen und Halbach[1]

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Brain-derived neurotrophic factor (BDNF), as a member of neurotrophin family, has neuroprotective and neurotrophic properties. BDNF has crucial roles during development, but also plays roles in the postnatal brain, especially in functions that are related to neuronal plasticity, learning and memory. Within the hippocampus the phenomenon of adult hippocampal neurogenesis can be observed. This form of adult neurogenesis is linked to learning and memory. Reduced levels of BDNF are known to impair memory functions.

Studies using different mouse models with reduced expression of BDNF revealed that reduced levels of BDNF seemed not to affect cell proliferation in the dentate gyrus (DG), but might affect survival and differentiation of newly borned cells into young neurons. Interestingly, the rate of adult hippocampal neurogenesis can be increased by enriched environment, especially by running. Running, among others, is capable of increasing BDNF-levels in the hippocampus. Thus, it might be possible that either running can rescue the effects of BDNF on adult hippocampal neurogenesis or that normal BDNF levels are essential for the running-induced increase in adult hippocampal neurogenesis. For getting insight into this topic, we analyzed different mouse models of BDNF-deficiency; mice with a deletion of BDNF only in projection neurons (conditional BDNF), heterozygous BDNF deficient mice (BDNF +/-), and heterozygous BDNF deficient mice with an additional deletion of BDNF in projection neurons in the CNS (double knockouts). All of the three BDNF mouse models survive into adulthood and were subjected to a voluntary wheel running program. As compared to heterozygous BDNF knockout and wildtype mice, the conditional knockout mice display reduced running distances and times and, unexpectedly an increase in their body weight. Concerning the effect of the running paradigm on adult neurogenesis, the different BDNF deficient mice display different levels of increased adult neurogenesis. However, the double knockouts failed to increase their rate of adult neurogenesis as compared to the levels observed in the other groups. This may indicate that low levels of BDNF are required for modulating the rate of adult hippocampal neurogenesis and that running is capable of increasing adult hippocampal neurogenesis.

Elucidating SYNGAP1 Isoform Functions in Human Neurodevelopment Using Cerebral Organoids

Ivanna Kupryianchyk-Schultz[1,2], Daniel Bauersachs[3], Ralf Kühn[3], Manuel Irimia[4], Sarah Shoichet[2], Agnieszka Rybak-Wolf[1]

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SYNGAP1, encoding Ras/Rap GTPase-activating protein, is a critical gene involved in synaptic signaling and neurodevelopment. Mutations in SYNGAP1 are associated with intellectual disability and autism spectrum disorder (ASD). However, the specific functions of its multiple isoforms, generated by alternative splicing and transcription start sites, remain poorly understood. Current methods for studying SYNGAP1 functions rely on animal models, which do not fully recapitulate human neurodevelopment. This project aims to elucidate the roles of different SYNGAP1 isoforms using human cerebral organoids as an alternative to animal models. Cerebral organoids, derived from pluripotent stem cells, offer a promising 3R approach by mimicking the human brain architecture and functionality. We will employ cutting-edge techniques including single-cell RNA sequencing, CRISPR-based gene perturbation, and BaseScope in situ hybridization to comprehensively profile SYNGAP1 isoform expression, manipulate their levels, and visualize their spatiotemporal distribution across different developmental timepoints: 15, 30, 60 days, roughly equivalent to 4, 10, 18 post-conceptional weeks of human development in vivo. By developing this innovative 3D model system, we aim to uncover isoform-specific roles of SYNGAP1 in human brain development. Our approach will not only advance mechanistic understanding of SYNGAP1 biology, but also exemplify how cerebral organoids can serve as a powerful alternative to animal use. This project showcases the potential of human cerebral organoids to replace animal experiments in studying neurodevelopmental disorders, aligning with the 3R principles (Replacement, Reduction, and Refinement). We will provide updates on the progress of this work.

High-throughput knockdown screening for modifiers of neuronal morphology in patient-derived neurons

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Leigh syndrome (LS) is a rare, incurable mitochondrial disease that results in intellectual disabilities and movement defects in children and is caused by mutations in genes that encode components of the mitochondrial respiratory chain. A particular locus of interest is mitochondrial complex IV assembly factor SURF1.
SURF1-deficient animals failed to recapitulate the neuronal pathology of human LS, commonly presented by neurodegeneration in the midbrain and basal ganglia, hindering the discovery of treatments. Therefore, we elected to utilise human models in the form of LS patient-derived induced pluripotent stem cells (iPSCs). These are then employed to generate neurons and brain organoids for modelling the disease, and we have identified an impaired capacity for neuronal outgrowth.
We therefore developed a high-content analysis approach for automatic quantification of neuronal outgrowth and branching. Next, we incorporated a genetic knockdown (KD) step into the established pipeline. For this, we devised a novel transfection protocol that enables efficient short interfering RNA (siRNA)-based knockdown in human neurons. Control iPSC lines and an isogenic line carrying mutant *SURF1* were engineered to express the transcription factor *Neurogenin 2*, enabling the rapid generation of pure inducible neurons (iNs) upon doxycycline exposure. A proof-of-concept siRNA KD screen was first performed in control iNs to confirm that the approach is capable of identifying known modulators of neuronal morphology.
Subsequently, a comprehensive siRNA KD screen will be conducted in LS iNs, focusing on the 'druggable genome', comprising approximately 800 genes that are known targets of FDA-approved medical drugs. For candidate genes whose siRNA knockdown could enhance the neuronal outgrowth capacity of LS iNs, we intend to evaluate the corresponding FDA-approved compounds using LS brain organoids, which we know exhibit defects in cellular organization and function.
In conclusion, our innovative KD screening approach has the potential to identify novel modifiers of neuronal outgrowth and branching and respective drugs that may be considered for repositioning to treat children affected by LS but also other diseases.

Adult neurogenesis in the mouse vomeronasal organ

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Neuronal turnover in olfactory epithelia plays a pivotal role in an animal's ongoing adaption to its environment. Thus, adult mammalian neurogenesis in olfactory epithelia persists throughout life. However, the precise physiological processes that govern adult neurogenesis in the vomeronasal organ (VNO) remain elusive. Here, we begin to characterize adult neurogenesis in the mouse vomeronasal sensory epithelium. We label newly generated vomeronasal sensory neurons (VSNs) by using a novel genetic approach: upon tamoxifen injection, VSN progenitor cells and immature neurons in *Id2CreER^{T2} :: Rosa26R-tdTomato* mice express tdTomato upon coincident *Id2* promoter activity. Descendants of these cells are thus labeled by red fluorescence. Using the *Id2* proliferation and differentiation marker as a VSN lineage tracer, we describe the proportion of newborn neurons within the VSN population. We identify the spatial distribution of individual newborn neurons along with their age-dependent migration patterns within the sensory epithelium. Furthermore, our results provide first insights into the turnover rate and lifespan of VSNs. Finally, by analysing marker protein expression in tdTomato-positive cells, we evaluate the differentiation and maturation state of newborn neurons at defined time points post tamoxifen injection.

Systematic analysis of the transcriptome and proteome of human iPSCs during differentiation into cortical neurons

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The differentiation of human induced pluripotent stem cells (iPSCs) into cortical neurons is a highly dynamic process, accompanied by significant changes in both the transcriptome and proteome. Here, we systematically characterize these changes using an integrated multi-omics approach, combining long- and short-read RNA sequencing with proteomics across three critical time points during differentiation. Our analysis revealed an expanded transcriptome complexity, including numerous novel isoforms and exons, validated through both splice junction mapping and peptide-supported proteomics data. We also assessed the proteome complexity by evaluating the protein-coding potential of these novel isoforms, identifying several previously unannotated open reading frames.

Further, we explored isoform switching events, revealing shifts in transcript usage and alternative polyadenylation that have implications for mRNA stability and translational regulation. Gene ontology and pathway enrichment analyses highlighted the functional consequences of these isoform changes, including their potential roles in cortical neuron development. We also investigated the correlation between mRNA and protein expression, identifying features such as untranslated region (UTR) lengths and non-sense-mediated decay (NMD) sensitivity that contribute to discrepancies between transcript and protein abundance. Finally, we integrated our findings with autism spectrum disorder (ASD) genetics, identifying overlaps between ASD-associated variants and novel transcript features, suggesting potential new mechanisms underlying neurodevelopmental disorders.

These findings provide a comprehensive resource for understanding the molecular events governing human cortical neuron differentiation and offer new insights into the genetic regulation of neuronal development and disease.

BAF Complex Modulates MGE-Derived GABAergic neuron Development

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The mammalian brain is capable of processing vast amounts of information, forming the basis for complex cognitive functions, emotional responses and behavior. The majority of these neurons are excitatory glutamatergic neurons, comprising about 80–85% of the cortical neuronal population. In contrast, inhibitory GABAergic (gamma-aminobutyric acid) neurons make up the remaining 15–20%. Despite their lower numbers, GABAergic neurons are highly diverse in terms of their morphology, electrophysiological properties and neurochemical profiles. The functional diversity of GABAergic neurons significantly amplifies the computing capabilities of inhibitory and disinhibitory motifs, allowing the inhibitory system to precisely regulate neural activity and adapt to different stimulus conditions. Consequently, dysfunctions within inhibitory circuits are strongly associated with neurodevelopmental disorders. Understanding the mechanisms of GABAergic neuron development is therefore crucial for developing therapies for conditions such as epilepsy, schizophrenia and autism.

GABAergic neurons can be classified based on their projection patterns. Local circuit neurons, referred as interneurons are the primary type found in the cerebral cortex. Conversely, GABAergic neurons with long-range projections are predominantly located in subcortical structures. Cortical and striatal GABAergic interneurons primarily derive from three transient ventral subpallial structures: the medial ganglionic eminence (MGE), the caudal ganglionic eminence (CGE) and the preoptic area (POA). The MGE is responsible for generating approximately 50–60% of cortical interneurons and the majority of striatal interneurons in mice, including those that express parvalbumin (PV) and somatostatin (SST). It is also a source of GABAergic prototypic neurons in the globus pallidus. Similar to the dorsal pallium, the germinative regions of the MGE also comprise a ventricular zone (VZ) and a subventricular zone (SVZ), each harboring distinct neural precursors or progenitors. Neurogenesis involves a series of tightly regulated processes. Several transcription factors (TF), such as DLX1/2, NKX2.1, LHX6 and SOX6 are crucial for the proper coordination of these steps in determining the neuronal output. Among them, NKX2.1 is particularly important for the precise specification of MGE-derived neurons, as well as for preserving the MGE’s identity by playing a role in inhibiting the development of neighboring cell types in the LGE and CGE.

In recent years, chromatin remodelers have emerged as key players in regulating neurodevelopment. They have the ability to reposition nucleosomes, thereby altering the accessibility of the DNA and influencing gene expression outcomes. Among these remodelers, the BAF chromatin remodeling

complex has been demonstrated to govern critical processes including the proliferation, differentiation and migration of cortical progenitors or post-mitotic neurons. De novo mutations in BAF complex contribute to certain cognitive and psychiatric disorders observed in humans such as Coffin-Siris Syndrome (CSS) and autism. Containing various subunits, BAF complex can assemble into diverse combinations, leading to customized complexes that cater to the specific requirements of various cells or tissues, thereby offering unique and specialized functions. In the dorsal telencephalon, the loss of BAF complex leads to a significantly thinner cortex attributed to increased apoptosis and disrupted cell-cycle progression of progenitors, in accompany with downregulation of genes essential for progenitor proliferation and differentiation. However, the role of BAF complex in regulating lineage progression programs that govern the production of GABAergic neurons remains unclear. The detailed mechanisms by which BAF complex interacts with aforementioned TFs are yet to be elucidated.

In this study, we first demonstrated the expression of the scaffolding subunits BAF155 and BAF170 in MGE-derived GABAergic lineages. Using a mouse model with conditional deletion of both BAF155 and BAF170 to abolish the function of BAF complex specifically in the MGE, we investigated how BAF complex influences GABAergic neurogenesis. Our results revealed that BAF complex exerted distinct regulatory effects across various cell types, as evidenced by cellular deconvolution of RNA-sequencing (RNA-seq) data and immunohistochemical analysis of BAF complex knockout brains. The ablation of BAF complex expanded the progenitor pool and induced dorsalization in the VZ. Moreover, cell death, deficits in proliferation and differentiation of cells in the SVZ and a fate switch towards adopting the characteristics of LGE-derived medium spiny neurons (MSN) upon loss of BAF complex greatly impeded the generation of GABAergic neurons. Furthermore, we observed defective neurite growth in MGE-derived cell cultures. The migration of GABAergic neuron was impaired, leading to a considerable number of cells failing to reach their cortical and subcortical destinations. Finally, our findings highlight that BAF complex regulates key genes crucial for GABAergic neuron differentiation at least partially through its impact on NKX2.1 expression and its interaction with NKX2.1. Thus, we are beginning to put together the puzzle of how BAF complex impacts the development of inhibitory neuronal lineages.

Molecular mapping of the neuroectoderm across phyla – conservation and divergence of brain regions between insects and vertebrates

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Gene expression has been employed for homologizing body regions across bilateria. The molecular comparison of vertebrate and fly brains has led to a number of disputed homology hypotheses. Data from the fly Drosophila melanogaster has recently been complemented by extensive data from the red flour beetle Tribolium castaneum with its more insect-typical development.

We revisit the molecular mapping of the neuroectoderm of insects and vertebrates to reconsider homology hypotheses. We claim that the protocerebrum is non-segmental and homologous to the vertebrate fore- and midbrain. The boundary between antennal and ocular regions correspond to the vertebrate mid-hindbrain boundary while the deutocerebrum represents the anterior-most ganglion with serial homology to the trunk. The insect head placode shares common embryonic origin with the vertebrate adenohypophyseal placode. Intriguingly, vertebrate eyes develop from a different region compared to the insect compound eyes calling organ homology into question. Finally, we suggest a molecular re-definition of the classic concepts of archi- and prosocerebrum.

Olfactory neuron regeneration in adult *Drosophila*

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Promoting adult neurogenesis has emerged as a potential intervention to rejuvenate neural circuits and restore associated functions in the aging brain. Establishing tractable models of adult neurogenesis can expedite the identification of compounds and their cellular mechanisms promoting regeneration of the aged nervous system. Expanding on our previous reports, here we present evidence for sustained neurogenesis in the adult *Drosophila* olfactory system. Particularly, by applying genetic tools and molecular techniques, we captured apoptosis and regeneration of Olfactory Sensory Neurons (OSN) in the antennae of adult flies over three weeks, indicating a sustained neuron turnover. Furthermore, automated segmentation and quantification of individual OSN reveals neuronal homeostasis over the same period, which is disrupted at later timepoints in an age-dependent manner. Finally, our ongoing efforts to leverage this model as a platform to identify compounds promoting regeneration of the aging nervous system will be presented. Ultimately, this approach has the potential to contribute to the development of regenerative therapies to treat otherwise irreversible neurodegenerative conditions.

C-terminal binding protein 1 is required for adult hippocampal neurogenesis

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The C-terminal binding protein 1 (CtBP1) is ubiquitously expressed, dual-function protein. In the nucleus, it nucleates corepressor complexes that control chromatin-modification and gene expression. In the cytoplasm, it regulates membrane trafficking processes by controlling the membrane fission reaction. Our previous study revealed an involvement of CtBP1 in the control of neural activity-regulated genes such as BDNF, Arc and Fos in the mature brain. The CtBP protein family has been proposed to regulate neuronal and glial differentiation. However, the role of CtBP1 in neurodevelopmental processes and in neurogenesis are still underexplored. Here, we investigated adult hippocampal neurogenesis in constitutive CtBP1 knock-out mice. Using BrdU pulse-chase experiments, we found a dramatic decrease in the number of newly generated neurons indicating an importance of CtBP1 in neurogenesis in adult hippocampus. To identify CtBP1-dependent expressional networks important for adult neurogenesis, we performed bulk RNA sequencing on neural progenitor cells (NPCs) that were derived from the neurogenic niches, hippocampal subgranular zone (SGZ) and subventricular zone (SVZ) of constitutive CtBP1 knock-out and wild-type mice, and cultured in vitro. We identified 41 significantly differentially expressed genes in SGZ and 647 in SVZ. GO analysis of these genes revealed significant enrichment for biological processes including NPC proliferation, migration and differentiation, as well as cell adhesion and cellular response to hypoxia. Multiple studies have shown that exercise changing the oxygen availability in the brain stimulates neurogenesis, promotes survival of adult born neurons and promotes plasticity. We asked whether exercise can improve hippocampal neurogenesis that we found reduced upon deletion of Ctbp1. Therefore, we investigated effect of voluntary wheel-running on adult hippocampal neurogenesis in constitutive CtBP1 mice. BrdU pulse-chase experiment showed an increased number of newly generated mature and immature neurons in running mice compared to non-running mice of both genotypes. However, we observed differential effect of running on proliferation of NPCs in CtBP1 compared to WT. Our findings highlight the importance of CtBP1 in the RGS proliferation, differentiation and maturation of newly born neurons, likely by impacting proliferation of early stages of neurogenic lineage and migration and adhesion capabilities of neuroblasts and immature neurons.

The role of MAST3 in neurodevelopment and disease

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The development of the human brain occurs early in human life and requires the coordination of key cellular events, including the generation, migration and differentiation of neurons. Genetic mutations that perturb these processes cause neurodevelopmental disorders, such as microcephaly, epilepsy, or autism spectrum disorders. Recent genetic studies have implicated MAST3 (microtubule-associated serine/threonine kinase 3) in a range of neurological conditions; however, the underlying molecular and cellular mechanisms are unknown. In this PhD project, I aim to remedy this deficit. To this end I have three goals. To: (1) Generate novel MAST3 mouse models that recapitulate disease causing mutations; (2) Undertake behavioral and anatomical characterization of these mouse lines; and (3) Investigate the molecular mechanisms underlying
MAST3-disease related phenotypes and unravel the function of this protein during brain development. Collectively, my doctorate will shed light on how MAST3 contributes to neurodevelopment and will provide insight into the pathophysiological mechanisms associated with MAST3 mutations.

In vitro models to explore mechanisms of hypoxia resistance in the naked mole-rat

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Naked mole-rats are eusocial animals living in uncommonly hypoxic and hypercapnic environment. To counteract this adverse habitat, they have evolved unique metabolic adaptations that allow them to completely recover from prolonged oxygen deprivation, without any organ impairment – an ability that sets them apart from other terrestrial mammals, including mice. The brain, being one of the most energy-demanding tissues, typically suffers rapid and severe damage in the absence of oxygen. To elucidate the molecular mechanisms that allow the naked mole-rats to survive bouts of hypoxia without any brain injury, we developed a novel in vitro model by differentiating naked mole-rat fibroblasts into neurons (induced Neurons, iNeurons) and establishing naked mole-rat astrocyte cultures. When exposed to extreme hypoxia, both naked mole-rat iNeurons and astrocytes demonstrated greater survival than their mouse counterparts, reaffirming a remarkable hypoxia resistance at the cellular level, that was consistent with our previous in vivo observations (Park, Reznick et al., 2017). Transcriptomic and proteomic analyses will be carried out in order to identify species-specific genes and pathways involved in hypoxia resilience. Given that mitochondria, as bioenergetic organelles, are crucial for maintaining most of the brain functions and are particularly vulnerable to oxygen deprivation, we will use Trasmission Electron Microscopy (TEM) and Focused Ion Beam Scanning Electron Microscopy (FIB-SEM), to investigate mitochondrial functionality and dynamics of both naked mole-rat and mouse iNeurons under normoxic and hypoxic conditions. Our purpose is to identify neuron-specific mitochondrial features that may further explain this peculiar trait of naked mole-rats. The results obtained will contribute to novel therapeutic approaches for conditions characterized by severe oxygen deprivation, such as stroke and organ transplantations.

Poster Topic

T2: Axon and Dendrite Development, Synaptogenesis

T2-1A Input synapse distribution on the dendrites of an ensemble of five *Drosophila* flight motoneurons
Lion Huthmacher, Carsten Duch

T2-2A Basal forebrain cholinergic innervation of the visual cortex during postnatal development in ChAT-cre transgenic mice
Jude Ijuo Abeje, David Cabrera-Garcia, Christian Lohmann

T2-3A A Comparative Study of Neuronal Architecture in the Caudate Nucleus: Insights from Camels and Humans
Sami Zaqout, Juman Almasaad, Ziad Bataineh

T2-1B 3D mapping of parvalbumin interneuron-derived cortico-striatal axonal projections
Hadiseh Hosseinnia, Maria Lehning, Andrew Octavian Sasmita, Clarissa Menschel, Patrick Spisse, Robert Fledrich, Kristina Lippmann, Markus Morawski, Ruth M. Stassart, Markus H. Schwab

T2-2B Spectraplakin interacts with MTOCs to organize dendritic microtubules
Sebastian Rumpf, Matthew Davies, Neeraja Sanal, Ulrike Gigengack, Ines Hahn

T2-3B H-Ras induces exuberant *de novo* dendritic protrusion growth in mature neurons regardless of cell type
Sarah Krüssel, Ishana Deb, Seungkyu Son, Gabrielle Ewall, Minhyeok Chang, Hey-Kyoung Lee, Won Do Heo, Hyung-Bae Kwon

T2-4B Cyclase-associated protein: an actin regulator with multiple neuronal functions
Marco Rust, Sharof Khudayberdiev, Cara Schuldt, Anika Heinze, Felix Schneider

T2-1C Dynamic structural plasticity determines developmental maturation of the cochlear inner hair cell ribbon synapse
Roos Anouk Voorn, Noboru Komiyama, Vladan Rankovic, Seth Grant, Christian Vogl

T2-2C The Methylation-Independent Role of the DNA Methyltransferase 1 on Neuronal Development and Intracellular Trafficking
Georg Pitschelatow, Cathrin Bayer, Philip Wolff, Jana Egner-Walter, Claudia Palacios, Christoph Hamacher, Ke Zuo, Mineko Kengaku, Paolo Carloni, Marc Spehr, Geraldine Zimmer-Bensch

T2-3C Chemogenetic and optogenetic modulation of cortical pyramidal cells influences axonal pattern formation
Ina Köhler, Adriana Rehm, Burak Ceylan, André Haase, Steffen Gonda, Petra Wahle

T2-1D Interactions of antibodies to *Treponema pallidum* with the collapsin response mediating protein CRMP1 lead to impaired neurite outgrowth in SiMa neuroblastoma cells
Bernhard Reuss

T2-2D Role of type I interferon receptor in brain development
Luisa Demuth, Shirin Hosseini, Kristin Michaelsen-Preusse, Martin Korte

T2-3D Expression of synaptic proteins and development of dendritic spines in fetal and postnatal neocortex of the pig, the European wild boar *Sus scrofa*.
Eric Sobierajski, Katrin Czubay, Marc-André Schmidt, Sebastian Wiedenski, Sarah Rettschlag, Christa Beemelmans, Christoph Beemelmans, Petra Wahle

Input synapse distribution on the dendrites of an ensemble of five *Drosophila* flight motoneurons

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This project has two goals. The first one is the analysis of the patterns of cholinergic input synapses to the wing depressor flight motoneurons (MN1-5) in *Drosophila melanogaster*. The second goal is to unravel the mechanisms that lead to the correct input synapse patterning to the MN1-5 ensemble. We have previously characterized the anatomy (Vonhoff & Duch, J. Comp. Neurol., 2010; Vonhoff *et al*., Development, 2013; Ryglewski *et al*., Neuron, 2017) and the physiology (Hürkey *et al*., Nature 2023) of MN1-5. In brief, each of the 6 fibers of the dorsal longitudinal flight muscle (DLM) is innervated by one of the MNs1-5, which exhibit similar dendritic lengths (6000 µm), 4000 branches that intermingle in the same space of the flight motor neuropil and share common excitatory input. Similarly, all 5 MNs show nearly identical input-output operations with matching I/F curves that exhibit linearity within the working range of firing frequencies relevant for flight behavior. Consequently, all 5 MNs fire at identical frequencies during flight, which is highly relevant for flight power production. However, neither the numbers of cholinergic input synapses, nor their distribution on MN1-5 dendrites, nor the mechanisms that ensure an equal proportion of synaptic inputs to all 5 MNs are known.

To address these questions, we first turned to in-depth analysis of the available FlyEM connectomics data (Janelia, MANC) to resolve the organizational structures of input synapses to MN1-5 within the *Drosophila* flight motor neuropil. We find that MN1-5 each receive about 6000 cholinergic inputs from a total of 643 presynaptic neurons. Only 20% of these presynaptic partners synapse onto all members of the MN1-5 ensemble, but this subset contributes 80 to 90% of the total inputs. Presynaptic partners that target only a subset or even only one member of the MN1-5 ensemble contribute well below 1% of the inputs to those neurons. These data indicate that few main synaptic partners synapse to all 5 MNs, whereas all other synaptic partners may be considered noise. Given that the EM data originate from 1 fly, we confirmed most of these results by high resolution light microscopy and electrophysiological approaches. But what are the mechanisms that ensure even synapse number allocation to each member of the MN1-5 ensemble?

Based on synapse distributions to different dendritic domains of MN5 (Ryglewski *et al*., Neuron, 2017), we hypothesized competition during synaptotropic growth to result in equal amounts of excitatory inputs to each MN. Experimentally, we selectively decreased the competition competence of subsets of the MNs during development by targeted inhibition of dendrite growth (Dscam1 RNAi) and tested the consequences on dendrite size of the remaining non-manipulated MNs. We find that MN dendritic size remains unaltered in a background of reduced competition, thus rejecting our hypothesis. Surprisingly, un-manipulated neurons with normal dendrites show in-flight firing frequencies that are matched to their dendritically impaired competitors. This indicates across ensemble adjustments in synaptic inputs or membrane properties. Alternatively, this could also be explained by effects of postsynaptic dendrite reductions in subsets of MN1-5 onto the presynaptic neurons that innervate the whole MN1-5 ensemble. To address these questions, we now conduct life imaging of dendrite growth of MN1-5 during synaptic partner matching with their cholinergic input partners.

Basal forebrain cholinergic innervation of the visual cortex during postnatal development in ChAT-cre transgenic mice

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Cortical development and formation of synaptic connections occur within a critical developmental window. Both activity-dependent synapse development and cortical organization are subject to modulation by neuromodulators such as acetylcholine synthesized in the basal forebrain. Although basal forebrain cholinergic neurons are present at birth, the development of cholinergic projections to the visual cortex before the first visual stimuli (eye-opening) has not been well-characterized. This study investigated the distribution and density of cholinergic axons originating from the basal forebrain to the visual cortex (specifically layers 1 and 2/3) in transgenic (choline acetyltransferase) ChAT-Cre mice before eye-opening. Our results showed a dense population of ChAT+ cholinergic neurons in the basal forebrain, consistent throughout the second postnatal week. We found that axons projecting from the basal forebrain cholinergic neurons arrived in the visual cortex at the beginning of the 2nd postnatal week. Although cholinergic innervation continues throughout the second postnatal week in L1 and L2/3 of the visual cortex, we did not observe clear differences in the density of cholinergic axons between age groups or layers. Our results reveal that basal forebrain cholinergic axons are already present in the developing visual cortex before eye-opening. This outcome is particularly important for investigating how basal forebrain cholinergic neurons modulate spontaneous network activity in the visual cortex within this critical developmental window.

A Comparative Study of Neuronal Architecture in the Caudate Nucleus: Insights from Camels and Humans

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The caudate nucleus (CN) plays a crucial role in complex neural processes, yet its structural diversity across species remains underexplored. This study offers a unique comparative analysis of CN neurons in camels and humans, utilizing the Golgi staining method to examine a select population of neurons. Three primary neuron types were identified—rich-spiny (Type I), sparsely-spiny (Type II), and aspiny (Type III)—with distinct subtypes based on soma size, dendritic architecture, and spine distribution. Remarkably, camel CN neurons displayed significant morphological differences compared to humans, particularly in dendritic complexity and spine density. These findings highlight potential evolutionary adaptations in the neuronal structures of different species, contributing to a deeper understanding of neural diversity and its implications for brain function.

3D mapping of parvalbumin interneuron-derived cortico-striatal axonal projections

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Fast-spiking parvalbumin-expressing interneurons (PV-IN) provide robust GABAergic inhibition in cortical networks, a major prerequisite for synchronized network activity tightly linked to learning and memory. PV-IN follow a protracted trajectory during brain development, including complex axonal outgrowth, and (partial) axonal myelination. Combined with a substantial energy demand due to their fast-spiking behavior, these features render PV-IN vulnerable to various cell-autonomous and external insults during neurological disease conditions. While the local axonal arborization and integration of PV-IN into cortical microcircuits is well documented, there is an emerging appreciation of a subpopulation of cortical PV-IN with long-range axonal projections to subcortical regions, including the striatum. However, a comprehensive map of PV-IN-derived cortico-striatal projections is not available.
This research aims to bridge this gap in the current state of knowledge by a detailed mapping of PV-IN-derived cortico-striatal axonal projections, their local arborization, and associated myelin in the striatum which would serve as a framework for studies into regulatory mechanisms of axonal connectivity in the cortico-striatal pathway using mouse mutants lacking the EGF-like signaling factor Neuregulin (NRG)-1.
Taken together, a more detailed insight into the cortico-striatal connectome and NRG1-mediated signaling functions into the development of PV-IN-derived axonal projections will be instrumental for a better understanding of GABAergic network functions in the striatum during normal and disease conditions.

Spectraplakin interacts with MTOCs to organize dendritic microtubules

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Microtubule organisation in neurites is critical for both transport and many developmental processes, but the underlying mechanisms are incompletely understood. We previously showed that the uniform orientation of dendritic microtubules is required for their coordinated disassembly during developmental dendrite pruning of Drosophila peripheral neurons. Here we show that the large actin-microtubule crosslinker Shot/Spectraplakin is
required for dendrite pruning and microtubule orientation. These roles depend on its microtubule-bundling domain and are synergistically enhanced by actin manipulations. We further identify Rab11 and Wdr62 as components of a putative vesicular microtubule organising complex in dendrites that is required for orientation and pruning. During development, Shot is localised adjacent to these factors at dendritic tips. We propose that tip-loclaized Shot bundles microtubules emanating from dendritic microtubule organising centres to promote their correct orientation.

H-Ras induces exuberant *de novo* dendritic protrusion growth in mature neurons regardless of cell type

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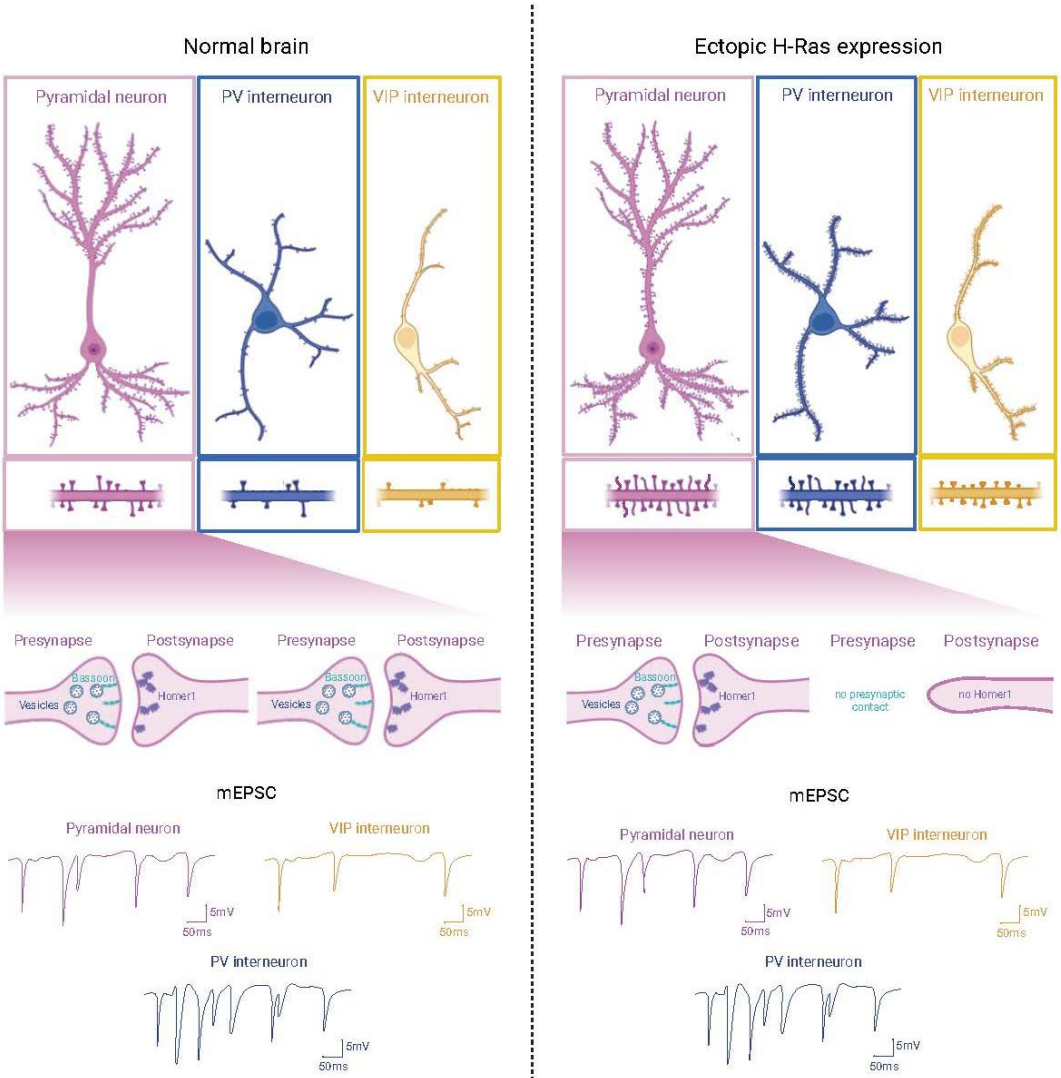
Dendritic protrusions, mainly spines and filopodia, correlate with excitatory synapses in pyramidal neurons (PyNs), but this relationship may not apply universally. We found that ectopic H-Ras expression increased protrusions across various cortical cell types, including layer 2/3 PyNs, parvalbumin (PV)-, and vasoactive intestinal peptide (VIP)-positive interneurons (INs) in the primary motor cortex. The probability of detecting protrusions correlated with local H-Ras activity, indicating its role in protrusion formation. H-Ras overexpression led to high turnover rates by adding protrusions. Two-photon photolysis of glutamate induced *de novo* spine formation in mature H-Ras expressing neurons, suggesting H-Ras's effect is not limited to early development. In PyNs and PV-INs, but not VIP-INs, spine neck lengths shifted to filopodia-like phenotypes. H-Ras primarily induced filopodia in PyNs and spines in PV- and VIP-INs. Increased protrusions in H-Ras-transfected PyNs lacked key excitatory synaptic proteins and did not affect miniature excitatory postsynaptic currents (mEPSCs), suggesting multifaceted roles beyond excitatory synapses.


Normal brain
Pyramidal neuron
PV interneuron
VIP interneuron
Presynapse
Postsynapse
Presynapse
Postsynapse
Bassoon
Vesicles
Homer1
mEPSC
Pyramidal neuron
VIP interneuron
5mV
50ms
PV interneuron
5mV
50ms
Ectopic H-Ras expression
Pyramidal neuron
PV interneuron
VIP interneuron
Presynapse
Postsynapse
Presynapse
Postsynapse
Bassoon
Vesicles
Homer1
no presynaptic contact
no Homer1
mEPSC
Pyramidal neuron
VIP interneuron
5mV
50ms
PV interneuron
5mV
50ms

Cyclase-associated protein: an actin regulator with multiple neuronal functions

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Cyclase-associated protein (CAP) is a multidomain protein that initially has been identified as an interaction partner of yeast adenylate cyclase. Subsequently, it became evident that CAP modulates not only adenylate cyclase activity, but also the actin cytoskeleton, and both activities are conserved from yeast to mammals. To date, most studies focused on CAP's actin function, and they established it as a key regulator for the assembly and disassembly of actin filaments (F-actin). However, surprisingly little is known about its cellular and physiological functions. This applies particularly to vertebrates that, different to most other species, own two CAP family members (CAP1, CAP2) with restricted, but partially overlapping expression pattern. The vertebrate brain is unique in that it is the only tissue in which both CAPs are expressed at substantial levels, and recent studies linked their dysregulation to human brain disorders, emphasizing the need to elucidate their neuronal functions.
By exploiting isolated neurons and gene-targeted mice, we identified CAP1 as a crucial regulator of the neuronal actin cytoskeleton that in cooperation with cofilin1 controls F-actin dynamics in axonal growth cones and dendritic spines, thereby regulating morphology and function of these neuronal compartments. Further, CAP1 acts as a transcriptional regulator in isolated neurons and mouse brain, which specifically represses a transcriptional program mediated by serum response factor (SRF) and its coactivator myocardin-related transcription factor (MRTF) in an actin-dependent mechanism. Finally, we found that CAP1 and CAP2 acquired overlapping functions in immature dendritic spines, in which they inhibit the actin regulator inverted formin 2 (INF2) to allow transition from immature, filopodia-like spines to mature, mushroom-like spines. Collectively, our studies established CAP1 and CAP2 as crucial regulators of neuron differentiation and function, and they provided important insights into CAP-dependent molecular mechanisms in neurons.

Dynamic structural plasticity determines developmental maturation of the cochlear inner hair cell ribbon synapse

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Sound detection occurs in the cochlea, where sensory inner hair cells (IHC) accurately convert auditory stimuli into a neurochemical signal. Presynaptically, cochlear IHCs harbor highly-specialized electron-dense specializations – so-called 'ribbons' – that facilitate ultrafast, temporally-precise and indefatigable exocytosis of ribbon-tethered synaptic vesicles (SV) onto postsynaptic spiral ganglion neurons (SGN). During synapse assembly and subsequent maturation, IHC ribbons increase in volume and SV tethering capacity. This volume accumulation of the developing ribbon is thought to result from the aggregation and fusion of multiple smaller precursors at the presynaptic active zone (AZ) in a maturation process that involves plastic structural remodeling. However, little is known of the exact processes underlying synapse assembly, nor the targeting of ribbons to the AZ, or the molecular composition of IHC ribbon precursors. Recently, we have been able to shed light on one of the molecular drivers of ribbon synapse formation and established that the microtubule (MT) cytoskeleton of IHCs is involved in the transport of ribbon precursors during presynaptogenesis. Moreover, we detected that highly frequent precursor fusion, as well as fission events take place along the MT network. This bi-directional plasticity appears to depend on MT-based transport, since pharmacological MT destabilization reduced the occurrence of plasticity events.

To now assess the developmental plasticity of the auditory ribbon synapse in more detail, we expanded our newly developed live-cell labeling and imaging techniques to allow for the triple-color, long-term examination of the live, developing mouse organ of Corti *in vitro*. In the present study, we traced ribbon precursor dynamics in cellular and synaptic context, and combined our live-cell imaging experiments with super resolution STED microscopy. We found that during early postnatal development, ribbon precursors are highly plastic, especially at the presynaptic AZ. In context of the forming synapse, we detected a concentrated clustering of – especially smaller – ribbon precursors, and a significantly increased frequency of bi-directional plasticity events. Hereby, ribbon material was both synaptically recruited and disassembled, and interestingly, also readily redistributed to other synapses. Specifically, we examined the exchange of smaller ribbon precursors between neighboring synaptic AZs, which included phases of active transport and relative stabilization in proximity to the novel synaptic contact. Furthermore, we found that inhibition of the spontaneously generated synaptic activity in the developing organ of Corti negatively impacted ribbon precursor mobility and plasticity at the AZ. The dramatic ribbon precursor remodeling at and between distinct presynaptic AZs, may thus relate to the differential states of activity at the proximal synapses.

In summary, our findings provide deep and novel insights into the developmental remodeling of ribbon precursors and the presynaptic AZ during synapse maturation in IHCs.

The Methylation-Independent Role of the DNA Methyltransferase 1 on Neuronal Development and Intracellular Trafficking

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The DNA methyltransferase 1 (DNMT1), the largest member of the DNMT family, being highly expressed in the developing and adult brain, is a prominent epigenetic key player that modulates various aspects of neuronal development as well as adult brain function. In addition to its well-described function catalyzing DNA methylation, non-canonical actions of transcriptional control have been reported for DNMT1, mediated e.g. by a crosstalk with histone-modifying complexes. In line with the functional diversity of DNMT1, different mutations in the DNMT1 gene are related with a variety of neurological symptoms. Of note, DNMT1 harbors the largest N-terminal domain of all DNMTs that provides multiple interaction sites with diverse proteins, enabling this functional diversity.

Alike other nuclear proteins, we and others detected DNMT1 in the cytosol, whereas discrete cytosolic functions have not been described yet. Surprisingly, we found numerous cytosolic proteins to interact with DNMT1, one of which was the indirect microtubule stabilizer DOCK7 (Dedicator of cytokinesis 7). Both proteins elicit similar effects on cortical neuron morphology and microtubule-dependent trafficking. Computational simulation and wet-lab approaches propose an interaction between DNMT1 and DOCK7 and identified specific interaction sites. This tempted us to deeper investigate a completely unexplored role of cytosolic DNMT1 in neuronal development through its interaction with DOCK7.

We provide evidence that cytosolic DNMT1 function modulates the morphological maturation of cortical neurons in concert with DOCK7. We verified the cytosolic co-localization of DNMT1 with DOCK7 by high-resolution microscopy. *In vitro* assays revealed that both DNMT1 and DOCK7 regulate subcellular processes such as the microtubule-based trafficking of diverse organelles and posttranslational microtubule modifications (PTMs), which underlie the morphological maturation of neurons. By the use of specific mutant constructs, we verified the relevance of cytosolic DNMT1 for the regulation of these processes.

In sum, diverse *in vitro* and wet-lab approaches in combination with computational simulations provide evidence for the unconventional role of cytosolic DNMT1-DOCK7 interactions in influencing the morphological maturation of cortical neurons and the underlying intracellular processes. This study not only illuminates the diverse functional spectrum of DNMT1 but also underscores its potential impact on disease phenotypes linked to specific DNMT1 mutations.

Chemogenetic and optogenetic modulation of cortical pyramidal cells influences axonal pattern formation

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The connectivity of the cortex is essential for its precise information processing. Thalamic input is the starting point for pyramidal cells and interneurons to process incoming information. This connectivity is established when early forms of network oscillations occur, representing the first cortical activity patterns. Therefore, initial cortical activity appears to influence the formation of pyramidal cell axons and consequently their connectivity. We aimed to investigate whether optogenetic and chemogenetic modulation of early activity at the single cell level is sufficient to alter the axonal maturation of layer 2/3 and 5/6 pyramidal cells, providing an idea of how early network activity is involved in axonal pattern formation. Here we compare two approaches and their effect on axonal maturation using cation-permeable channelrhodopsin-2 (ChR2) as an optogenetic tool to directly depolarise the cell. In contrast, activation of the Gq-coupled hM3Dq receptor with clozapine-N-oxide (CNO) leads to depolarisation via increased intracellular Ca^{2+} levels. ChR2 and hM3Dq were biolistically transfected into pyramidal cells in organotypic slice cultures of rat visual cortex. Pyramidal axons were analyzed for their primary collaterals and bouton terminaux, which were used as an approximation for neurotransmitter release sites. This analysis was conducted after treatment at three postnatal time points: DIV 10, 15, and 20. We found that repetitive optogenetic stimulation at 0.05 and 0.5 Hz increased the number of collaterals arising from the main axon of layer 2/3 pyramidal cells at DIV 10 and 15. The number of bouton terminaux arising from the main axon was not altered. In contrast, optogenetic stimulation did not affect layer 5/6 pyramidal cell axons. Chemogenetic stimulation of layer 2/3 pyramidal cells resulted in only a small increase in axonal collaterals at DIV 10, and no difference was observed at DIV 20. In contrast to the optogenetic results, hM3Dq activation increased the number of bouton terminals for layer 2/3 and 5/6 pyramidal cells. Our results suggest that the formation of collaterals and bouton terminaux appears to be differentially regulated and not solely coupled to the strength of depolarisation. Direct depolarisation via ion channel opening and indirect depolarisation via G protein-coupled signaling appear to modulate different aspects of axonal differentiation.

Interactions of antibodies to *Treponema pallidum* with the collapsin response mediating protein CRMP1 lead to impaired neurite outgrowth in SiMa neuroblastoma cells

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Syphilitic infections with the Gram negative spirochaete *Treponema pallidum* (*T. pallidum*), are on the rise again all over the world. If not successfully eradicated by antibiotic treatment, later in life they can affect the central nervous system, causing personality changes as they occur in mania, depression and psychosis (Friedrich et al., Psychopathology 2014, 47:3-9). Suggesting an autoimmune-mechanism for this, the present study reveals that antibodies to *T. pallidum* (α-*TPa*) are able to interact non-specifically with 60 different proteins on a human brain multiprotein array (HexSelect, Engine, Berlin, Germany), including the collapsin response mediating protein 1 (CRMP1), an important regulator of brain development and neurite outgrowth. Interaction of this protein with α-*TPa* was confirmed by Western blotting with a HEK293 transient overexpression lysate, and its expression in SiMa human neuroblastoma cells was demonstrated. Accordingly, preincubation of these cells with 10µg/ml of α-*TPa* resulted in a significant reduction in neurite length. Together with previous reports on changes in expression of CRMP1 and other members of this protein family in the brains of neuropsychiatric patients, results of the present study could well be of importance for a better understanding of at least some aspects of these disorders.

Role of type I interferon receptor in brain development

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Type I interferons are primarily known for their crucial role in the innate immune response. However, their functions extend beyond the scenario of infection. Previous work from our group demonstrated that signaling through the type I interferon receptor (IFNAR), specifically in a subtype of glia cells (astrocytes), critically modulates hippocampal synaptic plasticity and cognitive function in adult mice under physiological conditions. Given the involvement of cytokines, including type I interferons, in the regulation of neurogenesis and brain development, this study seeks to elucidate the role of IFNAR signaling in brain development. For this study, three-week-old IFNAR knockout (KO) mice and cell type-specific IFNAR knockout models in astrocytes (IFNARfl/fl GFAPCre+/-) and neurons (IFNARfl/fl SynCre+/-) were used. Golgi-Cox staining revealed a sex-specific reduction in spine density in the hippocampal CA1 region of female IFNAR KO mice, a trend that was also observed in the astrocyte-specific IFNAR KO but absent in male mice and the neuron-specific IFNAR KO animals. In the dentate gyrus a significant reduction in spine density was observed in the absence of type I interferon signaling regardless of the sex. Furthermore, immunostainings in female IFNAR KO mice showed a significant increase in the density of astrocytes in the respective hippocampal sub-regions. Single cell analysis of microglia and astrocytes provided evidence for altered synaptic pruning mediated by these cell types in specific hippocampal sub-regions, with variations noted between sexes and depending on which cell type lacked type I interferon signaling. In summary, this research highlights the important physiological role of the type I interferon receptor in brain development and opens up new insights in sex-specific as well as cell-type specific effects of this signaling pathway.

Expression of synaptic proteins and development of dendritic spines in fetal and postnatal neocortex of the pig, the European wild boar *Sus scrofa*.

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Synapse formation is a critical step in neuronal development. Current knowledge is primarily based on altricial rodents where synapse formation and maturation proceed largely postnatally. In precocially born mammals such as guinea pigs, presynapse and spine formation start well before birth. Here, we analyzed the developmental expression of proteins associated with synapse formation and maturation together with the development of basal dendritic spines of pyramidal neurons of the visual and somatosensory cortex of the pig, an emerging translational model for human neurodegenerative disorders.

A total of 23 selected proteins was quantified with Western blots. Most were detectable from midgestation embryonal day (E) 65 onwards. About half reached the adult expression level seen in postnatal day (P) 90 pig cortex already two weeks before birth (gestation 114 days) in somatosensory, albeit not yet in the visual cortex. For instance, major molecular components of synaptic plasticity such as GluN2B, CamKIIα, α-actinin-2, synaptopodin, and T286 phosphorylated CamKIIα were expressed at E100 in the somatosensory cortex. Dendritic spine type quantification with DiI-labeled material revealed increased total dendritic protrusions from E70 onwards. The increase was steepest in the somatosensory cortex, which had a proportion of mushroom spines at E110 equal to the proportion present at P90. Together, matching the ungulate life history, a rapid development of functional synaptic connectivity in the prenatal somatosensory cortex serves the somatomotor abilities essentially required by the newborn nest-fledgling. Results support the “cascading” model of sequential maturation of cortical areas, and in precocial species, the cascade starts well before birth.

Poster Topic

T3: Developmental Cell Death, Regeneration and Transplantation

T3-1A Histone deacetylase 8 (HDAC8) controls hypoxia-induced conversion of sensory Schwann cells into repair cells
Nadège Hertzog, Mert Duman, Maëlle Bochud, Valérie Brügger-Verdon, Maren Gerhards, Félicia Schön, Franka Dorndecker, Robert Fledrich, Ruth M. Stassart, Devanarayanan Sankar, Joern Dengjel, Sofía Raigón López, Claire Jacob

Histone deacetylase 8 (HDAC8) controls hypoxia-induced conversion of sensory Schwann cells into repair cells

Nadège Hertzog[1], Mert Duman[1,2], Maëlle Bochud[2], Valérie Brügger-Verdon[2], Maren Gerhards[1], Félicia Schön[1], Franka Dorndecker[1], Robert Fledrich[3], Ruth M. Stassart[3], Devanarayanan Sankar[2], Joern Dengjel[2], Sofía Raigón López[1], Claire Jacob[1]

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In contrast to the central nervous system, the peripheral nervous system (PNS) has an amazing regenerative capacity after lesion. Indeed, Schwann cells (SCs), the PNS myelinating glial cells, are highly plastic and react to injury by demyelinating and converting into repair cells that foster axonal regrowth, guide axons back to their former target, and then remyelinate the regenerated axons. The conversion into repair SCs is largely controlled by the transcription factor c-Jun. However, the mechanisms that induce c-Jun upregulation after injury are partially understood.

In our study, we show that ablating histone deacetylase 8 (HDAC8) in adult SCs enhances c-Jun phosphorylation and upregulation early after lesion and accelerates the regrowth of sensory axons and sensory function recovery. After lesion, the interruption of oxygen supply creates a hypoxic environment, which is known to upregulate c-Jun phosphorylation and expression and is characterized by hypoxia-inducible-factor α (HIF1α) upregulation. Additionally, we found that HDAC8 stabilizes the E3 ubiquitin ligase TRAF7, which destabilizes HIF1α, resulting in a delayed conversion into repair SCs. Our study emphasizes the function of HIF1α in SCs after injury and demonstrates that downregulating HDAC8 improves SC plasticity and promotes sensory axons regeneration and functional recovery by stabilizing HIF1α.

Interestingly, we found that HDAC8 ablation specifically promotes the regrowth of sensory axons and sensory function recovery and that HDAC8 is detected only in SCs ensheating sensory axons. This indicates that a specific subtype of SCs ensheating only sensory axons can be identified using HDAC8 as a marker and that the conversion of SCs into repair SCs is controlled by different mechanisms in motor and sensory SCs.

Poster Topic

T4: Neurotransmitters, Retrograde messengers and Cytokines

T4-1A How organelle communication shapes neuron function: triple organelle contact sites
Margret Bülow, Darla Patricia Dancourt Ramos, Marie König, Eleni Brüggemann, Nicole Kucharowski

T4-2A Unlocking Sleep: The Adenosine System's Role in Zaleplon's Mechanism
Jelena Martinovic, Marina Zaric Kontic, Ivana Gusevac Stojanovic, Dunja Drakulic, Ivana Grkovic, Natasa Mitrovic

T4-3A Peroxisome-Golgi Interaction in Neuropeptide Secretion
Nicole Kucharowski, Marie König, Margret H. Bülow

T4-1B Examining Cymbopogon citratus Potential for Synaptic Function Through AMPA Receptor Modulation
Belal Rahhal

T4-2B Extracellular pH is brain state dependent
Verena Untiet, Zuzanna Bojarowska, Yang Xue, Felix Ralf Michael Beinlich, Nicolas Cesar Petersen, Hajime Hirase, Maiken Nedergaard

T4-1C Cortical serotonin and the role of the 5-HT3 receptor
Patricia Przibylla, Christina Buetfering, Jakob von Engelhardt

T4-2C Dissecting dopamine deficiency: developmental, physiological and behavioral characterization of catecholamine-free zebrafish larvae
Susana Paredes-Zúñiga, Rebecca Peters, Kristine Østevold, Gerard Arrey, Dennis Frank, Wolfgang Driever

How organelle communication shapes neuron function: triple organelle contact sites

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Organelle communication of endoplasmic reticulum (ER), mitochondria and peroxisomes integrates multiple metabolic and signaling functions. We focus on the impact of organelle contact sites on the function of neurons *in vivo*. We found that phospholipid transfer at ER-mitochondria contact sites drives hydrogen peroxide formation and is required for dopaminergic neuron function and thereby locomotion in *Drosophila* (Paradis et al., 2022). Dynamic contacts between ER and mitochondria enable the exchange of calcium and phospholipids. Disturbed contact formation impairs mitochondrial dynamics and is a molecular hallmark of Parkinson's disease (PD). PD is also characterized by impaired mitochondrial respiratory complex I activity and dopaminergic neurodegeneration. Here we found that the ER protein Cystein-rich with EGF-like domain (Creld) regulates mitochondrial dynamics and function. Loss of Creld leads to mitochondrial hyperfusion and reduced ROS signaling in Drosophila melanogaster, Xenopus tropicalis and human cells. We used electron and super-resolution microscopy to analyze mitochondria-ER contact sites (MERCs): Creld mutants show enhanced, but less functional ER-mitochondria contacts. Lipidomics analysis of subcellular fractions revealed that phospholipid transfer at MERCs is reduced. This impairs mitochondrial respiratory complex I activity. Using optogenetics we show that the resulting low hydrogen peroxide levels are linked to disturbed neuronal activity and lead to impaired locomotion, but not neurodegeneration, in Creld mutants. MERCs recruit a third organelle, the peroxisome. Peroxisomes are important regulators of hydrogen peroxide homeostasis and shape the fatty acid profile of a cell (Bülow et al., 2018; Sellin et al., 2018). Creld interacts with the peroxisome biogenesis factor Pex19 on the protein level, and loss of Creld blocks peroxisome function in dopaminergic neurons. We conclude that Creld regulates ER-mitochondria-peroxisome communication and thereby hydrogen peroxide formation, which is required for normal neuron function.

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Bülow et al. Mol Biol Cell. 2018 Feb 15;29(4):396-407

Unlocking Sleep: The Adenosine System's Role in Zaleplon's Mechanism

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Background: A growing body of evidence suggests that the sleep-wake regulatory system relies on the neurotransmitters glutamate and GABA. The fine-tuning of this system can be achieved by controlling the presynaptic release of these neurotransmitters; however, it is generally believed that plasticity in the brain is primarily governed by regulating the expression and function of glutamate and GABA receptors. Zaleplon (Zal), a non-benzodiazepine hypnotic used for short-term management of insomnia, acts as a positive allosteric modulator by binding to the interface of the α and γ subunits of the $GABA_A$ receptor.

We previously reported molecular changes in the GABA/glutamate neurotransmitter systems in the hippocampus of naïve adult male rats following therapeutic doses of Zal (Martinovic et al., 2023). The finding of enhanced GABAergic signaling is not surprising, as decreased GABA neurotransmission is associated with the insomnia. However, we unexpectedly observed increased protein levels of components involved in glutamatergic signaling in the zaleplon-treated rats. The underlying mechanism still needs to be clarified, and adenosine has emerged as a promising candidate due to its crucial role in sleep regulation, particularly by facilitating sleep onset through A1 receptors.

Aim: In our ongoing study, we investigate the role of the adenosine system in the mechanism of action of zaleplon.

Material and methods: Upon completion of the Zal treatment (0.625 mg/kg ip, for five consecutive days), hippocampi from the same experimental groups (5 brains/group) were isolated for preparations of total RNA which was used in RT-qPCR analysis. Also, rat's brains were isolated for preparation of hippocampal synaptosomes for Western blot analysis.

Results and discussion: While mRNA levels of A1R remain stable, prolonged Zal administration leads to a significant reduction in protein levels of this receptors ($t(8) = 3.3703$, $p = 0.0098$). It might be assumed that decrease in A1R protein abundance was not due to downregulation of A1R-mRNA, but rather possible translocation of this receptor from synapse, which may contribute to the observed increase in proteins associated with glutamatergic signaling (vGlut, NR1, NR2A, NR2B). Specifically, glutamate released from the presynaptic membrane activates astrocytes, prompting them to release adenosine that bind to A1 receptors. This interaction may result in presynaptic inhibition of glutamate release and a decrease in the activation and surface expression of postsynaptic NMDA receptors, thereby mitigating neuronal hyperexcitability. However, these findings raise critical questions that we are currently investigating in our ongoing study regarding the role of adenosine system in the Zal mechanism of action.

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Peroxisome-Golgi Interaction in Neuropeptide Secretion

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Peroxisomes are vital organelles that interact with the ER, mitochondria, lysosomes, and lipid droplets. We generated a *Drosophila melanogaster* mutant for the peroxisome assembly factor Pex19 and found that the mutation leads to delayed development, early lethality and lipotoxicity due to hyperactive mitochondrial metabolism[1]. We showed that Pex19 mutants accumulate very-long-chain fatty acids (VLCFA), but more strikingly show a depletion of medium-chain fatty acids (MCFA). Dietary administration of MCFA rescues both development and lethality of Pex19 mutants[2].

Here we show that the VLCFA/MCFA imbalance affects distinct lipid classes, especially membrane lipids. We used click chemistry and lipidomics to trace the incorporation of orthogonal alkyne myristate in sphingolipids, with consequences for the membrane lipids. This leads to impaired neuropeptide secretion of insulin-like peptides (dilps) from insulin-producing cells (IPCs) upon nutrient stimuli, reduced insulin levels in the hemolymph and impaired insulin signaling.

To unravel the role of peroxisomes in vesicle secretion, we performed Expansion Microscopy and found a nutrient-dependent interaction of peroxisomes with the Golgi apparatus. In sum, we propose a role for peroxisomes in neuropeptide secretion by maintaining membrane lipid homeostasis.

1 Bülow MH et al. (2018), Mol Biol Cell, 15;29(4):396-407

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Examining Cymbopogon citratus Potential for Synaptic Function Through AMPA Receptor Modulation

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Background: Herbal medicine has always played a crucial therapeutic role in the history of civilizations, and lately, more and more plant extracts have been integrated into current medical practice. Such natural products have a variety of pharmacological activities, including the treatment and modulation of neurological disorders. Among many medicinal plants reported for their efficacy, Cymbopogon citratus stands out for its therapeutic properties and has been used since ancient times. The current research focused on one of the most important ionotropic glutamate receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), which takes part in synaptic plasticity-the ground for learning and memory. AMPA receptors allow an influx of cations to neurons on glutamate activation, facilitating excitatory neural signaling. The regulation of these receptors due to kinetic processes such as desensitization and deactivation provides the required regulation to maintain the correct neural performance. Dysregulation in AMPA receptor activity has been implicated in several neurological conditions, such as epilepsy and even neurodegenerative diseases, including Alzheimer's. Given the current interest in plant-based therapeutics, this study investigates the effect of Cymbopogon citratus water extract on the activity and kinetics of both homomeric (GluA1, GluA2) and heteromeric (GluA1/2, GluA2/3) AMPA receptor subunits in order to explain the potential neuromodulatory role.
Methods: The powdered Cymbopogon citratus was allowed to steep in water (about 25 g in 500 mL), shaken at room temperature, and stored for 7 days. The resultant extract was filtered, evaporated under vacuum, lyophilized in cryo-desiccator, and stored at 4°C until use. An aqueous extract of the same plant was evaluated for the presence of major classes of bioactive phytochemicals. HEK293t Cells transiently expressing AMPA receptors were used to assess electrophysiological responses to the plant extract (800 µg/mL) using the whole-cell patch-clamp technique. Statistical comparisons between the groups were performed by one-way ANOVA, with significance set at p-value < 0.05.
Results: Our initial screening of Cymbopogon citratus uncovered a wealth of beneficial phytochemicals, including tannins, phenols, saponins, polysaccharides, and flavonoids. When we applied the aqueous extract at a concentration of 800 µg/mL, we noticed a slight reduction in whole-cell current for the AMPA receptor subunits, although it was not significant enough to be considered as inhibition. The whole-cell patch clamp technique analysis revealed that the extract had a notable impact on receptor kinetics. The GluA2 subunit showed a remarkable 1.6-fold increase in deactivation rate and a significant 2-fold decrease in desensitization rate ($p < 0.01$). Besides, heteromeric subunits showed a 1.5-fold decrease in desensitization and increased the deactivation rate by 1.2-fold ($p < 0.05$).
Conclusion: Cymbopogon citratus extract alters AMPA receptor kinetics by modulating AMPA GluA2-containing receptors. Our findings highlight the potential of traditional herbal medicine to develop therapeutic techniques for neurological illnesses and call for additional research into clinical applications.

Extracellular pH is brain state dependent

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The regulation of extracellular pH (pHe) in the brain is essential for maintaining healthy neuronal function. Low pHe levels have been linked to neuropsychiatric disorders such as schizophrenia and bipolar disorder [1], underscoring its clinical significance. However, the *in vivo* dynamics of pHe remain largely unexplored. Astrocytes play a key role in pHe regulation through bicarbonate buffering and managing lactate production and clearance [2,3]. Recent findings indicate that pHe increases during heightened neuronal activity, such as that induced by foot shock in anesthetized mice [4]. However, anesthesia, like isoflurane, is known to alter pHe, potentially confounding results [5].
In this study, we investigate pHe during natural brain state transitions in freely moving mice. By using viral delivery of pH sensors combined with fiber photometry, EEG, EMG, and the complementary technique of microelectrode recordings, we measure both relative and absolute changes in brain pHe without the influence of anesthesia. Our results reveal that pHe is lowest during rapid eye movement (REM) sleep and increases during wakefulness, reflecting the brain's dynamic response to natural state changes.
This research highlights the importance of studying extracellular pH in the context of natural behaviors to better understand the interactions between neuronal and glial cell types within the brain's extracellular environment.

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Cortical serotonin and the role of the 5-HT3 receptor

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The hormone 5-hydroxytryptamin (5-HT), better known as serotonin, is one of the main neurotransmitters in the brain. Serotonin is involved in functions ranging from regulating mood, motor processes as well as complex behaviours. Serotonergic neurons in the raphe nuclei send out axons to different brain areas including branches terminating in cortex. The effect of serotonin is then conveyed via a variety of serotonin receptors. When exactly serotonin is released in cortex and how the activation of the different serotonin receptors affect cortical activity is largely unknown.

In this study, we are investigating how serotonin changes neuronal activity in cortical circuits in general and which network changes are mediated via the serotonin receptor 5-HT3 in particular. While most serotonin receptors are metabotropic receptors, the 5-HT3 receptor is a ligand-gated ion channel. It is unclear whether the signal generated by this receptor is unique and how it differs from the information conveyed by serotonin binding to metabotropic receptors.

We are using two-photon calcium imaging in behaving mice to record the activity of serotonergic axons coming from dorsal raphe as well as cortical neurons and their response to the release of serotonin. The signal of serotonergic axons is recorded while the mouse is involved in a range of behaviors including resting, running, grooming as well as receiving rewards and sensory stimulation. We then analyze which of these behaviors drive release of serotonin in somatosensory cortex. We find strong positive and negatively correlated running modulation in serotonergic axons.

In addition, we stimulate serotonergic axons optogenetically and record the activity of cortical neurons including neurons expressing the 5-HT3 receptor. Axon stimulation results in increased activity of cortical neurons. The different time scales of increased activations suggests that both, ionotropic and metabotropic serotonin receptors are involved in mediating the effects of serotonin on cortical activity. Further experiments using subunit-specific antagonists that block serotonin receptors are performed to investigate the contribution of the different serotonin receptors to the cortical response to serotonin release.

Our investigations should allow us to better understand when and how serotonin is released in cortex in an awake behaving mouse and help us to understand the effect of serotonin release on cortical networks.

Dissecting dopamine deficiency: developmental, physiological and behavioral characterization of catecholamine-free zebrafish larvae

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The catecholamines dopamine, noradrenaline and adrenaline have conserved roles in control of physiology and behaviors of vertebrates. Adrenergic dysfunctions may cause heart failure, while dopaminergic impairments are linked to neuropathologies including Parkinson's disease, cognitive problems, schizophrenia and drug abuse. So far, most of our knowledge about catecholamine systems derives from pharmacological approaches or neuronal ablation. In this work we generated a genetic zebrafish model completely devoid of catecholamines by combining mutations in all genes involved in L-DOPA synthesis: the two tyrosine hydroxylase genes (*th* and *th2*) in neurons, and tyrosinase (*sdy*) in melanocytes. Catecholamine-deficient zebrafish larvae are viable and develop an anatomically normal organization of catecholaminergic tracts and projection targets. In contrast, physiological functions that depend on catecholamines are impaired, including larval hatching and cardiac function. Behavioral assays reveal that specific visually guided locomotor outputs are impaired in triple mutants. An in-depth investigation of visual habituation revealed that the components of the dark flash response, specifically latency, magnitude and response probability, were differently modulated by dopamine depending on whether habituation was long-term or short-term. Comparison of single and multiple *sdy*, *th* and/or *th2* mutant larvae reveals that the *th* locus has the strongest contribution to the mutant phenotypes. Overall, our results show that catecholamine depletion impairs the fine tuning of physiological and behavioral responses in a way that resembles mammalian dopamine-derived dysfunctions, making the catecholamine-free zebrafish a suitable model for the translational study of dopaminergic systems.

Poster Topic

T5: G Protein-linked and other Receptors

T5-1B Investigation of dopamine and serotonin receptors and their heteromers using GPCR-based fluorescent sensors
Ponlawit Wisomka, Nik Meisterernst, Andreas Reiner

T5-2B Decoding Octopamine's Role in *Drosophila melanogaster*: A Behavioral and Molecular Study of Trojan Exon Mutants
Alexandra Großjohann, Marvin Hahmann, Andreas S. Thum

T5-1C The optogenetic potential of the anomalous Gi/o-coupled vertebrate ancient opsin from the flashlight fish Anomalops katoptron
Lennard Rohr, Philip Althoff, Ori Berman, Caroline Naber, Caroline Güers, Melanie Mark, Peter Soba, Alexander Gottschalk, Moran Shalev-Benami, Till Rudack, Ida Siveke, Stefan Herlitze

T5-1D Microglial-Neuronal Interactions in the Recovery Phase of Ischemic Stroke
Charlotte Catharina Oldenburg, Marie-Luise Brehme, Lynn Bitar, Tim Magnus, Thomas G. Oertner

T5-2D Expression of Cirl1 and Cirl3 Adhesion GPCRs in the Dorsal Root Ganglia in Different Peripheral Neuropathy Models
Mariam Medhat Sobhy Atalla, Maria Georgalli, Abdulrahman Sawalma, Annemarie Sodmann, Robert Blum, Heike Rittner

Investigation of dopamine and serotonin receptors and their heteromers using GPCR-based fluorescent sensors

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G protein-coupled receptors (GPCRs) are important targets for the treatment of various neurological and psychiatric disorders. Classical examples are dopamine (DA) and serotonin (5-HT) receptors, which exert a wide range of neuromodulatory functions. The functional diversity of these receptor families arises from the existence of different receptor subtypes that couple to different downstream effectors. The proposed heteromerization of various class A GPCRs, including DA and 5-HT receptors, would provide additional possibilities for crosstalk and pharmacological targeting. One example are D_2R and 5-$HT_{2A}R$ heterodimers for which altered signaling properties have been reported [1] and might play a role in schizophrenia.

Here, we made use of recently developed GPCR-based fluorescent sensors to investigate the effects of possible heteromerizations on the receptor level, i.e. on the ligand affinities and efficacy of the individual receptor subunits. For this we used the human D1 receptor-based sensor dLight1.3b [2] and the D2 receptor-based sensor $GRAB_{DA1h}$ [3]. We expressed these sensors in HEK293T cells and characterized their response towards different doses of dopamine (DA), which is a full agonist, and partial agonists using plate-reader assays. In addition, we used fluorescence microscopy to investigate the real-time responses of these sensors upon agonist application and removal. Coexpression of dLight1.3b with 5-$HT_{1A}R$ or 5-$HT_{2A}R$ did not result in significant alterations in DA binding, neither in the absence or presence of 1 µM 5-HT, thus not giving any direct evidence for heteromer formation. However, coexpression of $GRAB_{DA1h}$ with both 5-$HT_{1A}R$ or 5-$HT_{2A}R$ resulted in a loss of fluorescence signals. Current experiments aim at elucidating whether this is caused by cotrafficking/internalization of D_2R and 5-$HT_{2A}R$. We further aim at investigating the consequences on G_q/G_i downstream signaling cascades, using Ca^{2+} imaging and GIRK (G protein-coupled inwardly rectifying potassium channel) assays, respectively. This work demonstrates that GPCR-based fluorescent sensors can be useful tools for determining the pharmacological properties and binding/unbinding characteristics of various agonists. Moreover, potential heteromerization of various receptor subtypes deserves further investigation.

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Decoding Octopamine's Role in *Drosophila melanogaster*: A Behavioral and Molecular Study of Trojan Exon Mutants

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The invertebrate equivalent of Noradrenaline, Octopamine, has been reported to play a role in a wide spectrum of physiological and behavioral processes. Accordingly, in adult and larval *Drosophila melanogaster* Octopamine and its related receptors are analyzed to gain further mechanistic insight into the nervous system. The novel genetic tool 'Trojan Exon' by Diao et al. (2015) utilizes a Minos mediated integration cassette (MiMIC) and viral T2A factor to promote the translation of a reporter protein product and the related gene of interest from a single transcript. Depending on the insertion site in the gene, a truncated, mutated version of the protein results. However, the expression of the reporter should correspond to the endogenous expression of the related gene. Behavioral analysis of 7 new Trojan Octopamine and Octopamine receptor lines available at Bloomington stock center shows that only some of the described effects can be reproduced at the developmental, behavioral and anatomical level. Our molecular analysis reveals that in some cases, this was based on the absence of the Trojan Exon or additional inserts at the Trojan Exon insertion site. Overall, we recommend the Trojan Exon method as a possible alternative to perform gene-behavior correlations in *Drosophila melanogaster* . However, before experiments can be performed, the molecular organization of the respective constructs must be verified, as some lines may contain genetic alterations.

The optogenetic potential of the anomalous Gi/o-coupled vertebrate ancient opsin from the flashlight fish Anomalops katoptron

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Vertebrate ancient long opsin, or VAL-opsin, is a light-sensitive protein that is also found in tissues outside the eyes of vertebrates. Its blue-green light sensitivity and wide distribution in retina, brain, testis and skin in non-mammalian ver-tebrates suggest an important role in light-dependent physiological processes beyond vision. However, many aspects of their physiological properties and specific functions remain to be elucidated. Here we characterized the VAL opsin from the flashlight fish Anomalops katoptron according to its activation spectra and optogenetic potential in HEK293 cells, mouse brain slices and Caenorhabditis elegans. We found that this VAL opsin couples to the Gi/o pathway and acts as a bistable or monostable receptor depending on the retinal compound present. In line with this, we show that VAL opsin modulates neuronal activity in cerebellar Purkinje cells, where UV/blue light reduces, and green-red light increases neu-ronal activity. In addition, in vivo expression and activation of VAL opsin in neurons innervating body muscles of C. elegans immediately induces a strong Gi/o dependent inhibition of movement. Thus, VAL opsin reveals retinal-dependent, powerful Gi/o pathway activation to inhibit neuronal function.

Microglial-Neuronal Interactions in the Recovery Phase of Ischemic Stroke

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Stroke remains one of the most common cause of death world-wide. Morphologically, the stroked area can be divided into a necrotic infarct core and a periphery, called the penumbra, where cells survive the initial stroke, but may die later if blood flow is not re-established fast enough. During and after stroke, microglia – the immune cells of the central nervous system – sense a perturbance in the brain parenchyma, migrate into the affected area and release all kinds of pro- and anti-inflammatory cytokines, growth factors and matrix-metalloproteases. Mediation of these ambivalent processes makes it difficult to investigate what role microglia play in the recovery phase of stroke.

We utilize a Cre-dependent DREADD (Designer Receptor Exclusively Activated by Designer Drugs) system to manipulate G protein signaling exclusively in microglia. We show that Gq activation in microglia triggers Ca^{2+} transients and leads to a retraction of cellular processes. The DREADD system allows effective manipulation of microglia without side effects or co-activation of other cell types, helping to decipher mechanisms of stroke recovery directly related to microglia. To study the effects of microglia activation on neurons and synapses, we use oxygen-glucose deprivation (OGD) of organotypic hippocampal slice cultures to mimic stroke conditions *in vitro*. OGD (20 min) affects both microglia morphology and the density of spines on CA1 pyramidal cell dendrites, and these effects are modulated by pre-activation of Gq. In summary, our methodological approach helps us to understand the impact of microglia on neuronal survival and synaptic function in the stroke penumbra.

Expression of Cirl1 and Cirl3 Adhesion GPCRs in the Dorsal Root Ganglia in Different Peripheral Neuropathy Models

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Background and Aims

Neuropathic pain is a debilitating condition resulting from lesions in the somatosensory system. Gi/o coupled G protein-coupled receptors (GPCRs) play a critical role in both the transmission and interruption of pain signals, rendering them highly relevant for pain research. Gi-coupled receptors, such as opioid receptors, are among the most potent analgesics. Previous investigations in Drosophila demonstrated that the aGPCR dCirl modulates mechanotransduction and antinociception [1,2]. In rodents, studies have shown a decrease in Cirl1 clusters in non-peptidergic (NP) neurons within the dorsal root ganglia (DRG) during the acute pain phase of chronic constriction injury (CCI)-induced peripheral neuropathy [2]. In our study, we hypothesized that bortezomib-induced peripheral neuropathy (BIPN) similarly causes a transient down-regulation of Cirl1 and Cirl3 in the acute phase, followed by recovery in rats, which may parallel the proteins' roles in antinociception. We also utilize the CCI model in mice to further investigate this hypothesis and similarly examine human samples to provide translational insights.

Methods

Bortezomib-induced peripheral neuropathy (BIPN) was modelled in male Wistar rats by administering a mild dose of Bortezomib (BTZ) twice weekly for two weeks, replicating a single cycle of BTZ treatment as used in multiple myeloma patients [3]. In the BIPN model, tissues were collected at three key time points: 12 days, signifying peak hypersensitivity; 18 days, marking the onset of pain resolution; and 25 days, indicating the end of resolution.
To further investigate neuropathic pain mechanisms, we employed a chronic constriction injury (CCI) model in mice, in which the sciatic nerve was ligated with three silk sutures. Tissues were harvested at defined time points corresponding to peak hypersensitivity (1 week) and the onset of pain resolution (4 weeks), providing a temporal framework to compare the progression of neuropathic changes across models. Pain-related behaviours were assessed through the electronic Von Frey test for mechanical hypersensitivity.
Human DRG samples were obtained from forensic medicine sources or patients who had sustained avulsion injuries for translational comparison. Given the lack of specific antibodies for Cirl subtypes, RNAscope labelling was used to detect Cirl1 and Cirl3 RNA within the dorsal root ganglia (DRG). This approach was combined with immunofluorescence to identify non-peptidergic (NP) and neurofilament (NF) neuronal subtypes [4]. To quantify Cirl1 and Cirl3 clusters across neuronal populations unbiasedly, we applied the deep learning tool Deepflash, which automatically segmented DRG neuronal subpopulations [5]. Furthermore, the FISH-quant analysis pipeline was modified to enable automatic, objective quantification of Cirl clusters across full DRG sections [6].

Results

Throughout all the timepoints investigated, Cirl1 and Cirl3 were observed in all DRG neurons. The pattern of Cirl3 clusters suggested its potential presence in neurons and satellite glial cells whereas Cirl1 was mostly in neurons Unbiased quantification in the BIPN model revealed that the average number of Cirl1 clusters was ~50 per NP neuron and ~100 per NF neuron. A slight downregulation of Cirl1 clusters was observed in the BTZ-treated group at the 25-day time point compared to the vehicle control in the NF neurons. Cirl3 clusters showed an average of ~50 per NP neuron and ~60 per NF neuron, with no significant alterations across time points or treatment groups. In the CCI model, analysis is still underway. In human DRGs from patients with plexus avulsion injuries, a downward trend in CIRL1 expression was noted compared to healthy controls, though no statistically significant difference was found.

Conclusions

Both Cirl isoforms are expressed in sensory neurons, but more clusters are in NF positive sensory neurons than in nociceptive neurons, possibly due to their larger size which hadn't been corrected for. Cirl3 is also present in non-neuronal cells in the DRG. The slight downregulation of Cirl1 clusters seen in BTZ-treated rats may be attributed to the use of a mild model that induces subtle changes. The lack of significance in the human tissues could be due to variability across patient samples, limited sample size, and the diverse underlying mechanisms of neuropathy in these human cases. Further investigation with larger sample sizes and refined neuropathy models may help clarify these trends and establish a clearer connection to neuropathic pain mechanisms.

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Poster Topic

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

T6-1A Distinct Subcellular Compartmentalization of Kv4.3 Channels in the Hippocampal CA1 Interneurons and its Impact on the Perisomatic Inhibition
Laxmi Kumar Parajuli, Shantanu Durgvanshi, Nithya Sethumadhavan, Marco Ross, Akos Kulik, Claudio Elgueta

T6-2A TRPV4 channels mediate Na^+ influx and promote cellular ATP loss during energy deprivation in mouse cortex
Nils Pape, Christine R. Rose

T6-3A Structural and functional insights into GluK2/GluK5 kainate receptor gating
Laura Moreno Wasielewski, Alexa Strauss, Nandish Khanra, Sophie Lenze, Joel Meyerson, Joshua Levitz, Andreas Reiner

T6-4A Molecular Determinants of Cesium- and Glycine-dependent Glycine Receptor Activation
Steffen Fricke, Magnus Harnau, Elina Zeller, Jochen Meier

T6-1B Probing the role of ion channel degeneracy for robust neuronal excitability
Selina Hilgert, Carsten Duch, Stefanie Ryglewski

T6-2B Effects of energy deprivation on cellular ATP levels and ion homeostasis in human cortical brain organoid slices (cBOS)
Louis Anton Neu, Laura Petersilie, Sonja Heiduschka, Nils Pape, Alessandro Prigione, Christine R. Rose

T6-3B Circadian and ultradian rhythms in the spontaneous activity of insect olfactory receptor neurons
Aditi Vijayan, Mauro Forlino, Katrin Schröder, Huleg Zolmon, Martin Garcia, Monika Stengl

T6-4B Investigation of glutamate accumulation and neuronal depolarization in metabolic stress conditions
German Lauer, Tim Ziebarth, Hanna Praast, Andreas Reiner

T6-1C Pharmacological inhibition of $Ca_V2.1$-α2δ1 interface suppresses neuronal firing and reduces synaptic density in hippocampal network
Arthur Bikbaev, Corinna Werkmann, Lea Wazulin, Lea Driesang, Abderazzaq El Khallouqi, Ana Carolina Palmeira do Amaral, Martin Heine

T6-2C Allosteric modulation of $GABA_AR$ by sesquiterpenes representing a distinct fraction in volatile oils and plant extracts
Julian Leopold Nausester, Anna-Lena Wießler, Christian Boehm, Andrea Buettner, Carmen Villmann

T6-3C A secreted protein controls surface expression of a postsynaptic ion channel
Sven Kuspiel, Dominik Wiemuth, Stefan Gründer

T6-4C CKAMP59 (aka Shisa7) is probably an auxiliary subunit of the AMPA receptor complex
Benedikt Grünewald, Samy Al-Qut, Alexander Hammen, Jakob von Engelhardt

T6-1D Transient Neonatal Hyperexcitability Induces Persistent Network Alterations in *Scn2a* p.A263V Mouse Model of Epilepsy
Yana Reva, Katharina Ulrich, Hanna Oelßner, Daniil Kirianov, Mohamad Samehni, Birgit Engeland, Ricardo Melo Neves, Dirk Isbrandt

T6-2D Decoding reelin's impact on cholinergic signaling: a novel perspective on neural modulation
Marie-Luise Kümmel, Eckart Förster, Max Wulf, Katrin Marcus-Alic

T6-3D Modulation of Rat and Human Acid-Sensing Ion Channel 3 by the Thyroid Hormone T3
Lu Qin, Dominik Wiemuth, Stefan Gründer

T6-4D Preliminary study of analgesic effect of bumetanide on neuropathic pain in patients with spinal cord injury*A*
Leila Zarepour

Distinct Subcellular Compartmentalization of Kv4.3 Channels in the Hippocampal CA1 Interneurons and its Impact on the Perisomatic Inhibition

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Inhibitory GABAergic interneurons (INs) in the hippocampus comprise a diverse set of cells with distinct morphological, molecular, synaptic, and physiological properties. These cells tightly regulate principal cell activity, enhancing local computational complexity and supporting hippocampal functions such as spatial navigation, pattern separation, memory formation, network synchronization, and oscillatory activity generation. IN-IN connectivity and inhibition are particularly relevant for generating gamma oscillations, which play a critical role in hippocampal function. Here we show that Kv4.3 channels are selectively expressed in a subset of interneurons in the CA1 pyramidal cell layer of the hippocampus. Confocal microscopy and serial section pre-embedding immunogold labeling demonstrated that Kv4.3 immunoreactivity is most pronounced at the sites of somatic inhibitory contacts. Strikingly, Kv4.3 channels are preferentially localized towards the plasma membrane portion of an interneuron soma that does not make direct physical contact with the surrounding cells in the CA1 pyramidal cell layer. In contrast to the distribution pattern in the soma, such subcellular compartmentalization of Kv4.3 was not observed in the dendritic processes of an interneuron. Rather, Kv4.3 immunogolds were scattered over the dendritic plasma membrane. SDS-digested freeze-fracture replica labeling (SDS-FRL) and serial section pre-embedding immunogold labeling showed that the distribution pattern of Kv4.3 in the soma is not a common organizational feature of Kv channels in the hippocampal CA1 as other Kv subunits expressed in somatodendritic domains in the CA1, namely Kv2.1 and Kv4.2, displayed different distribution pattern than that of Kv4.3. In order to further obtain quantitative parameters on the precise number of the inhibitory contact sites, Kv4.3 clusters and the density of Kv4.3 channels in a given cell, volume reconstruction of a number of Kv4.3 labeled interneurons were carried out by SEM array tomography. Notably, using detailed biophysical models based on the realistic morphology and the distribution pattern of Kv4.3 obtained from the volume EM data, we demonstrate that the association of Kv4.3 channels with GABAergic synapses significantly modulates somatic inhibition in these IN populations. Our results show that both the synaptic features of interneurons and the compartment specific subcellular localization of ion channels thereof are crucial determinants of neuronal computation in the hippocampus.

TRPV4 channels mediate Na^+ influx and promote cellular ATP loss during energy deprivation in mouse cortex

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The vertebrate brain has an exceptionally high energy need, most of which is consumed for the generation and maintenance of ion gradients across cell membranes. During an ischemic stroke, intracellular ATP concentrations rapidly decline, resulting in a breakdown of ion gradients, cellular depolarization and cellular damage. In the present study, we employed the genetically encoded sensor ATeam1.03^{YEMK} as well as the sodium indicator dye ING-2 to analyze the pathways driving the loss of ATP and the accumulation of intracellular Na^+ upon transient metabolic inhibition in neurons and astrocytes of mouse neocortical tissue slices.

We demonstrate that brief chemical ischemia, induced by combined inhibition of glycolysis and oxidative phosphorylation, results in a transient decrease in intracellular ATP and an accompanied increase in intracellular Na^+. Neurons experienced both a larger relative ATP decline and Na^+ increase and showed less ability to recover their ATP levels from prolonged (>5 minutes) metabolic inhibition than astrocytes. Blocking voltage-gated Na^+ channels or NMDA receptors ameliorated the ATP loss neurons and astrocytes, while blocking glutamate uptake aggravated the overall reduction in neuronal ATP, underlining the central role of excitatory neuronal activity in the cellular energy loss. Unexpectedly, pharmacological inhibition of transient receptor potential vanilloid 4 (TRPV4) channels significantly reduced the ischemia-induced ATP decline as well as the accompanied Na^+ increase in both cell types. Despite the close association of TRPV4 with NMDA receptors, co-inhibition of the two revealed this effect to be at least partially independent of the former's influence on glutamate receptors for both ATP and Na^+ changes. TRPV4 activation via its selective agonist furthermore revealed the channel's previously disregarded role in promoting Na^+ influx.

Taken together, our results show that brief metabolic inhibition results in a decline in cellular ATP, accompanied by an increase in intracellular Na^+, to which neurons exhibit a higher vulnerability than astrocytes. While pathways of glutamatergic signaling were expected to play a large role in energy consumption during ischemic conditions, our data revealed an unexpected strong contribution of TRPV4 channels to loss of cellular ATP. This ATP consumption is most likely a direct consequence of TRPV4-mediated Na^+ influx, which could be observed under metabolic inhibition as well as direct stimulation. TRPV4 activation thus provides a hitherto unacknowledged contribution to the cellular Na^+ dynamics and cellular energy loss during energy failure, generating a significant metabolic cost in ischemic conditions.

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Structural and functional insights into GluK2/GluK5 kainate receptor gating

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Kainate receptors belong to the family of ionotropic glutamate receptors (iGluRs), which are ligand-gated ion channels that both mediate and modulate excitatory signal transmission in the central nervous system. The GluK2/GluK5 heteromer is one of the most abundant kainate receptor subtypes in the brain. Experiments have shown that GluK2/GluK5 heteromers assemble with preferential 2:2 subunit stoichiometry [1] and previous cryo-EM data revealed the overall architecture of this complex in the resting and desensitized state [2]. In order to obtain further insight into gating of these heteromers, new structural data was obtained in the presence of a GluK5-selective agonist, namely 5-iodowillardiine (5-IW). 5-IW drives activation by binding to only the GluK5 subunits, which, however, is not sufficient to cause desensitization [3]. The structures of these partially occupied GluK2/GluK5 heteromers show two novel states – one pre-active state with partial rearrangements of the ligand binding domains (LBDs) but a closed ion channel pore, as well as a partially desensitized state with one intact and one ruptured LBD dimer. Detailed structural analyses were performed to identify the interfaces between the four LBDs and amino acid exchanges were performed to investigate possible inter-subunit interaction sites. The GluK2/GluK5 variants were expressed in HEK cells and investigated using ultra-fast perfusion patch-clamp experiments, which allow for rapid ligand application and analysis of their gating kinetics, including activation, desensitization, deactivation, and recovery. These functional measurements revealed that some interaction sites, including those that mediate inter-dimer interactions, influence channel gating more drastically than others. For example, altering residues that are unique to GluK5 caused substantial slowing of deactivation, slower desensitization, and a more than twice as fast recovery upon glutamate application. Especially the effects on deactivation warrant further investigation, since slow deactivation kinetics are a unique feature of GluK2/GluK5 heteromers that may be of physiological relevance [4].

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Molecular Determinants of Cesium- and Glycine-dependent Glycine Receptor Activation

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Cesium was recently identified as an agonist of the neurotransmitter receptor for glycine (GlyR). Atomistic molecular dynamic simulations on GlyR α3 suggested the amino acids D141, E192, and D194 with negatively charged side chains as possible binding sites for cesium in the neighborhood of the RNA editable P185 site.

To test this hypothesis, we mutated these positions to code for alanine with a neutral, hydrophobic side chain or lysine with a positively charged side chain. The recombinant, mutated and RNA-edited GlyR α3L-185L channels were expressed in HEK293T cells and analyzed using whole cell patch clamp electrophysiology. The results show that D141 mutations had no considerable effect on GlyR currents, E192 is critical for cesium-dependent and D194 for general GlyR activation. While E192A had no effect on GlyR activation, E192K almost completely abolished GlyR activation up to 5 mM cesium. Moreover, D194 mutations drastically reduced potency of glycine and cesium as GlyR agonists. However, normalization of cesium to glycine-evoked currents removed the effect of D194 mutations on cesium-dependent GlyR activation indicating a higher impact on GlyR activation by glycine per se.

Thus, E192 and D194 are considered essential for GlyR activation with E192 affecting cesium-dependent receptor activation compared to D194 affecting general activation and preponderantly glycine-evoked currents.

Probing the role of ion channel degeneracy for robust neuronal excitability

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Correct neural network function depends on the intrinsic excitability and synaptic connections of neurons. Despite environmental challenges the participating neurons have to perform robust physiological behavior. How do neurons maintain robust intrinsic excitability in the face of external and internal challenges? Beside the well accepted concept of homeostatic compensation that coregulates e.g. ion channel expression, the role of ion channel degeneracy (ion channels with functional overlap) for robust neuronal excitability is still under debate as experimental proof is sparse. Combining Drosophila genetics and in situ whole cell patch clamp recording, our work provides the in vivo results that show that ion channel degeneracy renders neuronal excitability robust.

As model we use the five identified flight motoneurons, MN1-5, that innervate the dorsal longitudinal flight muscle in *Drosophila melanogaster*. MN1-5 all show robust slow tonic rate coding, also called type 1 excitability. In the behaviorally relevant working range of the neurons linear input output computation for smooth frequency changes are essential for adequate wing power control during flight. We have previously studied the ion channel complements of these neurons and applied various ion channel manipulations. The intrinsic excitability as well as the network activity during flight are impressively robust to a variety of ion channel perturbations. However, the underlying ion channels that tune the intrinsic excitability of MN1-5 are imprecisely regulated as current amplitudes of different ion channels can vary up to 300% (e.g. Shaker (Kv1) or Shab (Kv2)). We hypothesize that the imprecise expression of many different ion channel types renders excitability more robust to perturbation than the precise expression of few ion channels, or in short, ion channel heterogeneity increases excitability robustness. To test this, we reduced heterogeneity without affecting excitability. We did this by reducing the isoform variability of the voltage gated calcium channel cacophony (Cav2). This manipulation did not affect the total calcium currents, the calcium signals, the spike shape, or input-output computation. This allowed us to test how reduced heterogeneity affects the robustness of MN excitability against perturbations. As perturbation we used genetic and pharmacological manipulations of Shab potassium channels.

The key finding is that blockade of Shab current alone does not alter MN firing responses to input, whereas upon reduced calcium channel isoform heterogeneity, the same perturbation of Shab significantly alters MN spike shape and input-output computation. To our knowledge, these are the first in vivo experiments that demonstrate a role of ion channel degeneracy in maintaining neuronal excitability robust. In addition, we used temperature paradigms from 18°C to 30°C to mimic external perturbation as occurring under normal behaviorally relevant conditions. Reducing heterogeneity significantly narrowed the temperature range at which motoneurons were resilient against manipulation of Shab. This further underscores our conclusion that heterogeneity renders neural excitability more robust to perturbations.

Effects of energy deprivation on cellular ATP levels and ion homeostasis in human cortical brain organoid slices (cBOS)

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The brain has a high energy demand. Although it represents only 2% of the body mass, it requires 20% of the body's total oxygen consumption and utilizes 25% of the available glucose. During an ischemic stroke, however, glucose and oxygen supply are reduced or interrupted, leading to a rapid decrease of intracellular ATP levels which results in the breakdown of ion gradients and ultimately in cell damage. To better understand the cellular mechanisms driving cell death upon ischemia in the human brain, new model systems are required. Recently, 3D brain organoids, derived from human induced pluripotent stem cells (hiPSCs) have been established. These also allow the preparation of slices maintained in air-liquid interphase cultures, which can be kept for prolonged periods and avoid the necrotic core often seen in 3D organoids.
Here, we employed cortical brain organoid slices (cBOS) derived from hiPSCs to study the consequences of energy deprivation on cellular ATP levels and ion homeostasis in neurons and astrocytes. To this end, we expressed multiple genetically encoded biosensors using adeno-associated viral vector (AAV) delivery, namely the FRET-based sensor ATeam1.03^{YEMK} (ATeam) to measure cellular ATP levels, as well as the ratiometric sensor pHRed and the single wavelength sensor GCaMP for imaging of pH and Ca^{2+}, respectively. The promotors hGFAP or hSYN were used to enable cell-type specific expression of the sensors in astrocytes and neurons. Energy deprivation was induced by brief periods of pharmacological inhibition of glycolysis and mitochondrial respiration ("chemical ischemia"). Our data show that brief chemical ischemia resulted in a rapid decrease in intracellular ATP levels in both cell types in cBOS. The amplitude of this decrease was higher in neurons as compared to astrocytes. ATP levels recovered to values close to the initial baseline within about 10 minutes after washout of the blockers. In addition, our experiments revealed that in neurons intracellular Ca^{2+} levels transiently increased and that cells showed a long-lasting acidification in response to the chemical ischemia.
Taken together, our results demonstrate that human neurons and astrocytes undergo a decline in ATP and a transient loading with Ca^{2+} and protons upon brief energy deprivation. Moreover, they indicate that cBOS are well-suited to study the immediate consequences of metabolic failure on cellular energy and ion homeostasis in an intact minimal network of human brain cells.

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Circadian and ultradian rhythms in the spontaneous activity of insect olfactory receptor neurons

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We examine general membrane properties by a comparative study of spontaneous activity of olfactory sensory neurons (ORNs) in the hawkmoth *Manduca sexta* and the Madeira cockroach *Rhyparobia maderae*. We hypothesize that insect ORNs evolved membrane properties to tune into and resonate with meaningful environmental/social rhythms acting as autonomous multiscale oscillators coupled to but not forced by transcriptional translational feedback loop (TTFL)- based clocks.
Insect ORNs are endogenous circadian clocks that express daily/circadian rhythms in clock gene expression constituting a TTFL clockwork. Currently, it is not known whether daily rhythms in sensitivity and temporal resolution of ORNs of *M. sexta* or *R. maderae* are under mandatory control of this circadian TTFL-clockwork. To study circadian control, we recorded action potential (AP) activity with extracellular *in-vivo* long-term tip-recordings from pheromone-sensitive trichoid sensilla on the antennae of both insect species. Already in the absence of pheromone stimuli, multiscale rhythms in spontaneous AP activity were observed in the hawkmoth. To determine whether/how both ultradian and circadian AP rhythms of ORNs are linked and whether they are controlled via the circadian TTFL clockwork we employed pharmacology and RNAi studies combined with electrophysiology and computational modelling. We found interlinked multiscale rhythmicity in ORNs´ spontaneous activity and searched for responsible pacemaker channels, focusing first on ORCO, the olfactory receptor coreceptor. With antagonists of the spontaneously opening non-specific cation channel Orco we could delete the circadian oscillation and affected ultradian activity rhythms in hawkmoth ORNs. Comparing WT animals with insects with knocked-down TTFL clockwork we search for changes in multiscale rhythms. Aditionally, we present a novel conductance-based theoretical model that incorporates ORCO as a pacemaker ion channel with linear conductance dependent on cAMP concentration. Our model takes into account that cAMP concentrations express daytime-dependent rhythms with maximal concentration during the insect ´s activity phase. By using stochastic differential equations based on the microscopic Markovian states of ion channels, our model can reproduce the observed spike distribution with its circadian oscillations. With these studies we attempt to decipher the ORNs´ multiscale clocks underlying temporal encoding.
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Investigation of glutamate accumulation and neuronal depolarization in metabolic stress conditions

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During metabolic stress, such as ischemic stroke, insufficient energy supply leads to ATP deprivation, resulting in ion imbalances and depolarization of cells. This results in uncontrolled glutamate release and impaired glutamate uptake by astrocytes. However, the role of elevated extracellular glutamate concentrations in metabolic stress conditions remains unclear. We here used the green-fluorescent glutamate sensor **SF-iGluSnFR(A184V)**[1] to investigate the glutamate dynamics during **chemical ischemia** (5 mM sodium azide, 2 mM 2-deoxy-D-glucose, no glucose) in cortico-hippocampal slice cultures from mice. Wide-field imaging after AAV-mediated expression of SF-iGluSnFR(A184V) confirmed synchronous network activity but also revealed spontaneous, local glutamate release events with plume-like properties, reminiscent of recent reports from a migraine mouse model[2]. Plumes were relatively large in size, heterogenous and comparably long-lasting. During chemical ischemia plumes increased in size, duration, and frequency. Silencing the network activity with TTX had no effect on plume occurrence, but inhibition of glutamate transporters with TBOA caused a strong increase in plume frequency. Vice versa, blocking AMPA and NMDA receptors with AP5 and GYKI 53655, respectively, strongly suppressed plumes under baseline conditions and during ischemia. The data, moreover, suggests that plumes might be the main driver of glutamate accumulation during chemical ischemia. Current experiments aim at addressing how much the elevated glutamate concentrations contribute to neuronal depolarization by either activating iGluRs or, indirectly, by activating other ion channels downstream (secondary conductances). We focus here on the calcium-binding transient receptor potential melastatin 4 (**TRPM4**) channel, which has been shown to be activated during ischemic events[3] and to interact with **NMDA** receptors[4]. For this we perform functional experiments on TRPM4 overexpressed in HEK cells as well as in organotypic slice cultures during chemical ischemia.

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Pharmacological inhibition of $Ca_V2.1$-α2δ1 interface suppresses neuronal firing and reduces synaptic density in hippocampal network

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Computational performance of the brain is a function of synaptic connectivity. Presynaptic calcium channels (Ca_V) play a pivotal role in triggering presynaptic neurotransmitter release and therefore are crucial for successful synaptic transmission. Calcium channels are multi-subunit complexes, with auxiliary α2δ subunits being important for trafficking and modulation of biophysical properties of the pore-forming α1 subunit. Gabapentin and pregabalin are prototypical members of gabapentinoid family of drugs that bind to α2δ1 and α2δ2 isoforms and inhibit their interaction with the α1 subunit of high voltage-activated calcium channels. Gabapentinoids have been widely used for treatment of several diseases and conditions, such as convulsive epilepsy and chronic pain. However, to achieve therapeutic efficacy classical gabapentinoids generally require rather prolonged treatment often associated with adverse side effects such as somnolence and dizziness.

In this study, we analyzed the acute and chronic effects of novel gabapentinoid mirogabalin (MGB) on neuronal activity and synaptic connectivity in murine hippocampal cultures. First, we examined the impact of MGB on the spontaneous network activity in hippocampal cultures grown on 120-channel microelectrode arrays (MEAs). We found that a single application of MGB, but not gabapentin, led to strong acute suppression of the neuronal firing in hippocampal networks. This effect of MGB was developmental stage-dependent, with the strongest suppression being evident in mature 3-week-old cultures as compared to 1- or 2-week-old ones. Given the reports on developmental expression profile of $Ca_V2.1$ (P/Q-type calcium channels), next we tested the MGB effect on the network activity under conditions of $Ca_V2.1$ knockout. We found that application of MGB in mature hippocampal cultures lacking $Ca_V2.1$ induced no marked change in the mean firing rate, when compared to respective pre-application baseline levels. In an additional set of hippocampal cultures grown on MEAs, we induced lentiviral overexpression of the α2δ1 subunit during first developmental week and examined the effect of MGB upon reaching mature state. We observed statistically stronger MGB-induced decrease of the mean firing rate in α2δ1-overexpressing cultures, when compared to age-matched naïve control cultures. These data demonstrate that MGB triggers acute suppression of neuronal firing in hippocampal networks, which is associated with the inhibition of interaction between the $Ca_V2.1$ and α2δ1 subunits. To examine the effect of MGB on the synaptic density, we applied MGB in developing 1- or 2-week-old hippocampal cultures and performed immunostaining after 21 days in vitro. We found that in both groups MGB treatment led to significant decrease in the density of glutamatergic synapses, while the effect on inhibitory synaptic density was rather moderate.

Our data demonstrate that MGB acutely affects neuronal network activity and can trigger long-term changes in the structural synaptic connectivity. Taken together, these findings render MGB as promising pharmacological tool for treatment of such syndromes and conditions associated with upregulation of α2δ subunits and/or deficits in synaptic pruning, as chronic and neuropathic pain or autism spectrum disorders.

Allosteric modulation of $GABA_AR$ by sesquiterpenes representing a distinct fraction in volatile oils and plant extracts

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γ-Aminobutyric acid type A receptors ($GABA_AR$) are the most abundant inhibitory ligand-gated ion channels in the central nervous system (CNS). The pentameric $GABA_AR$ can be composed out of a set of 19 different subunits, which are arranged in a clockwise manner e.g. α-β-α-β-γ. The number of subunits expressed underlies a large variability of $GABA_AR$ subunit compositions in the CNS. Moreover, depending on the composition of subunits, $GABA_ARs$ display either phasic or tonic inhibition. An imbalance between excitatory and inhibitory signal transduction is linked to neurophysiological and mood disorders. Treatment options targeting $GABA_AR$ have the aim to enhance the inhibitory function, however, most substances lack specificity to distinct subunits or even subunit compositions, respectively. Hence, the underlying mechanisms and differences between $GABA_AR$ compositions and their specific targeting still need further investigation.

Numerous naturally occurring substances, such as terpenoids in plant extracts, are known to modulate $GABA_AR$. So far, most studies used direct application of these substances at relatively high dosages. In this project, we focus on the long-term effects of physiologically relevant dosages of terpenoids on phasic and tonic $GABA_AR$ compositions. We investigate known synaptic and extra-synaptic $GABA_AR$ compositions to evaluate subunit-specific effects.

We address these questions using transiently transfected HEK293 cells and readouts from primary hippocampal neurons and neurons of the olfactory bulb. Following a pre-treatment of the cells at various time points, functional analysis is carried out via whole-cell electrophysiological measurements. In parallel, alterations in subunit expression levels are monitored using Western blot analysis and immunocytochemical stainings.

Recordings from transfected HEK293 cells are performed in a top-down approach starting with volatile oils, plant extracts, extract subfractions, and finally single substances (e.g. caryophyllene) or metabolized single terpenoids (e.g. caryolanol) or combinations of single substances.

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A secreted protein controls surface expression of a postsynaptic ion channel

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Acid-sensing ion channel 1 (ASIC1) is a trimeric ion channel, activated by a fast drop of extracellular pH. ASIC1a is expressed in the CNS and localized at the postsynapse. It has been shown that ASIC1a can be activated by protons co-released during neurotransmitter exocytosis, thereby contributing to synaptic transmission. ASIC1a has been reported to significantly influence various physiological and pathophysiological processes, including fear related behavior, long-term potentiation and stroke.

We hypothesized that identifying accessory and regulatory proteins of ASIC1 could provide further insights into its function. In a knock-out controlled proteomic screen from mouse brain we identified secreted protein 1 (SEP1) as novel interaction partner of ASIC1. In HEK cells, SEP1 strongly potentiates current density of ASIC1a (3-5 fold, $p<0.001$) without altering its biophysical properties. Furthermore, SEP1 increases surface expression of ASIC1a. Neurons from SEP1-/- mice exhibited a complete loss of ASIC currents, suggesting that SEP1 is indispensable for functional ASIC1a. In hippocampal slice recordings, we demonstrated that SEP1 knockout impairs long-term potentiation (LTP). SEP1 is secreted and, when applied from extracellular, enhances ASIC1a current amplitudes in cells lacking SEP1, indicating a transcellular effect of SEP1. Additionally, we demonstrated that SEP1 is endocytosed and trafficked to the endoplasmic reticulum. In summary, SEP1 represents a new interaction partner of ASIC1a, crucial for the function of the channel.

CKAMP59 (aka Shisa7) is probably an auxiliary subunit of the AMPA receptor complex

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Core subunits of ionotropic receptors such as AMPA receptors (AMPARs) interact with auxiliary subunits that control their surface trafficking, localization and function. CKAMP59 (CKAMP = Cystine-knot AMPAR Modulating Protein; aka shisa7) was identified by sequence similarity to CKAMP44 (aka shisa9), along with CKAMP39 (aka shisa8) and CKAMP52 (aka shisa6) as a potential AMPAR auxiliary subunits. CKAMP59 was found to reduce GluA2-mediated currents when co-expressed in HEK293T cells (Farrow et al, 2015). Deletion of CKAMP59 abolishes LTP and strongly impairs contextual fear-conditioned memory but seems to have little influence on basal AMPA-mediated transmission (Schmitz et al, 2017). Surprisingly, the group of Wei Lu provided evidence that CKAMP59 interacts with GABAA receptors (GABAARs) and influences GABAergic synaptic transmission and at the same time did not affect AMPA receptors (Han et al, 2019).

Due to these contradictory data, it is currently not possible to decide whether CKAMP59 influences the function of AMPA and/or GABA receptors. It is possible that some contradictions are due to different methods and the investigation of different variants of CKAMP59. We are trying to resolve these contradictions using a combination of electrophysiological and biochemical methods and also to identify potential mechanisms by which CKMAP59 may influence AMPA function.In HEK293T cells, we find that CKAMP59 co-expression reduces GluA1- and GluA2-mediated currents independent of the splice variant examined. Furthermore, the data indicate that CKAMP59 is involved in post-translational modifications of the receptor. These modifications in turn may influence the surface abundance of the AMPAR. Furthermore, we can demonstrate in hippocampal CA1 neurons that CKAMP59 has an impact on basal neuronal transmission, as we found a reduction of the AMPAR/NMDAR-ratio after stimulation in the stratum lacunosum-moleculare of CKAMP59-deficient mice. Interestingly, we do not find corresponding changes upon stimulation in the stratum radiatum. In mEPSC recordings, we detect only subtle changes in the kinetics of the AMPA-mediated currents when CKAMP59 is missing.

The data on the influence on the GABAergic synaptic transmission indicate that CKAMP59 may only a small influence on the GABAR function.

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Transient Neonatal Hyperexcitability Induces Persistent Network Alterations in *Scn2a* p.A263V Mouse Model of Epilepsy

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Rapid changes in the nervous system during development render it more susceptible to variations in neuronal excitability, which could lead to life-long consequences. Developmental and epileptic encephalopathies (DEEs) are characterized by early-onset seizures that frequently continue into adulthood, co-morbid with intellectual disability, autism, and developmental delay. We investigated the impact of the *SCN2A* p.A263V pathogenic variant found de novo in patients with conditions ranging from benign self-limiting epilepsies to severe DEEs, using *Scn2a* p.A263V mouse model.

Using *in vivo* local field potential (LFP) recordings in neonates and adults, and electrocorticogram (ECoG) video telemetry we examined network changes across developmental stages. Previously, *in vitro* recordings revealed a gene-dose dependent increase in excitability of hippocampal CA1 and CA3 pyramidal neurons in heterozygous and homozygous mutants at P10-P14, which normalized by P24-P26. Consistent with these findings, *in vivo* recordings showed spontaneous electrographic seizures as early as P3. At P6-P7 100% of homozygous (*mut/mut*) and 50% of heterozygous (*+/mut*) mice exhibited seizures during 1-2h-long recording periods. Seizure frequency was higher in *mut/mut* mice (2.7 [1.8 4.8] seizures/hour, median [Q1 Q3]) compared to *+/mut* mice (0.9 [0.8 2.2] seizures/hour). Moreover, *mut/mut* animals spent 26% of the recording time seizing, with each seizure lasting approximately two minutes in both genotypes.

In adulthood, only homozygous mutants displayed seizures, with a frequency of 0.7 [0.3 2.1] seizures per day. Notably, 18% of *mut/mut* mice died during ECoG recording, invariably during the postictal depression phase following a seizure event. Overall survival analysis revealed increased mortality in *mut/mut* mice but not in *+/mut* mice, with strain-dependent effects. Analysis of cortical network activity across different sleep/wake states in adult ECoG recordings revealed more pronounced changes in *mut/mut* animals, including increased theta power during slow-wave sleep and decreased theta oscillation frequency during wakefulness. Mid-gamma power during wakefulness was increased in both *mut/mut* and *+/mut* mice, showing a gene-dose-dependent effect.

Since neonatal data indicated that at P7 seizures likely originated in the hippocampus, we next closely examined adult hippocampal activity. Homozygous mutants exhibited minor but consistent changes in hippocampal interictal activity, including a decrease of 5.4 [4.5 6.0] Hz in mid-gamma oscillation frequency across all layers, increased theta phase shift across hippocampal layers, and altered theta-gamma modulation in the dentate gyrus middle molecular layer, suggesting disrupted entorhinal cortical input.

Our data suggests that transient neonatal hyperexcitability driven by the *Scn2a* p.A263V mutation may contribute to lasting, gene dose-dependent network dysfunction. These findings point to the early postnatal period as a potentially critical window during which altered excitability could lead to persistent network changes. Future experiments will explore whether targeting this developmental period with genetic approaches such as CRISPRi or antisense oligonucleotides to modulate *Scn2a* levels might help normalize network activity and prevent seizures in adult animals.

Decoding reelin's impact on cholinergic signaling: a novel perspective on neural modulation

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The extracellular matrix protein reelin, named after the reeler-mutant mouse, exerts a multitude of key functions in both the developing and adult brains of mammals. In addition to the well-studied effect of reelin on the migration of neuronal cells during embryonic development, several studies have demonstrated an effect of reelin on neuronal signaling in the mature brain. By modulating both excitatory glutamatergic and inhibitory GABAergic neuronal signaling, reelin affects central neurophysiological processes such as learning and memory formation.
To obtain further insights into the complex impact of reelin on neuronal signaling processes we investigated the direct effect of Reelin on acetylcholine-induced calcium signals, which had not yet been examined. By using the calcium imaging technique on primary neurons, cell lines and rodent brain slices we could show that reelin modulates acetylcholine-induced calcium signals in a receptor-specific manner. Furthermore, reelin increases the nuclear distribution of specific transcription factors, alters the level of different epigenetic protein modifications and modulates the proteome of the treated neurons.
Given that the cholinergic system plays a pivotal neuromodulatory role in the human brain, the direct effect of reelin demonstrated here may have a profound impact on several neuronal signaling processes throughout the brain. Further investigation of the underlying molecular mechanisms will not only complement our knowledge of how reelin affects neuronal signaling, but it will also contribute to a better understanding of how the neuronal cholinergic signaling is regulated in our brain.

Modulation of Rat and Human Acid-Sensing Ion Channel 3 by the Thyroid Hormone T3

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Acid-sensing ion channels (ASICs) are proton-gated Na^+ channels predominantly expressed in the nervous system. Among these, ASIC3 is a critical subunit within the peripheral nervous system, playing an essential role in nociception. The thyroid hormone triiodothyronine (T3) is fundamental in the regulation of metabolism and growth, exerting its effects primarily through binding to nuclear receptors and modulating protein expression. However, few studies have addressed its direct effects on ion channels.

We expressed rat ASIC3 (rASIC3) in HEK cells and found that micromolar concentrations of T3 strongly potentiated the proton-activated currents of rASIC3 (EC_{50} > 100 µM at pH 7.0). Mechanistically, T3 increased the apparent proton affinity of rASIC3, shifting activation curves to a more alkaline pH, resulting in the potentiation of both transient and window currents. Strikingly, for human ASIC3 (hASIC3), T3 induced strong sustained currents at neutral and even alkaline pH, but smaller currents at acidic pH. Even though the affinity of T3 for hASIC3 was not high (EC_{50} > 100 µM at pH7.4), nanomolar concentrations of T3 (10-100 nM) were sufficient to induce ASIC currents. Similar to proton-induced currents, hASIC3 currents elicited by T3 were selective for Na^+ (E_{rev} = +31.2 mV) and inhibited by amiloride (IC_{50} = 264.6 µM), a canonical ASIC inhibitor.

While plasma concentrations of free T3 typically range in the picomolar level, we hypothesize that T3 release from thyroid follicular cells could result in local concentrations high enough to activate ASICs. Our findings indicate that ASIC3 is expressed in a human thyroid follicular epithelial cell line. We are currently investigating the effects of T3 on ASIC3 activity in this cell line.

Preliminary study of analgesic effect of bumetanide on neuropathic pain in patients with spinal cord injury*A*

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Background/objective
The current study evaluated the analgesic effects of bumetanide as an adjunctive treatment in managing neuropathic pain following spinal cord injury. The peripheral expression level of Na-K-Cl-cotransporter-1 (NKCC1) and K-Cl-cotransporter-2 (KCC2) genes in polymorphonuclear lymphocytes (PMLs) assessed as a possible biomarker indicating central underlying mechanisms.
Methods
This open-label, single-arm, pilot trial of bumetanide (2 mg/day) is an add-on treatment conducted in 14 SCI patients for 19 weeks. The whole duration consisted of three phases: pre-treatment (1 month), titration (3 weeks), and active treatment (4 months). Ultimately, nine patients completed the study. The primary outcome variables were the endpoint pain score measured by the numeric rating scale (NRS), and the short-form McGill Pain Questionnaire. Secondary endpoints included the Short-Form Health Survey that measures the quality of life. Blood samples were collected and used for determining the expression of NKCC1 and KCC2 genes in transcription and translation levels.
Results
Bumetanide treatment significantly reduced average pain intensity according to the NRS and the short form of the McGill Pain Questionnaire scores. The baseline expression of KCC2 protein was low between groups and increased significantly following treatment ($P < 0.05$). Through the current study, pain improvement accompanied by the more significant mean change from the baseline for the overall quality of life.
Conclusion
These data might be a piece of preliminary evidence for the analgesic effect of bumetanide on neuropathic pain and could support the potential role of the upregulation of KCC2 protein and involvement of GABAergic disinhibition in producing neuropathic pain.

Poster Topic

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T7-7D Comparing the Ca^{2+}-binding kinetics of Synaptotagmin 1 and 2 at cortical synapses
Simone Brachtendorf, Grit Bornschein, Abdelmoneim Eshra, Jens Eilers, Stefan Hallermann, Hartmut Schmidt

The impact of CAR on glutamatergic synapses

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The cell adhesion protein CAR (Coxsackievirus and Adenovirus Receptor) is expressed in various organs, with particularly high levels in the brain during development and perinatal stages with a reduction in the adult brain. This transmembrane protein is detected in cortex and hippocampus where it colocalize with dendritic and axonic markers. The intracellular PDZ motif of CAR has been shown to interact with PSD95, a key component of the dendritic scaffolding in glutamatergic synapses. The precise action of CAR in the complex machinery of the synapse is yet to be defined. Here we propose a modulatory role of CAR in the stability of the glutamatergic synapses using a CAR knockout (KO) mouse model. The perinatal CAR KO hippocampus exhibits an increased proportion of pyramidal CA1 neurons and interneurons. Additionally, the glutamatergic receptors of the NMDA family changed its subunit proportions in the CAR-KO hippocampus. NMDA-2B receptor, that mediates long-term potentiation (LTP), is increased in the absence of CAR and the NMDA-2A/2B ratio is change in the KO hippocampus from perinatal to adult stages. Moreover, the Neurexin family and postsynaptic proteins are also altered in the absence of CAR. These molecular data suggest potential alterations in electrophysiological properties of the CAR-KO neurons, including a decreased in capacitance and sodium and potassium currents in primary hippocampal CAR-KO neurons. We are currently investigating the effects of CAR deletion on short term plasticity (STP) and in learning and memory. These results aim to contribute to the understanding of synaptic plasticity and its relevance to neurological disorders that affects memory formation.

Presynaptic ATP decreases during physiological-like activity

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Recent evidence indicates that ATP concentrations remain stable during neuronal activity due to activity-driven ATP production. However, the mechanisms of activity-driven ATP production remain controversial. To stabilize the ATP concentration, feedforward mechanisms are required that may rely on calcium or the sodium potassium pump. On the other hand, conventional feedback mechanisms could be triggered by changes in the concentration of the adenine nucleotides. To test the theoretical possibility of feedback mechanisms, we carefully quantified the ATP concentration in presynaptic terminals during synaptic activity in acute brain slices from mice stably expressing a genetically encoded ATP sensor. We first focused on a large presynaptic terminal in the cerebellum, which is specialized for high-frequency synaptic transmission. At physiological temperature and metabolite concentrations (glucose 3 mM, lactate 1 mM and pyruvate 0.1 mM), the resting ATP concentration was 3.3 mM. During strong, presumably non-physiological stimulation, the ATP concentration during activity decreased within a few seconds and recovered within ~20 s. When ATP production was blocked, the rate of ATP decrease was increased several-fold, demonstrating activity-dependent ATP production. Weaker stimulation resembling physiological activity at this synapse (200 action potentials at 20 Hz for 10 s or at 100 Hz for 2 s) caused a decrease in ATP concentration of ~200 µM. To investigate whether such an ATP decrease also occurs at conventional small synapses, we performed similar experiments on cultured hippocampal neurons. Stimulation of 200 action potentials at 10 Hz induced a similar decrease in the ATP signal. Our data reveal a small reduction in ATP concentration in presynaptic terminals during physiological-like activity and provide quantitative constraints on the possibility of feedback mechanisms controlling activity-dependent ATP production.

Regulation of mitochondrial Ca^{2+} homeostasis and neuronal activity by mitochondrial fission factor in AgRP neurons

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Orexigenic agouti-related peptide-expressing neurons (AgRP neurons) are located in the arcuate nucleus of the hypothalamus and play an essential role in systemic energy and glucose homeostasis. AgRP neurons promote hunger, increase food intake, and reduce energy expenditure. These neurons receive neural and hormonal signals from the periphery and modify their activity according to the changing needs of the organism. The activity of AgRP neurons is in part modulated via dynamic adaptations of their mitochondrial network. Mitochondrial dynamics, or cycles of mitochondrial fusion and fission, are known to occur upon changes in the intracellular environment. The shape, size, and function of mitochondria are regulated by numerous molecules, including the mitochondrial fission factor (MFF). Located at the outer mitochondrial membrane, MFF mediates mitochondrial fragmentation. Here, we focus on the role of MFF in AgRP neurons. For that, an AgRP-specific MFF knock-out mouse line was used. We show that mice lacking MFF in AgRP neurons have larger mitochondria in AgRP cell somata and their PVN-projecting axons than control littermates. Patch clamp recordings have shown an increased excitability of AgRP neurons in the $MFF^{\Delta AgRP}$ group.

The increase in mitochondrial size in MFF-deficient neurons resulted in greater mitochondrial Ca^{2+} uptake capacity, higher mitochondrial membrane potential, and enhanced NADH production. These changes collectively boosted neuronal excitability and increased neurotransmitter release. The study reveals that MFF-dependent mitochondrial fission is vital in coordinating mitochondrial Ca^{2+} handling, energy production, and neuronal activity in AgRP neurons. Precise regulation of intracellular Ca^{2+} is crucial for controlling spike frequency and synaptic transmission. There is a clear link between the larger mitochondria seen in MFF-deleted AgRP neurons and increased neuronal excitability and asynchronous synaptic transmission. The larger mitochondria enhance Ca^{2+} uptake, lowering free intracellular Ca^{2+} and reducing the activation of inhibitory Ca^{2+}-activated K^+ currents. This leads to reduced spike frequency adaptation and greater excitability, particularly during high Ca^{2+} load conditions. While the increased mitochondrial size improves Ca^{2+} uptake, it also disrupts the precise localization of mitochondria near synaptic release sites. This misalignment impairs the mitochondria's ability to quickly clear Ca^{2+} from active zones during high-frequency activity, resulting in more asynchronous and less precise synaptic transmission. Despite the increased neurotransmitter release, the reduced precision in

synaptic transmission can lead to less effective signaling at high action potential frequencies.
Since AgRP neuron activation is known to increase food intake, the increased excitability and synaptic drive observed in MFF-deficient AgRP neurons likely contribute to heightened feeding behavior. These findings suggest that MFF-mediated mitochondrial fission is crucial for regulating mitochondrial function, neuronal activity, and feeding behavior in AgRP neurons. The study also highlights the broader significance of mitochondrial dynamics in controlling energy metabolism and neuronal excitability, offering potential insights into how disruptions in these processes could impact metabolic disorders.

Adolescent alcohol drinking compromises excitation/Inhibition balance in adult mouse dentate gyrus granule cells

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Excessive alcohol consumption during adolescence is regarded as a risk factor for the development of alcoholism later in life, but the pathophysiological mechanisms that render the adult brain susceptible to alcohol are largely unknown. Notably, key players of glutamatergic and GABAergic neurotransmission are among the molecular targets of alcohol in the brain. Here we used a mouse model of heavy adolescent drinking to examine how binge drinking in early ages affects excitatory and inhibitory synapses in the adult hippocampus, with particular emphasis on the well-known anatomical and functional segregation of the hippocampus along its longitudinal (dorsal-ventral) axis. To gauge acute and persistent alcohol-induced changes in the excitatory/inhibitory (E/I) balance, we measured excitatory and inhibitory postsynaptic currents using whole-cell voltage-clamp recordings from dentate gyrus (DG) granule cells in dorsal and ventral hippocampal slices from adult mice. Compared to alcohol-näive control mice, heavy drinking in the dark (7 pm to 7 am, 20% alcohol; two-bottle choice paradigm) for two weeks during adolescence produced a long-lasting reduction in excitatory and inhibitory synaptic events, predominantly in ventral DG granule cells. In both dorsal and ventral hippocampal slices from alcohol-näive adults, acute alcohol exposure tilted the E/I balance by enhancing synaptic inhibition and reducing synaptic excitation. Interestingly, when slices from adult mice with an earlier drinking experience were re-exposed to alcohol, inhibitory synaptic drives were less augmented in both regions, whereas excitatory synaptic inputs onto granule cells were less suppressed only in ventral DG. Our study demonstrates that heavy adolescent drinking has lasting consequences on how excitatory and inhibitory synaptic inputs are weighted in the DG. Furthermore, early binge drinking alters the impact of acute alcohol exposure on synaptic functions in adulthood, thereby possibly promoting relapse. Our data also reveal a gradient of susceptibility to early alcohol drinking along the longitudinal axis of hippocampus, with the ventral hippocampus emerging as a key player in mediating its sequelae.

A specific association of presynaptic K^+ channels with Ca^{2+} channels underlies K^+ channel-mediated regulation of glutamate release

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K^+ channels are powerful regulator for cytosolic Ca^{2+} level both at resting state and during action potentials (APs). Given that neurotransmitter release is highly dependent on presynaptic Ca^{2+} levels, it is crucial to understand how K^+ channels contribute to the regulation of release. It is generally believed that presynaptic K^+ channels regulate spontaneous release and AP-evoked release by regulating resting membrane potential (RMP) and AP duration (APD), respectively, but direct experimental evidence is lacking. Furthermore, it remains to be elucidated whether different K^+ channels use common mechanisms to regulate transmitter release. To investigate these issues, we used blockers for three different types of K^+ channels, M-type (K_V7), D-type (K_V1), and high-voltage activated K_V3 channels, and examined their effects on electrical properties (RMP and APD) and synaptic currents (mEPSC and eEPSC for spontaneous and evoked glutamate release, respectively) in hippocampal excitatory autapses. Blockade of K_V7 and K_V1 depolarized the RMP by 2.3 and 3.4 mV, respectively, while increasing the mEPSC frequency by 1.7 and 1.6 folds, respectively. Their effects on mEPSC frequency were greater than the effect expected from RMP depolarization, which was a 1.4-fold increase by 10 mV when depolarization was induced by current injection. Blockade of KV3 had no effect on RMP or mEPSC frequency. Blockade of KV7, KV1, and KV3 increased APD by 1.1, 1.1, and 1.4 fold, respectively, while increasing the eEPSC amplitude by 1.4, 1.7, and 1.5 fold, respectively, showing that their effects on eEPSC amplitude and APD are not proportional. Interestingly, 10 mM EGTA in the pipette solution selectively abolished the effects of K_V7, not K_V1 or K_V3, blockade on the glutamate release without affecting the effects on RMP or APD. Furthermore, the effects of K_V7 blockade were completely abolished when L-type Ca^{2+} channels (LTCCs) and calmodulin (CaM) were inhibited, suggesting that K_V7 may regulate LTCC activities and thus Ca^{2+}/CaM activation. On the other hand, the effects of K_V1 blockade on mEPSCs and eEPSCs were completely abolished when phospholipase C (PLC) was inhibition, suggesting the K_V1-depedent regulation of PLC that may facilitate vesicle priming. The effects of K_V3 blockade were not affected either by CaM inhibition or by PLC inhibition, suggesting that increased Ca^{2+} influx by K_V3 blockade facilitates the release directly. Taken together, our study shows that different K^+ channels regulate glutamate release through different mechanisms.

Elucidating the Nano-architecture of the presynaptic proteome

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Synapse is crucial for neuronal communications. In our brain, there are billions of neurons and each of them can form up to 1000 synapses that are highly diverse. This synapse diversity is the foundation of complex brain functions. Till now, understanding synapse diversity remains a great challenge because we have no idea how different functional synaptic states are encoded by the synaptic proteome. There are at least two major factors that determine synapse diversity: the subcompartmentalized proteome and the regulatory proteins around the core release machinery. My project is designed to answer these two, to resolve the synaptic proteome in a subcompartment-specific manner and in a subtype-specific manner to identify cellular processes that shape synaptic function.

Characterisation of Magi-family synaptic scaffolding proteins in human iPSC derived neurons

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Synapse formation is critical for the wiring of neural circuits in the developing brain. The composition and function of synapses has predominantly been studied in rodent cells. Here we show a novel protocol to generate human iPSC-derived neurons, with relatively rapid synaptic development. We use this protocol to provide an initial characterisation of MAGI-family synaptic scaffolding proteins in human neurons. In rodents the synaptic scaffolding protein S-SCAM/MAGI2 plays a critical role in the assembly and maintenance of synapses and interacts with signalling proteins facilitating synapse to nucleus signalling. Of the three MAGI protein family members MAGI2 shows the highest neuronal expression, while MAGI1 is expressed more evenly throughout cell types and tissues. Loss of MAGI2 results in altered gene expression for multiple important synaptic proteins. Increased expression of S-SCAM/MAGI2 in the human brain on the other hand is associated with schizophrenia. We show that MAGI1 can be found at both inhibitory and excitatory synapses in human neurons and can substitute for the loss of MAGI2. We analyse how S-SCAM/MAGI2 levels at the synapse can influence gene expression.

The Role of Stim and Orai variants in neurotransmission

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Neurotransmission relies on increasing presynaptic cytosolic Ca^{2}+ levels, primarily through voltage-gated Ca^{2}+ channels. The role of additional Ca^{2}+ sources in vesicle release are unclear. The Endoplasmic Reticulum (ER) acts as a major regulator of Ca^{2}+ homeostasis, functioning as both a sink & a source of Ca^{2}+. Contacts between the ER & other membranes, like the plasma membrane (PM), are frequently observed in neurons, including the presynapse. These sites serve as signalling hubs, where ER Ca^{2}+ depletion is detected by Stim proteins in the ER membrane. They activate Ca^{2}+-selective Orai channels in the PM, refilling ER stores & initiating downstream Ca^{2}+-dependent processes in a process called Store-operated Ca^{2}+ Entry (SOCE). Several Stim & Orai (splice) variants with distinct properties exists, complicating understanding their specific physiological roles. We previously identified the neuronal splice variant Stim1B, which localizes to presynaptic boutons & supports short-term enhancement during high-frequency stimulation in an Orai-dependent manner following overexpression. This suggests that the abundances of SOCE components in the presynapse may contribute to functional synaptic heterogeneity. However, the specific contribution of these variants and their potential interaction partners are less well understood. Using a mouse strain that is lacking c-terminal Stim1 splice variants we investigated the impact on glutamate release, vesicle exocytosis and presynaptic cytosolic Ca^{2}+ handling in hippocampal neurons at physiological temperatures. Additionally, a TMT-mass spectrometry screen in hippocampal neurons revealed several potential interaction partners of Stim1B involved in presynapse maintenance and the vesicle cycle. To uncover their endogenous localization and distribution, we employ a CRISPR/Cas9-mediated knock-in strategy, simultaneously examining the localization of Stim1 & Stim1B within the same neuron, as well as Orai2, the main Orai homologue expressed in neurons.

The Coxsackievirus and Adenovirus Receptor - A new Target for Improved Synaptic Transmission

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The Coxsackievirus-adenovirus receptor (CAR), is a transmembrane protein belonging to the Immunoglobulin superfamily (IgSF) that was initially identified as a receptor for viruses associated with myocarditis, pancreatitis, and meningoencephalitis. While CAR is predominantly expressed during heart and brain development, its expression decreases after birth but can be upregulated under pathological conditions, such as myocardial infarction. In the brain, CAR is highly expressed during the pre- and neonatal stages across various cell types, including neurons, astrocytes, and microglia. However, the precise role of CAR within the complex mechanisms of synaptic function remains still unclear. Given that cell adhesion proteins of the IgSF are known to significantly influence critical neuronal processes, such as neurite outgrowth, synaptic plasticity, and synaptic signal transmission, CAR may represent a promising target for therapeutic strategies aimed at stroke recovery under hypoxic conditions as well as the enhancement of memory development.

We investigate the role of CAR in neurons by creating a knockout (KO) model using genetically modified human-induced pluripotent stem cells (iPSCs) via CRISPR/Cas9 technology, which are then differentiated into NGN2-mediated glutamatergic neurons. We assess neuronal functionality and phenotypic differences through electrophysiological techniques, while integrating transcriptomic and proteomic analyses to elucidate the effects of CAR.

By using multi-electrode array (MEA) recordings, significant differences in neuronal activity were observed between CAR knockout (KO) and wildtype (WT) neurons under normal, untreated conditions. Specifically, CAR KO neurons exhibited a markedly higher frequency of action potentials compared to WT neurons. Additionally, when subjected to conditions of chemical induced hypoxia through the administration of $CoCl_2$, CAR KO neurons maintained their elevated activity levels, suggesting a potential enhancement in viability under hypoxic conditions. Future investigations will employ patch-clamp techniques to further explore the influence of CAR on sodium, potassium, and calcium channels in context with synaptic transmission. Alongside these functional tests, preliminary differences in the transcriptome were identified using next-generation sequencing (NGS).

While these findings suggest that the absence of CAR may enhance synaptic transmission and plasticity, the specific functions and mechanisms underlying these effects are still unclear. Furthermore, CAR may be involved in the development of neurological disorders affecting memory. This emphasizes the need for further research to elucidate these relationships and their potential implications for therapeutic strategies.

Octopamine-induced diacylglycerol signaling rapidly enriches active zone with Unc13 for enhanced presynaptic signaling

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Monoamines are neuromodulators that impact mood, arousal and behavior. They can tune neuronal activity through synaptic plasticity via G-protein coupled receptors (GPCRs). Here we use the *Drosophila melanogaster* neuromuscular junction (NMJ) as our model synapse to understand how octopamine, the invertebrate homolog of norepinephrine, rapidly potentiates evoked release at the presynapse on a timescale of few minutes. We show that this depends on the presynaptic expression of the octopamine receptor in the mushroom bodies (OAMB), a receptor known for its involvement in memory and learning in the adult fly brain. Confocal microscopy revealed that the presynaptic scaffolding protein Bruchpilot (BRP) and the essential active zone release site protein Unc13A had increased intensity of immunolabelled signals within one minute incubation. We observed no changes in calcium responses with octopamine incubation using GCaMP calcium recordings. Furthermore, we assessed the involvement of a phospholipase C (PLC) dependent pathway in this potentiation and found that 1-minute octopamine potentiation was attenuated by pharmacological and presynaptic knockdown of the PLC in electrophysiological experiments. Knockdown of presynaptic Unc13A blocked octopamine potentiation, and loss of the N-term of the Unc13 protein delocalizes Unc13 from the plasma membrane. This truncated Unc13 was observed to be rapidly translocated to the plasma membrane via phorbol esters, which are a diacylglycerol (DAG) analogs, and showed similar translocation during 1-minute octopamine incubation. Furthermore, octopamine receptor blocker epinastine blocked translocation of Unc13 to the plasma membrane. The Unc13 protein has a C-terminal C1 domain that binds to DAG and phorbol esters. DAG production comes from PLC hydrolyzing phosphatidyl-inositol-4,5-bisphosphate ($PI(4,5)P_2$) which occurs downstream of a G-coupled protein receptor pathway (GPCR); thus, this process could be pointing to the Gq protein as a driver for this rapid octopamine potentiation process. Unc13A C1 domain point mutations showed enhanced neurotransmission, short term synaptic depression and reduced Unc13 confocal signals following immunostaining, while it is blocking octopamine-induced potentiation. This could mean that the point mutation constitutively “turns on” the Unc13 protein while homeostatically reducing Unc13 protein levels. Live single molecule imaging of a full length endogenously tagged Unc13 revealed that octopamine incubation showed a reduction in Unc13s movements as well as a compaction of the protein at the active zone. Our data points to a mechanism for rapid potentiation of presynapse via a monoamine induced Gq/PLC pathway that compacts Unc13 at the active zone by DAG-mediated recruitment via its C1 domain.

Characterizing the interface of biomolecular condensates at the synapse

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Chemical synapses facilitate neurotransmitter release. Neurotransmitters are stored in synaptic vesicles (SVs), which undergo calcium-regulated exocytosis for release. SVs are recycled through endocytic and sorting processes, demanding precise metabolite balance and organelle organization, including the endoplasmic reticulum and mitochondria. Recent findings suggest that synaptic bouton represents a multiphase system where SV clusters, the active zone, and the endocytic sites all represent examples of biomolecular condensates. How the interfaces between biomolecular condensates and membrane-bound organelles are regulated remains unclear. We first turned to synapsin 1, a highly abundant presynaptic protein, shown to form condensates with SVs. Our data indicate that synapsin condensates wet lipid bilayers in a charge-dependent manner. Furthermore, using graphene-based sensors, we discovered the accumulation of an electric double layer at the interface of synapsin condensates, suggesting their potential to act as mesoscale capacitors. To further assess condensate-to-membrane interactions, we systematically examined the properties of proteins that accumulate at membrane contact sites (MCS), the specialized regions that facilitate the interaction of membrane-bound organelles in cells. Specifically, we focused on a charged region of PDZD8, an ER-resident protein, shown to localize at MCS with mitochondria and endosomes/lysosomes. Using the minimal reconstitution system with purified proteins and model membranes, single-molecule tracking, and ultrastructural analyses, we demonstrated that PDZD8 undergoes phase separation, forming condensates that interact with the lipid surfaces. We further observed that PDZD8 condensates can induce membrane deformations in their vicinity and stitch distinct membranes together, mimicking the physiological morphologies of the proteins at MCS. Together, these data point to the crucial role of charge at the interface of condensates in regulating condensate-to-membrane interactions.

The SNARE protein SNAP25a is intrinsically disordered and highly dynamic in its pre-fusion conformation

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SNARE (soluble N-ethylmaleimide-sensitive-factor attachment receptor) proteins are central for synaptic vesicle fusion at the neuronal synapse. The assembly of the SNARE complex provides the energy necessary for membrane fusion. Structural transitions of the SNARE proteins and their membrane interactions are, however, not well understood at the molecular level. We studied SNAP25a (synaptosomal-associated protein of 25kDa), one of the three SNARE proteins, by NMR spectroscopy to obtain new structural insights in the pre-fusion state: SNAP25a is mostly intrinsically disordered and shows high internal flexibility. Two α-helices form the N-terminal part of the first SNARE motif of SNAP25a, but the remainder of the protein is intrinsically disordered, including the second SNARE motif. We hypothesize that the SNAP25a N-terminus may act as a nucleation site for initiating SNARE zippering. [1]

To assess the internal dynamics of the SNARE protein SNAP25a with residue-specific resolution, we recorded NMR relaxation experiments [2] at different magnetic field strengths, between 600 and 1200 MHz. The field-dependent NMR measurements reveal novel insights into the structural dynamics of the intrinsically disordered state of SNAP25a at the picoseconds to nanoseconds timescale.

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Role of Synaptotagmin 7 in regulating presynaptic function at the *Drosophila* neuromuscular junction

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Calcium contribute to several fundamental presynaptic functions, such as synaptic vesicle (SV) release, recycling, replenishment of the readily releasable pool (RRP) and synaptic plasticity. To properly coordinate these processes presynaptic calcium signals must be read out by calcium sensing proteins like the family of synaptotagmin proteins. While the fast and low affinity synaptotagmin 1 is well known to ensure synchronous SV release, in mammalian synapses the slower but higher affinity synaptotagmin 7 (Syt7) is thought to regulate short term plasticity, RRP replenishment and asynchronous release (Huson and Regehr, 2020). Although the underlying mechanisms are not yet fully understood, recent studies in mammals suggest participation in SV docking as another function of Syt7 (Vevea et al., 2021; Wu et al., 2024). At the *Drosophila* larval neuromuscular junction (NMJ), on the other hand, it has been suggested that many of the functions of mammalian Syt7 may not be conserved in flies (Guan et al., 2020). However, we find considerable conservation of Syt7 function between mammals and flies. Here we present a model in which Syt7 together with the voltage gated calcium channel (VGCC) Dmca1D (homolog to vertebrate Cav1) regulates SV replenishment, short-term facilitation and asynchronous release at the *Drosophila* larval NMJ.

First, we found that Syt7 is required for the fast phase of RRP recovery after high frequency stimulus induced pool depletion. Knockdown of Cav1 further speeds up fast RRP recovery, whereas knockdown of the plasma membrane bound calcium ATPase (PMCA) completely inhibits Syt7 mediated fast RRP replenishment, thus indicating that this function of Syt7 is inhibited by calcium. Therefore, Cav1 channel activation promotes SV recycling (Krick et al., 2021) and decreases fast RRP replenishment (this study), so that synapse function at lower transmission amplitudes can be maintained at high rates for prolonged times. Second, short-term facilitation as observed upon Cav1 channel knock down is abolished upon concomitant Syt7 knockout, indicating that Syt7 is required for short term plasticity as reported for mammals. Third, we found that Syt7 is required for asynchronous release, again as reported for mammalian synapses. At the *Drosophila* larval NMJ, asynchronous release becomes apparent upon reducing expression levels of basigin, an essential cofactor for the PMCA. Reduced calcium extrusion in basigin mutants could trigger asynchronous release by providing more calcium for Syt7. Since asynchronous release has been shown to ensure robust firing during stimulation bursts in vertebrates (Luo and Südhof., 2017), the high frequency activation of *Drosophila* larval muscles during crawling could be affected by asynchronous release as well.
Taken together these findings suggest an interplay of Syt7 and presynaptic Cav1 in the orchestration of short-term plasticity, SV recycling, RRP replenishment and asynchronous release that cooperatively tunes these presynaptic mechanisms to behaviorally relevant activity patterns.

Alternative *Cacophony* splice isoforms mediate fast versus graded synaptic transmission in *Drosophila*

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Synaptic vesicle release in chemical synapses is induced by calcium influx into the active zone (AZ) through voltage gated calcium channels (VGCCs), whereby synapses of fast action potential (AP)-triggered and graded membrane potential-dependent vesicle release are distinguished. Therefore, VGCC must meet the requirements of the particular type of synapse.
In vertebrate central synapses, fast transmitter release is predominantly mediated by Cav2.1 and Cav2.2 channels, while graded synaptic transmission, e.g. in photoreceptors and cochlear hair cells, is guaranteed by specialized active zones and L-type Cav1 channels (Zhang et al., Front. Synaptic Neurosci. 2022).

Cacophony (*Cac*), the only Cav2 homolog in *Drosophila*, mediates fast synaptic transmission, for example at the *Drosophila* neuromuscular junction (NMJ). Contrary to the vertebrate situation, graded transmission in the *Drosophila* visual system from photoreceptors to lamina interneurons is mediated by *Cacophony* (Cav2) rather than Cav1. Therefore, *Cac* channels have to fulfil the physiological requirements to induce transmitter release as triggered by APs but also for membrane potential-dependent graded transmitter release.
Cacophony undergoes extensive alternative splicing, producing at least 18 isoforms, which mediate calcium currents with varying properties. Alternative splicing of mutually exclusive exon pairs affects channel properties such as activation voltage and channel localization at the AZ (Bell et al., eLife, 2024). We focus on the mutually exclusive exon pair IS4A or B, that encodes the fourth transmembrane segment (S4) of the first homologous domain (I), that is part of the voltage sensor.

We find, that *Cac* isoforms that express exon IS4B mediate transmission at the NMJ but do not trigger release at the photoreceptor to lamina graded synapse. *Vice versa*, *Cac* isoforms containing alternative exon IS4A fail to induce vesicle release at the NMJ but are necessary for transmission at the graded photoreceptor to lamina synapse.

Immunohistochemical and confocal data show strong expression of *Cac* isoforms containing exon IS4B but not IS4A in the active zone of the NJM but not at graded synapses in the visual system. Contrary to that, isoforms containing exon IS4A but not IS4B are expressed in photoreceptor terminals in the lamina but not at the NMJ. This is further supported by electrophysiological data as two electrode voltage clamp recordings at the NMJ demonstrate that genomic excision of exon IS4B by CRISPR/Cas9, abolishes transmission at the fast synapse, but is normal after excision of exon IS4A.The other way around, electroretinograms indicate that exon IS4A is mandatory for graded transmission from photoreceptor terminals since excision of IS4A leads to loss of the lamina response.

Based on our data, we propose that differential expression of *Cac* isoforms with either one or the other mutually exclusive exon IS4A or IS4B provides functional diversity of *Cacophony* suited for fast versus graded synapses in *Drosophila*.

Neuronal membrane shape regulation through interplay of the cytoskeleton and BAR-domain proteins

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During our lifetime neurons undergo extensive membrane remodeling: starting from neurodevelopment and the growth of axons and dendrites, through synapse formation followed by selective pruning, during sustained neurotransmission, and, finally, during learning and memory formation. These complex events have to be orchestrated by elaborate signaling cascades, and locally specialized membrane remodeling machineries that are able to couple local intracellular scaffolds (like at the presynaptic active zone or the postsynaptic density) to membrane shape, trafficking organelles, and to the cytoskeleton. In this work we studied how pre- and postsynaptic plasma membrane dynamics is regulated by various classes of BAR domain proteins that couple membrane shape to the control of the assembly and disassembly of the actin cytoskeleton. To this aim we combine work in human induced neurons (iNeurons), cultured murine neurons, with a bottom-up minimal in vitro system consisting exclusively of model membranes and purified proteins.

Controlling the Formation of Molecular Nanoclusters in the Postsynapse

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Memory and learning are shaped by long-term changes in synaptic strength, though the exact molecular mechanisms are not fully understood. A key component in this process is the postsynaptic density (PSD), a complex protein assembly that regulates synaptic function and adaptability. Despite its dynamic nature and variability in structure, the PSD remains stable over time. Recent studies propose that Liquid-Liquid Phase Separation (LLPS) might be involved in PSD organization, but further research is needed to confirm this. To explore the mechanisms governing PSD organization, we took a two steps.

First, we developed a novel simulation framework named PyRID. Second, we constructed a minimal coarse-grained patchy model of the PSD, which consists of interacting multivalent proteins. Using this model, we investigated the collective phase behavior of the PSD. Through molecular dynamics simulations, we observed the emergence of multiclusters that share characteristics of PSDs, particularly when using fixed concentration boundary conditions. This boundary condition was chosen because protein synthesis in dendritic spines plays a significant role in response to varying neuronal activity. In addition to the multicluster phase, we also identified two other distinct phases—Large Cluster and Small Cluster phases—which appeared with specific changes in parameters.

Beyond using the patchy molecular dynamics approach, I collaborated with Dr. Zwicker's group at the Max Planck Institute for Dynamics and Self-Organisation in Göttingen to apply the Cahn-Hilliard equation, a phase field method that incorporates boundary reactions. The results from this model supported our findings, reinforcing the idea that the interplay between boundary concentration and interaction strength is key in the formation of different phases within the system.

Moreover, I validated our simulation results in collaboration with Dr. D'Este and Prof. Hell at the Max Planck Institute for Medical Research in Heidelberg. We compared our model with experimental data obtained through the MINFLUX imaging technique, which provided the spatial locations of PSD95 proteins within dendritic spines under various treatments, mimicking different neuronal activity states. Our analysis shows that PSDs organize into phases similar to those observed in our simulations. This indicates that neuronal activity can influence the PSD's state, specifically the Multicluster, Large Cluster, and Small Cluster phases, further validating our model's assumptions and conclusions.

Neuromodulation of the endbulb of Held to Bushy Cell synapse in the cochlear nucleus by serotonin and norepinephrine

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Despite relying on a common and conserved set of proteins, synapses vary greatly in initial synaptic strength and plasticity, even within the same circuitry or set of pre- and postsynaptic neurons. Neuromodulation is a candidate mechanism to explain some of this variability. Neuromodulators such as monoamines can differentially regulate presynaptic function as well as neuronal excitability. Variability is found also for calyceal synapses of the auditory pathway that are endowed with high synaptic vesicle (SV) release probability (Pvr) and large postsynaptic currents enabling reliable and temporally precise transmission of auditory information. Here we investigated whether the calyceal endbulb of Held – bushy cell (BC) synapse in the anteroventral cochlear nucleus (AVCN) in modulated by Norepinephrine (NE) and Serotonin (5-HT).

Using immunostaining for vesicular 5-HT and NE transporters we found evidence for 5-HT- and NE-releasing varicosities in the AVCN, juxtaposed to both endbulbs and BCs. Furthermore, we detected immunofluorescence for 5-HR7 receptors and 2C-adrenergic receptors in BCs. Using electron microscopy of the cochlear nucleus, we found evidence for putative monoaminergic varicosities in the dorsal cochlear nucleus (DCN). Voltage-clamp recordings from mouse BCs revealed an increase in frequency of miniature excitatory postsynaptic currents (mEPSCs) upon application of NE but not 5-HT. Evoked synaptic transmission was unaffected by the application of NE and 5-HT. Likewise, we did not observe effects of NE or 5-HT on low-voltage-activated K+ (K+LVA) and hyperpolarization-activated mixed cation (HCN) channels during application.

In summary, we report evidence for the presence of monoaminergic innervation in the cochlear neurons but find limited evidence for functional neuromodulation at the endbulb of Held synapse.

Analysis of the function of Nlgn2 at different GABAergic synapse subtypes in the mPFC

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Dysfuncion of inhibitory neurons leads to a disturbance of the fragile balance between excitation and inhibition (E/I) and is a main reason for neurological and psychiatric disorders. Despite the importance of these effects, the underlying mechanisms are poorly understood, limiting the development of therapeutic approaches. γ-aminobutyric acid receptors type A (GABAARs) are key regulators of neuronal activity and are recruited and anchored at inhibitory synapses by organizer complexes,which are composed of transsynaptic adhesion proteins such as Neuroligin2 (Nlgn2). Nlgn2 mutations were shown to cause impaired inhibitory synaptic transmission, GABAAR clustering and psychiatric disorder phenotypes. The specific distribution as well as the precise function and the underlying mechanisms are yet to be understood. Especially the role of Nlgn2 in different GABAergic synapse subtypes in the medial prefrontal cortex (mPFC) is of major interest, since its impairment was shown to be associated with a variety of neurological and psychiatric disorders. Aim of this study is to determine if Nlgn2 functions equally at parvalbumin (PV), somatostatin (SOM) and vasoactive intestinal peptide (VIP) expressing GABAergic synapse subtypes or if there is a specificity for perisomatic synapses formed by PV-positive neurons. By optogenetically activating specific presynaptic neurons and performing simultaneously electrophysiological recordings of the postsynaptic neuron in mouse brain slices, the function of Nlgn2 at the different GABAergic synapse subtypes can be analyzed. Resolving the longstanding question at which GABAergic synapses Nlgn2 exerts its effect is critical for understanding how mutations in Nlgn2 shape neuronal circuits in disease.

Dynamic interactions between presynaptic calcium channel subunits

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At presynaptic boutons, neurotransmitter release is triggered by calcium influx through voltage gated calcium channels (VGCCs). These key transducers of membrane depolarization into intracellular calcium transients are multi-subunit complexes composed of a pore-forming α1 subunit, that associates with an intracellular β and a mainly extracellular (GPI-anchored) α2δ auxiliary subunit. The α2δ subunits promote forward trafficking of the channel to the cell surface, modulate biophysical properties of the channels and have a strong impact on synaptogenesis, which is independent from their functions as channel subunits. Genetic alterations of α2δs are implicated in neurological disorders including autism spectrum disorder, schizophrenia and epilepsy emphasizing their importance. This promotes the question can we identify α2δ subunits that are essential for channel function and/or synaptogenesis?

Although the importance of α2δ subunits in regulating proper neuronal function is evident, the question how they translate their effects on VGCCs, and network development remains. The localization and association of the pore forming subunit, and its auxiliary subunit is still elusive and challenging to study due to a lack of suitable specific α2δ subunit antibodies and the at least partial redundancy of the subunits.

Here we aim to elucidate how α2δ subunits influence VGCC organization and synaptic function by visualizing endogenous α2δs using a CRISPR-Cas9 mediated labeling approach. Single particle tracking in primary hippocampal cultures revealed distinct dynamics of the pore-forming and the auxiliary α2δ-subunits at the cell surface, indicating rather weak and transient interactions. Furthermore, we modulate this interaction pharmacologically to understand the association of VGCC subunits and their significance for network activity. In parallel we probed subunit organization within knock-in models of *Drosophila melanogaster*, where we also used CRISPR based strategies to introduce endogenous labels for the α2δ subunit straightjacket and the Cav2 α1 subunit cacophony. Immunohistochemical and single particle tracking experiments propose that both subunits have only limited interaction on the cell surface, supporting a channel independent synaptogenic function of α2δ subunits in the cell membrane.

Functional and Morphological Characterization of VIP+/ChAT+ Neurons may act as “disinhibitors” in L2/3 of mouse barrel cortex

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The aim of our work is to find out how GABAergic interneurons (INs), in particular the vasoactive intestinal polypeptide(VIP) -expressing INs, are involved in information processing in the barrel cortex (part of the primary somatosensory cortex). We are primarily interested in their electrophysiological and morphological profile and with which functional consequences different subtypes of VIP cells influence their respective cortical target cells. It has already been shown that VIP cells synaptically inhibit two other classes of INs, the somatostatin (SST)- and the parvalbumin (PV)-expressing INs, and thus form a disinhibitory circuit motif. Based on recent studies, VIP cells can be divided into at least three main molecular subgroups: calretinin (CR)-, cholecystokinin (CCK)- and choline acetyltransferase (ChAT)-expressing VIP cells. In the first part of the study, we focused on VIP+/ChAT+ cells, as little is known about this subtype in terms of their functional properties and connectivity. We performed intralaminar (L2/3 to L2/3) paired patch-clamp recordings from VIP+/ ChAT+ to PV+ and SST+ INs in the mouse barrel cortex using acute brain slices from quadruple transgenic mouse lines: VIP-Flp/ChAT-Cre/SOM-Flp/Ai193 and VIP-Flp/ChAT-Cre/PV-Flp/Ai193. Action potentials (AP) of presynaptic VIP+/ChAT+ INs were recorded in the current clamp (CC) at -65 mV using a potassium gluconate-based intracellular solution (IS). Inhibitory inputs to postsynaptic SST INs were recorded in a voltage clamp (VC) at 0 mV using a cesium-based IS. In contrast, postsynaptic PV+ INs were recorded in (VC) at -70 mV using a potassium-based chloride-symmetric IS. All solutions contained 0.5% biocytin to allow post-hoc morphological identification. VIP+/ChAT+ INs show 73 % continuous fitting behaviour and 27 % irregular spikes. They have a medium to high input resistance (250-800 MΩ), a low rheobase (15-80 pA), and high AP amplitudes (+30 to +40 mV). Morphologically, they show a bipolar or bitufted dendritic pattern. With paired recordings, we demonstrated an intralaminar connection success rate of 59.3% of VIP+/ChAT+ to SST+ INs, and 18.5% for their reciprocal interconnection. In contrast, the connectivity rate of VIP+/ChAT+ to PV+ INs was only 25 % for investigated pairs. In the preliminary analysis of short-term plasticity between VIP+/ChAT+ and postsynaptic SST+ INs, both facilitation and depression were observed. These results indicate a significant connectivity of VIP+/ChAT+ cells to SST+ INs in L2/3, which could previously only be identified for the entire VIP population. In further experiments, the two other VIP subpopulations CR+ and CCK+ cells will be investigated in analogue experiments. The aim is to establish a correlation between electrophysiological data and morphological characteristics of the molecularly distinct VIP subtypes and to describe their connectivity patterns to other GABAergic interneurons in more detail. From this we want to derive conclusions about information processing and their impact to maintain the balance of excitation and inhibition in the barrel cortex.

Cellular calcium loading in human cortical brain organoid slices (cBOS) exposed to ischemic conditions

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Rodents are a major model system in neuroscience to study neurological and neurodevelopmental disorders. However, the translation of results generated in rodent models to humans is limited due to species-specific differences. Further model systems are therefore needed to fill this gap and to improve translation of findings to clinical trials. Human induced pluripotent stem cells (hiPSCs) coaxed in three-dimensional (3D) structures, known as brain organoids, are a promising human model system to study the brain and its (dys)functions. However, long-term cultures of these result in the development of a necrotic core in the inner part due to insufficient nutrient and oxygen supply. Air-liquid interphase cultures of whole-brain cerebral organoids (ALI-COs) were recently introduced to overcome this limitation.
Here, we present the generation of cortical brain organoid slices (cBOS) derived from regionalized brain organoids that can be maintained in prolonged cultures at the air-liquid interface. Propidium iodide staining revealed the absence of regions with increased cell death in cBOS under control conditions. Well-organized and connected structures of astrocytes and neurons with putative dendritic spines were identified via immunofluorescence labelling. Whole-cell patch-clamp experiments showed subthreshold synaptic inputs and neuronal action potential firing. Calcium imaging demonstrated spontaneous intracellular calcium transients and evoked network activity upon disinhibition. The presence of ionotropic glutamate receptors was identified by bath applications of glutamate. Pharmacological inhibition of the glycolysis and oxidative phosphorylation (“chemical ischemia”) led to a transient increase in intracellular calcium concentrations. The calcium loading in cBOS after metabolic inhibition increased with a prolonged time in culture. Imaging of changes in neuronal and astrocytic ATP revealed immediate decreases in intracellular ATP after metabolic inhibition.
In conclusion, our results demonstrate that cBOS provide a powerful platform to study cellular (dys)function of human neural cells in intact minimal networks. In addition, molecular pathways leading to cellular damage after ischemic conditions can be elucidated and thereby novel cellular targets for therapeutic approaches can be identified.

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3D MINFLUX combined with DNA-PAINT reveals the orientation and arrangement of Bassoon at the active zone of hippocampal neurons

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Neurotransmitter release and membrane retrieval occur locally inside presynaptic terminals. These processes require precise spatial and temporal coordination relying on an intricate molecular machinery constituting the presynaptic active zone. However, the exact nano structural organization of this machinery is not yet fully elucidated. Scaffolding proteins like Bassoon are suggested to extend as filamentous structures from the presynaptic plasma membrane into the synaptic terminal, and synaptic vesicles are positioned both within and outside the network formed by these filaments. This raises questions as to whether these scaffolding structures exhibit static or dynamic properties, necessitating a more detailed understanding of the nanoscale organization of the active zone machinery. To investigate the nanoscale organization of the active zone super-resolution techniques like STED and STORM microscopy have been used. However, these techniques have limitatons, especially in their 3D spatial resolution capacity. Compared to these techniques, 3D MINFLUX microscopy offers superior 3D precision of localization below 10 nm, allowing one to refine the organization of the active zone machinery. In this study we used 3D MINFLUX combined with DNA-PAINT to analyze the positioning and orientation of the scaffolding protein Bassoon in presynaptic terminals of glutamatergic synapses from hippocampal neurons, achieving a localization precision of 5 nm. This approach allowed us to spatially resolve the N-terminal and the C-terminal region of Bassoon and confirm and refine the notion that Bassoon exhibits a specific orientation at the active zone, with the C-terminal region directed toward the synaptic cleft and the N-terminal region positioned toward synaptic vesicles. Moreover, the location of individual Bassoon molecules was determined in 3D at 5 nm resolution, yielding an unprecedented image of the distribution of Bassoon at the active zone.

Spatio-temporal dynamics of lateral Na+ diffusion in apical dendrites of mouse CA1 pyramidal neurons

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During glutamatergic synaptic transmission, influx of Na+ through voltage- and ligand-gated ion channels drives the depolarization of the postsynaptic cell. The immediate clearance of Na+ from its point of entry is crucial as prolonged elevated intracellular Na+ concentrations lead to persistent depolarizations and impair Na+-dependent secondary transport processes. Prior research has shown that local dendritic Na+ increases are mainly cleared via rapid lateral diffusion, while recovery from global Na+ increases requires the Na+ pump. The biophysical characteristics of Na+ diffusion along spiny dendrites, however, are largely unknown.

In order to address this issue, we employed multi-photon Na+ imaging combined with whole-cell patch clamping of CA1 pyramidal neurons of organotypic mouse hippocampal slices. rapid Fluorescence Lifetime Imaging Microscopy (rapidFLIM) of the Na+ sensor ING-2 revealed an average baseline Na+ of 9.5 mM in primary and secondary apical dendrites. Dendritic Na+ decreased significantly following inhibition of action potential firing by tetrodotoxin, indicating that it is shaped by neuronal activity. To study the diffusion of Na+, we performed line scans of glutamate-induced changes in SBFI fluorescence intensity along dendrites at high temporal resolution. These experiments demonstrated that local glutamate iontophoresis resulted in local increases in dendritic Na+. The amplitude of glutamate-induced Na+ transients rapidly declined with increasing distance from the stimulation site and their latency increased, indicating lateral diffusion along dendrites from the site of influx. Moreover, we found that Na+ also efficiently diffused from dendrites into adjacent dendritic spines. Diffusional dynamics were independent from spine density and dendrite diameter, with apparent diffusions coefficient (Dapp) strongly decreasing over time. Mathematical simulations indicated that the observed spread of Na+ along dendrites followed normal diffusional characteristics and were best replicated using the previously reported diffusion coefficient of 600 µm2/s. Simulations furthermore indicated that dendritic Na+ clearance is mainly mediated by diffusion in the initial stages following the influx. In subsequent phases and with reduced concentration gradients, extrusion through the Na+-pump becomes more relevant.

Taken together our study thus shows that diffusional dynamics of Na+ are largely governed by normal diffusion and are independent from dendrite diameter and spine density. Moreover, they demonstrate that fast lateral diffusion is the main mechanism for the rapid clearance of Na+ from its site of influx, while at later stages, extrusion via the NKA is the dominating mechanism.

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A model investigation of synaptic transmission tuned via the Unc13 protein

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Short-term synaptic plasticity is a fundamental mechanism in neural computation, influencing sensory adaptation and working memory on timescales ranging from milliseconds to minutes. At the molecular level, the interplay between two processes of vesicle release establishes the depressing and facilitating components at a single presynaptic site. The composition of short-term plasticity is driven by the expression of the gene variants (M)Unc13A and (M)Unc13B, which are evolutionary conserved across invertebrate and vertebrate species. In this study, we introduce a modification of the well-established Tsodyks-Markram model for short-term plasticity, incorporating independent facilitating and depressing model components. Our model is constrained through in vivo intracellular recordings within the olfactory pathway of Drosophila melanogaster, enabling accurate reproduction of postsynaptic responses towards dynamic presynaptic stimulation patterns. With our refined model, we emphasize the importance of the interplay between (M)Unc13A- and (M)Unc13B-dominated synapses in fine-tuning transmission dynamics. Moreover, analysis of the tuned parameter sets allows for comparison between different knock-down experiments, providing direct biological interpretability of the model parameters. Our findings contribute to the understanding the molecular basis of short-term plasticity in olfactory processing. Further, our fitted model can be utilized in future studies to design large scale neural network models covering a highly realistic representation of protein-dependent short-term plasticity.

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Synapsin condensates are molecular beacons for actin organization at the synaptic bouton

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Neuronal communication relies on precisely maintained synaptic vesicle (SV) clusters, which assemble via liquid-liquid phase separation (LLPS). This process requires synapsins, the major synaptic phosphoproteins, which are known to bind actin. The reorganization of SVs, synapsins and actin is a hallmark of synaptic activity, but their interplay is still unclear. Here we combined the reconstitution approaches and super-resolution imaging to dissect the roles of synapsin-SV condensates in the organization of the presynaptic actin cytoskeleton. Our data indicate that LLPS of synapsin initiates actin polymerization, allowing for SV:synapsin:actin assemblies to facilitate the mesoscale organization of SV clusters along axons mimicking the native presynaptic organization in both lamprey and mammalian synapses. Understanding the relationship between the actin network and synapsin-SVs condensates is an essential building block on a roadmap to unravel how coordinated neurotransmission along the axon enables circuit function and behavior.

Estimates of quantal synaptic parameters in light of more complex vesicle pool models

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Chemical synapses transmit information by releasing neurotransmitters that are stored in synaptic vesicles (SVs). Within presynaptic terminals the transmitter-filled vesicles are thought to be organized in discrete pools. In fact, the subdivision of SVs into discrete pools is a leading concept of synaptic physiology. It accounts for various phenomena of release and plasticity. To explain specific properties of transmission and plasticity, it has been suggested initially that the readily releasable pool (RRP) of SVs is subdivided into two parallel pools differing in their release probability (Wölfel et al., 2007). More recently, evidence was provided that sequential pools with a single RRP, uniform release probability and with ultra-rapid replenishment of the RRP during high-frequency synaptic activity equally well or even better accounts for most aspects of transmission and plasticity (Miki et al., 2016; Doussau et al., 2017). It was further suggested that a fraction of the presynaptic release sites (*N*) is initially unoccupied by vesicles and furthermore that the number of release sites itself changes with rapid dynamics in an activity-dependent manner. Indeed in most recent papers sequential models dominate over parallel models (Miki et al., 2016; Doussau et al., 2017; Bornschein et al., 2019; Aldahabi et al., 2024), even at synapses like the calyx of Held where originally parallel RRPs were proposed (Lin et al., 2022). On the other hand, there are also recent papers showing that parallel and sequential models may equally well describe several aspects of synaptic transmission (Eshra et al., 2021; Weichard et al., 2023). Notably, all of these papers rely on rather complex modeling to derive their conclusions about the organization of SV pools. Here we propose a framework that identifies specific signs of the presence of series-connected SV pools, using a combination of two experimental electrophysiological standard methods, cumulative analysis (CumAna) and multiple probability fluctuation analysis (MPFA). These signs differentiate between different sequential models and the alternative model with parallel RRPs, without necessitating complex modeling. Our suggestions are aided by simulations that predict the outcome of CumAna and MPFA experiments for different arrangements of SV pools.

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Munc13-3 tightens vesicle docking at a central synapse

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Synaptic vesicle docking and priming determine synaptic strength and plasticity (Aldahabi et al. 2024) and were described as a reversible multi-step process (Kusick et al. 2020, Neher 2024). The presynaptic Active Zone (AZ) protein Munc13-3 has been shown to increase release probability, enable synaptic transmission, and is thought to be a powerful vesicle super-priming factor (Ishiyama et al. 2014). We performed electron tomography on high-pressure frozen and freeze substituted acute cerebellar slices from Munc13-3$^{-/-}$ and littermate mice to investigate structural correlates for its known functional effects. Surprisingly, synaptic clefts at Parallel Fiber to Purkinje Cell (PF-PC) synapses are broadened in KO mice at the edges of the cleft. Additionally, although both control and KO PF-PC synapses possess a large pool of morphologically docked and undocked vesicles, Munc13-3$^{-/-}$ synapses show a significant reduction in those closest to the membrane accompanied by an increase in more loosely docked vesicles. Vesicles in a tightly docked state, located at a distance of 2 nm or less from the AZ surface, are in Munc13-3$^{-/-}$ further away from the membrane than in littermates (see Figure). Furthermore, these vesicles are also smaller and have a reduced lateral extent of the electron-dense material (EDM), which links them to the presynaptic membrane in preparation for fusion. While the EDM extension increases at the edge of control synapses, this effect was absent in Munc13-3$^{-/-}$ mice. Our ultrastructural data imply a role of Munc13-3 at a late priming step of vesicles at PF-PC active zones, with a particular focus on the AZ edge.

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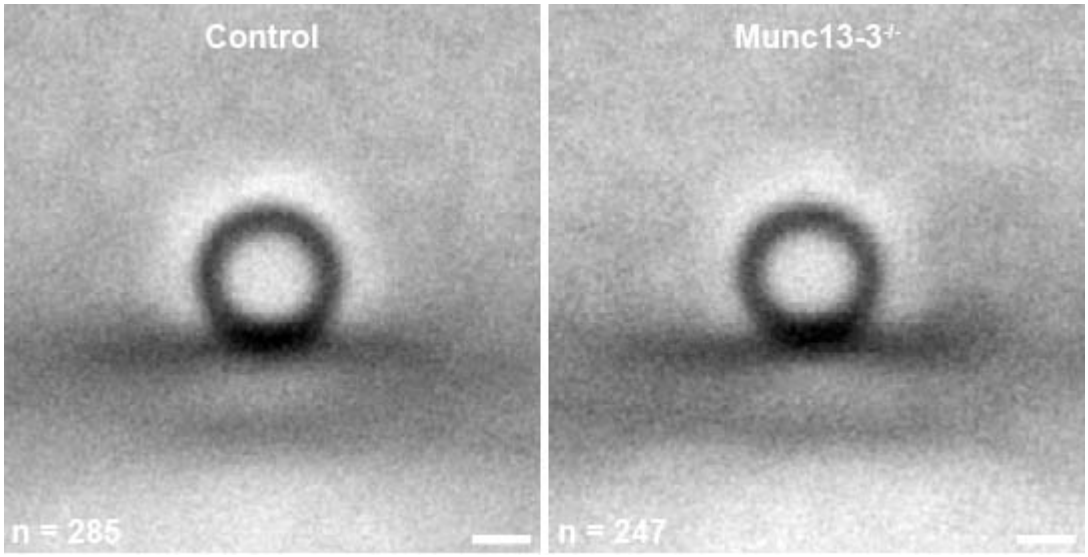

Averaged tightly docked vesicles. Vesicles are further away from the active zone, smaller and have less associated EDM in Munc13-3 KO synapses (scale bar: 20 nm).

Defining the electrochemical properties of synaptic condensates

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Synapsins form condensates that cluster synaptic vesicles (SVs) at the presynapse. Recently, we discovered that synapsin condensates accumulate electric potential at their interfaces. Yet, how other synaptic proteins and membranes affect this process remains unknown. For example, α-synuclein, another highly abundant intrinsically disordered protein that is present at the presynapse, was shown to be recruited to SV condensates altering their material properties and slowing down the rate of synapsin/SV condensate formation. We hypothesize that the positively charged intrinsically disordered region of synapsin will be modulated by the negatively-charged C-terminal tail of α-synuclein. To characterize the electrochemical properties of synapsin condensates, we employ the hyperspectral imaging combined with phasor analysis of ACDAN, an environment-sensitive probe that reports on dipolar relaxation within the condensate nano-environment. Co-incubation of synapsin with α-synuclein in an equimolar ratio resulted in reduced dipolar relaxation. Interestingly, the core of the synapsin/ α-synuclein condensates showed higher hydrophobicity than the surface, suggesting discreet molecular arrangements at the condensate interface. These interfacial alterations may likely be the cause of the distinct wetting properties of synapsin condensates on neutral and negatively charged membranes. Currently, we are employing electric fields to determine the changes that α-synuclein induces in the electric and material properties of synaptic condensates. Together, our study highlights the tunability of electrical and material properties of synapsin/SV condensates by α-synuclein. This modulation offers new insights into the regulation of SV clustering and the architecture of the presynapse, which are particularly important in the context of aging and synucleinopathies.

Comparing the Ca^{2+}-binding kinetics of Synaptotagmin 1 and 2 at cortical synapses

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The Ca^{2+} sensitivity of transmitter release is a major determinant of synaptic fidelity and plasticity. Synaptotagmin (Syt) 1 and 2 are the main Ca^{2+} sensors triggering action potential-mediated release in the brain. Ca^{2+} binding to Syt2, the dominant isoform in the hindbrain, has been studied in detail [1,2]. But for Syt1, the dominating sensor in the forebrain, similar quantitative detail from brain synapses is currently not available.

For quantification of the Ca^{2+}-binding kinetics of both isoforms in the context of their intact release machineries we combined Ca^{2+}-uncaging [1–3], two-photon G/R Ca^{2+}-imaging and patch-clamp electrophysiology. Syt1 and Syt2-triggered release was quantified in pairs of connected layer 5 pyramidal neurons (L5PNs) in the S1 somatosensory cortex of mature mice and in pairs of connected cerebellar Purkinje cells (PCs) of young mice, respectively. Expression of Syt1 in L5PN and Syt2 in PC boutons was ensured by antibody staining. To quantify the local intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) at the release sensor, $[Ca^{2+}]_i$ was uniformly elevated in presynaptic terminals from a caged Ca^{2+} compound by brief UV-laser flashes. Changes in the presynaptic G/R fluorescence were measured at individual boutons by point-mode two-photon imaging and converted to $\Delta[Ca^{2+}]_i$ based on cuvette calibrations. The corresponding EPSCs were recorded and synaptic delays and deconvolution-based release rates were quantified.

Syt1-mediated release typically started at $\Delta[Ca^{2+}]_i$ above ~4 µM and peak release rates increased until $\Delta[Ca^{2+}]_i$ of ~30 µM, saturating thereafter with no substantial further increase up to $\Delta[Ca^{2+}]_i$ of ~100 µM. Synaptic delays decreased concomitantly. Syt1-triggered release had a steep Ca2+-dependency in a dynamic range between $\Delta[Ca^{2+}]_i$ of 10 to 30 µM that was covered by action potential-evoked release and could be described by a Hill function (EC50, 20 µM; Hill coefficient, 3.57). In contrast Syt2-triggered release showed a much shallower Ca^{2+}-dependency in particular in the dynamic range, without saturation at the higher $\Delta[Ca^{2+}]_i$. Established models of Syt2-triggered release [1,2] well described our Syt2 data from PCs but these models could not be fit to the curve of Syt1-mediated release from L5PN boutons. We developed a kinetic model of Syt1-triggered release that well fit our data and reproduced the significant differences to Syt2-triggered release. Our results suggest that Syt1 optimizes the release machinery of L5PN synapses for high reliability at moderate local Ca2+ elevations and for high plastic controllability, in particular within the dynamic range.

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Poster Topic

T8: Synaptic Plasticity, LTP, LTD

T8-1A Altered Inhibitory Circuits: The Effects of iTBS900 on GABAergic Synapses in CA1 Pyramidal Neurons
Ramya Rama, Martin Mittag, Peter Jedlicka, Andreas Vlachos

T8-2A Experience-dependent modulation of oxytocin neurons during postpartum
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T8-3A Calcium mediated presynaptic homeostatic plasticity at the *Drosophila* NMJ
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T8-5A An iPSC derived human neuronal 3D model system for studying dendritic spine pathology in psychiatric disease
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T8-1B All-optical investigation of the role of CaMKII on long-term plasticity in the hippocampus
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T8-2B Plasticity of Electrical Synapses between L1 Interneurons in the medial Prefrontal Cortex
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T8-3B The Role of Mechanics for Neuronal Plasticity
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T8-4B Marking active neurons using immediate early genes: FOS vs NPAS4
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T8-5B The role of the endoplasmic reticulum in synaptic plasticity
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T8-6B Role of neuronal activity on microglia-mediated synapse refinement and circuit stabilization following incomplete spinal cord injury
Fritz Kagerer, Nina Heinrichs, Almir Aljovic, Florence Martine Bareyre

T8-1C Casein kinase 2 controls functional and structural synaptic plasticity at the Drosophila NMJ
Lena Maria Lion, Zeeshan Mushtaq, Jan Pielage

T8-2C Mapping Multisite Network-wide Synaptic Transmission and LTP in the Hippocampal Network
Shahrukh Khanzada, Xin Hu, Brett Addison Emery, Hayder Amin

T8-3C Diurnal varations in the contribution of mGlu5 receptors to hippocampal synaptic plasticity
Janna Maria Aarse, Denise Manahan-Vaughan

T8-4C Mechanisms for activity dependent adjustments of quantal size at the *Drosophila* NMJ
Kristina Vanessa Kolb, Carsten Duch

T8-5C Frequency-dependent synaptic plasticity and NMDAR subunit content are distinct in supra- and infrapyramidal blade of the dentate gyrus in freely behaving animals
Christina Strauch, Juliane Böge, Olena Shchyglo, Valentyna Dubovyk, Denise Manahan-Vaughan

T8-1D Repetitive magnetic stimulation induced synaptic plasticity relies on cooperative pre- and postsynaptic activation
Christos Galanis, Maximilian Lenz, Mohammadreza Vasheghani Farahani, Andreas Vlachos

T8-2D Functional and molecular mechanisms underlying plasticity-mediated CNS recovery after spinal cord injury in adulthood and aging
Adna Smajkan, Julie Fourneau, Hannah Peedle, Florence Martine Bareyre

T8-3D Modulation of activity-dependent synaptic plasticity by the AMPAR interacting-protein PRRT2
Eric Jacobi, Muhammad Aslam, Jakob von Engelhardt

T8-4D Interaction of actin dynamics and spine geometry as a synaptic tag
Mitha Thomas, Michael Fauth

T8-5D Effects of adolescent stress on synaptic transmission and plasticity in the adult mouse dentate gyrus
Nadja Treiber, Fang Zheng, Christian Alzheimer

T8-6D Dendrodendritic inhibition of mitral cells
Joel Kappen, Daniela Hirnet, Christian Lohr

Altered Inhibitory Circuits: The Effects of iTBS900 on GABAergic Synapses in CA1 Pyramidal Neurons

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Intermittent theta-burst stimulation (iTBS) has emerged as a promising therapeutic tool in neurology and psychiatry. It is FDA-approved for the treatment of major depressive disorder (MDD), with short treatment durations and lasting effects on neural circuits. iTBS delivers bursts of high-frequency magnetic pulses in a pattern that mimics the brain's natural theta rhythm, altering neural plasticity and improve brain function. In previous studies, we demonstrated that 10 Hz rTMS induces long-term depression (LTD) of dendritic inhibition, with somatic inhibition remaining unaffected. In the present study, we investigated the effects of a pulse-matched iTBS protocol (iTBS900) on inhibitory synapses to better understand the mechanisms of action. Using entorhino-hippocampal slice cultures, we examined the iTBS-induced plasticity of GABAergic synapses on CA1 pyramidal neurons. Our experiments reveal that iTBS900 significantly reduces GABAergic synaptic strength 2–4 hours after stimulation. Further analysis revealed that iTBS900 predominantly affects somatic and proximal inhibition, as evidenced by sorting rise times in relation to mIPSC amplitudes, while leaving distal dendritic inhibition unaffected. Ongoing work, including multi-cell patch clamp recordings, immunohistochemistry, and electron microscopy, aim to confirm and extend these results. These results highlight the input-specific effects of different rTMS protocols: iTBS900 modulates inhibitory circuits differently than the dendritic inhibition reduction induced by 10 Hz rTMS. These findings provide new insights into rTMS-induced plasticity, paving the way for the design of protocols that could selectively target somatic versus dendritic inhibition in the human cortex.

Experience-dependent modulation of oxytocin neurons during postpartum

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Naturalistic behaviors in mammals are triggered by specific sensory cues and strongly influenced by hormonal levels. Mouse maternal behavior, such as nursing and pup retrieval behavior, is enabled by sensory stimuli (i.e. vocalizations and somatic touch) from the offspring and dependent on the release of the hypothalamic neurohormone oxytocin (Menon & Neumann, 2023; Valtcheva et al., 2023). Pup stimuli, however, are often aversive to virgin females (Lecca et al., 2023; Schiavo et al., 2020) and fail to activate the virgin oxytocin system (Valtcheva et al., 2023; Carcea et al., 2021). What experience-dependent changes in the postpartum hypothalamus contribute to the integration of sensory cues from the offspring to enable infant-oriented behaviors in new mothers remains unexplored. Here, we investigate the synaptic and circuit mechanisms underlying the sensory-hormonal coupling in the postpartum hypothalamus necessary for maternal behavior. We specifically tested experience-dependent synaptic plasticity at different sensory inputs (auditory and somatosensory) to hypothalamic oxytocin neurons in virgin and maternal mice to define the molecular and synaptic underpinnings which actively participate in refining maternal sensitivity to infant stimuli during the postpartum period. We combine in vitro whole-cell recordings coupled with optogenetic activation of different synaptic inputs in transgenic mice, viral tracing approaches and immunohistochemistry to study this.

Calcium mediated presynaptic homeostatic plasticity at the *Drosophila* NMJ

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At the presynaptic terminal of chemical synapses numerous calcium-dependent processes like synaptic vesicle (SV) release, SV recycling, and synaptic plasticity occur side by side and must be dynamically adapted to ensure synapse function. These mechanisms operate in parallel but have different though partially overlapping spatial and temporal requirements.
We recently showed that at the *Drosophila* neuromuscular junction (NMJ), an established model for glutamatergic synapse function, a separate regulation of different Ca2+-dependent processes is achieved through division of labor between two voltage-gated calcium channels (VGCCs). The Cav2 homolog, cacophony (cac), localizes to active zones and mediates evoked SV release, while the Cav1 homolog, DmCa1D, localizes outside the active zone and augments SV recycling and short-term plasticity. The calcium extrusion pump PMCA isolates these functions and ensures stable release probability by protecting the active zone from other presynaptic calcium signals (Krick et al., PNAS, 2021).
To preserve stable synaptic transmission, perturbations of synaptic strength, are precisely compensated for by homeostatic plasticity. At the *Drosophila* NMJ, presynaptic homeostatic plasticity (PHP) includes mechanisms that adjust release probability and are temporally and functionally divided into an initiation and a maintenance phase. Numerous well-studied mechanisms contribute to this; however, a key question remains: does the transition from the initiation to the maintenance phase permit the re-initiation of PHP in response to an additional perturbation?
Recent studies have shown that during the initiation phase of PHP, an increased number of cac channels are recruited to the active zone (Ghelani et al., Science Advances, 2023). We are currently investigating whether the number of cac channels remains consistent during the maintenance phase or if a reset occurs, which would then enable PHP re-initiation.
We have found that Cav1 and PMCA play critical roles in the initiation phase of PHP. PMCA activity regulates the functional coupling distance of Cav1 channels to SVs and thus also determines the impact of Cav1 mediated Ca2+ influx on synaptic transmission. However, to maintain PHP, Ca2+-signaling must outlast VGCC-mediated Ca2+-signals. Combining *Drosophila* genetics, electrophysiology and imaging techniques we are currently investigating whether signaling downstream of Cav1 and PMCA is necessary for the PHP maintenance phase. Here we focus on the role of ER Ca2+-signaling and the regulation of plasma membrane ER interactions. The tuning of store operated calcium entry (SOCE) via STIM-Orai channels and calcium induced calcium release (CICR) through ryanodine receptors may be key in maintaining increased release probability via elevated intracellular Ca2+ levels. This mechanism may also help to restore the adaptive potential of PMCA and Cav1 to compensate for additional future perturbations.

Postsynaptic cAMP signaling does not induce LTP at hippocampal synapses

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Cyclic adenosine monophosphate (cAMP) is a ubiquitous second messenger that, when elevated by forskolin, induces long-term potentiation (LTP) of synaptic transmission. The mechanism is thought to depend on activation of the PKA-CREB pathway in the postsynaptic neuron. However, most studies have relied on pharmacological tools whose actions are not confined to specific cells or synaptic compartments. Here, we use photoactivatable adenylyl cyclases to elevate cAMP in a single hippocampal CA1 neuron, in many presynaptic (CA3) or postsynaptic (CA1) neurons, or in all excitatory neurons to investigate cell-autonomous and network-level effects of cAMP.

Surprisingly, optogenetic elevation of cAMP in single postsynaptic CA1 neurons did not induce LTP despite strong PKA activation. On the other hand, increasing cAMP in all excitatory neurons induced LTP and strong FOS expression, similar to forskolin application. Furthermore, increasing cAMP in all excitatory neurons increased spiking and triggered the formation of new dendritic spines, whereas single-cell cAMP did not induce any change in these parameters.

Our results question the concept of a direct intracellular signaling pathway from cAMP via CREB activation to synaptic LTP. Rather, cAMP seems to increase neuronal excitability and boost transmitter release, processes that are conducive but not sufficient for LTP. If applied throughout an intact, synaptically connected network of neurons, these relatively subtle physiological changes drive bursting activity and thus create perfect conditions for LTP. The importance of astrocytic cAMP remains to be investigated.

An iPSC derived human neuronal 3D model system for studying dendritic spine pathology in psychiatric disease

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The majority of excitatory synapses in the cortex is located on dendritic protrusions of the postsynaptic neuron called dendritic spines. Dendritic spines consist of a bulbous head and a thin neck connected to the parent dendrite. Spines compartmentalize biochemical signals and contain molecular signaling complexes regulating neuronal plasticity.
Postmortem tissue samples from psychiatric patients show dendritic spine abnormalities. Furthermore, genome-wide association studies and transcriptomic analyses identified numerous risk genes implicating synaptic dysregulation in psychiatric disease.
There is a need for a human model system integrating the polygenetic complexity of psychiatric disease for studying dendritic spine pathology. In particular, exploring impairments in spine plasticity is highly important since neuronal plasticity is considered a fundamental mechanism underlying recovery from disease.
We generated human neuronal 3D cultures (“spheroids”) combining two types of cells derived from human iPSCs: 90% neuronal progenitor cells and 10% neurons induced by expression of the transcription factor neurogenin 2 of which 2% expressed in addition the fluorescent protein GFP for imaging. GFP labelled neurons were imaged by 2-photon microscopy for up to 6 months. The spine density of labeled neurons increased over the course of culturing reaching 0.109 ± 0.016 spines/µm (mean ± SEM, n = 5 cells) after 5-6 months. In time-lapse recordings, filopodia and spines display structural dynamics reminiscent of neuronal plasticity.
In conclusion, we developed and characterized a novel human model system for studying dendritic spine pathology and impaired plasticity in psychiatric disease as basis for better understanding disease etiology and for developing new therapeutic strategies.

All-optical investigation of the role of CaMKII on long-term plasticity in the hippocampus

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Synaptic plasticity, inducing long-lasting changes in synaptic efficacy and structure, is a major mechanism of information storage in the brain. Calcium–calmodulin-dependent protein kinase II (CaMKII) is one of the most important memory molecules that, through its autophosphorylation feature, transforms transient activation due to synaptic activity-related increases in calcium into longer-lasting changes in synaptic strength. Whether CaMKII is essential for both induction and/or maintenance of synaptic plasticity has been controversial. We took advantage of optogenetic tools to investigate the role of CaMKII in synaptic plasticity, by inducing synaptic plasticity and manipulating relevant signaling pathways at the same time. Specifically, we induced spike-timing-dependent plasticity (STDP) at Schaffer collateral synapses in rat hippocampal slice culture by independently stimulating the pre- and postsynaptic neurons expressing spectrally separated channelrhodopsins with violet and red light, respectively. The all-optical protocol induced timing-dependent long-term potentiation (tLTP) increased synaptic strength both acutely (about 30 minutes, early tLTP) and more interestingly, chronically (3 days, late tLTP). When we co-expressed a photoactivatable inhibitor of CaMKIIα (paAIP2) only in postsynaptic neurons to inhibit CaMKIIα during the during the 60 s induction protocol, acute tLTP was abolished. Unexpectedly, 3 days later the late tLTP was apparent and indistinguishable from neurons with active CaMKIIα during induction. STDP-induced CA1 neurons received significantly stronger input than their neighbors 3 days after stimulation, a delayed potentiation that appears to be independent of CaMKIIα activity during induction of early tLTP. Coincidently, expression of the immediate early gene c-Fos following induction was also independent of CaMKIIα activity. Interestingly, an inhibitor of protein kinase Mζ, applied 3 hours after STDP induction prevented late tLTP.
Rather than inhibiting CaMKII, 100 s illumination of photoactivatable CaMKII (paCaMKII) in CA1 neurons was sufficient to induce functional early LTP, structural and ultrastructural synapse alterations but no late LTP. By continuous longitudinal large-scale tracking of excitatory synapse and spine dynamics for about 14 hours after paCaMKII activation, we noticed that the structural plasticity developed in a spatially clustered manner. Together, these data suggest that activity-dependent potentiation of synaptic inputs has two phases: CaMKIIα is necessary and sufficient for the induction of early LTP. A second, CaMKIIα independent mechanism, dependent on the persistent activity of protein kinase Mζ, is responsible for the selective strengthening of inputs days later.

Plasticity of Electrical Synapses between L1 Interneurons in the medial Prefrontal Cortex

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The role of electrical coupling in neuronal processing throughout the mammalian CNS has become increasingly apparent over recent years. Electrical synapses undergo regulation and plasticity comparable to that of chemical synapses. Electrically coupled neurons form discrete networks that can have a defined resonance frequency and be synchronized or desynchronized by the strength of their electrical synapses. Formed by gap junctions, electrical synapses allow a bidirectional flow of ions between neurons. Particularly among clusters of interneurons (IN), they may facilitate the short-range spread of activity that can influence the subsequent inhibition of target cells. We previously demonstrated that electrical synapses between neurons in the thalamic reticular nucleus show use-dependent and Ca^{2+}-dependent plasticity. In this report, we provide conclusive evidence of functional electrical synapses between Layer 1 (L1) IN in the medial prefrontal cortex (mPFC).

The mPFC integrates and coordinates input from multiple brain regions, serving as an executive hub for the interplay between sensory, limbic, and motor information. L1 IN are essential for signal processing within the mPFC and electrical coupling may help to sort, jitter and integrate input signals to contribute to brain oscillations by (de-)synchronizing neuronal activity.

The electrical synapses identified in L1 of mPFC are asymmetric in their signal conduction, undergo use-dependent and frequency-dependent plasticity, and their coupling strength and low-pass filtering properties appear to be dependent on intrasomatic distance. The application of mefloquine, a Cx36 blocker, led to a decrease in transjunctional conductance (G_j), accompanied by an increase in the input resistance (R_{in}) of at least one of the coupled cells.

Previously, plasticity of Cx36-based electrical synapses was induced by (de-)phosphorylation or by Ca^{2+}/Calmodulin binding. Key pathways influencing the phosphorylation state include cAMP-dependent protein kinase A (PKA) and Ca^{2+}/Calmodulin-dependent protein kinase II. Their activity depends on intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and is modulated by group I or group II metabotropic glutamate receptors via $G_{i/o}$ or G_s proteins.

Electrical synaptic plasticity was examined under five stimulation paradigms, designed to simulate neuronal activity in the PFC-typical theta range, involving unilateral and bilateral communication between coupled neurons. All stimulation protocols resulted in a decrease in R_{in} of coupled cells. Changes in the coupling coefficient (CC) are probably determined, at least in part, by changes in the R_{in}, as changes in G_j were non-significant but followed similar trends. The plasticity range of electrical synapses appears to depend on their initial state, with stronger synapses being less modifiable, as reported previously. Changes in CC and G_j were also elicited when transiently shifting the membrane potential of one of the two cells to the range where voltage-dependent Ca^{2+} channels are known to be activated. Activation of $G_{i/o}$ by mastoparan led to high potentiation of G_j, indicating that plasticity of electrical synapses also

depends on metabotropic glutamatergic pathways, as previously shown in the thalamic reticular nucleus. Taken together, these findings indicate different mechanisms of electrical synaptic plasticity, acting on different time scales and in different directions, highlighting the importance of further research in this field.

The Role of Mechanics for Neuronal Plasticity

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The formation of learning and memory requires neuronal plasticity, which results in functional rearrangements of neuronal networks for the purpose of encoding meaningful information. While neuronal networks undergo significant rearrangements during the developmental period of the young brain, they are much less plastic in adulthood. This is because stable neuronal circuits are required for efficient brain function and persistent memory. A significant factor that inhibits structural rearrangements and neuronal plasticity in the mature brain is a specialized form of the extracellular matrix (ECM). This perineuronal ECM is a mesh-like structure comprising a backbone of hyaluronic acid and chondroitin sulphate proteoglycans, which interact with a range of secreted and membrane-associated molecules. The formation of the perineuronal ECM and the associated decline in neural plasticity occur concurrently with alterations in the mechanical properties of brain tissue, which may be attributed to the physical characteristics of the perineuronal ECM. Therefore, ECM-derived changes in the mechanical properties of the brain tissue may contribute to network stabilization and the decline in neuronal plasticity observed in the adult brain. Nevertheless, thus far, this potential link has not been tested directly. The objective of this study was to characterize the contribution of the ECM to the mechanical properties of the brain in coronal brain sections from adult mice using nano-indentation atomic force microscopy (AFM). Given the ECM is distributed inhomogeneously across the cortical layers, we used AFM to separately measure the stiffness of these cortical layers. In addition to the abundance of the ECM, layer-specific variations in cell density also contribute to local differences in the tissue's mechanical properties. To eliminate the contribution of the ECM to the measurements, the ECM was digested using chondroitinase ABC enzyme, and then the stiffness of the brain slices was then compared with that of the control sample. Subsequently, we characterized the ECM density using immunohistochemistry within the mouse cortex. It was observed that the cortex exhibited differing stiffnesses between layers with varying ECM compositions. Moreover, it was demonstrated that the removal of the ECM altered the mechanical properties of these cortex layers. Collectively, these results provided valuable insight into the impact of the ECM on brain mechanics and the role of the viscoelastic properties of the ECM in neuronal plasticity. Additionally, future studies will investigate the effects of different mechanical properties of OHA/GEL (oxidized hyaluronan) hydrogels on neuronal outgrowth.

Marking active neurons using immediate early genes: FOS vs NPAS4

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Nuclear calcium is known to induce expression of the short-lived immediate early genes (IEG) *FOS* and *NPAS4*. FOS protein expression or promoter activity is widely used to mark active neurons during memory formation and recall with the goal of identifying engrams. We find, however, that FOS protein level is not always high after neurons spike. The nuclear localized photo-convertible calcium indicator H2B-CaMPARI2 allows 'freezing' intracellular calcium levels at a particular period of time, which can be read out hours later and after fixing the tissue. Driving neuronal activity in organotypic hippocampal slice cultures with bicuculline increases both calcium and FOS, which are positively correlated across the population of neurons in the slice but are not correlated when the calcium and FOS levels in individual neurons are compared. FOS expression decreases on subsequent days following repeated stimulation of slices with bicuculline in the dentate gyrus and area CA1. This effect was mediated by persistent expression of the FOSB splice variant ΔFOSB and recruitment of HDACs. Thus, FOS protein level is highly dependent on the history of neuronal activity and many active neurons, in particular those recently active, will not express FOS. This leads to questions regarding the reliability of FOS as a marker for neuronal activity. Another of the IEGs, *NPAS4* is reported to be better correlated with membrane depolarization and calcium influx. We observe that after bicuculline there are many neurons which express either FOS or NPAS4 in addition to neurons expressing both proteins. Currently we are investigating the relationship between calcium, activity history and NPAS4 protein levels.

The role of the endoplasmic reticulum in synaptic plasticity

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The endoplasmic reticulum (ER) is a complex organelle. It is distributed throughout neurons, including the soma, axon and dendritic arbor, and is present in some dendritic spines. While it is well known for its role in protein synthesis and transport, and for its ability to release calcium, less is known about how it affects synaptic plasticity. It has previously been shown that ER is stably present in 10% of dendritic spines while a further 60% are visited by transient ER every 5 h, with functional consequences for plasticity (Perez-Alvarez et al., Nat Commun. 2020). Here we aim to investigate the role of the ER in dendritic spines, the conditions under which it acts as a calcium source or sink, and how this affects synaptic plasticity.

Using two-photon microscopy, we investigate dendritic calcium dynamics of neurons in rat organotypic hippocampal slice cultures. To examine the effects of SERCA pump accessory subunits, collectively known as “regulins”, on calcium responses, we use genetically targeted, low affinity ER calcium sensors. Sensors with different affinities allow us to quantify ER calcium responses while we manipulate calcium dynamics via pharmacology, electrophysiology and novel optogenetic approaches. Collectively, these tools allow us to disentangle the contribution of different calcium sources at dendritic spines during plasticity protocols.

Role of neuronal activity on microglia-mediated synapse refinement and circuit stabilization following incomplete spinal cord injury

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Spinal cord injuries can have devastating impacts on patients' lives. While complete injuries often result in permanent disabilities, incomplete spinal cord injuries (iSCI) may allow for some level of recovery and regained function. Previous findings of our lab suggest that this functional recovery depends on the formation of intra-spinal detour circuits bypassing the lesion. For example, we have demonstrated that the corticospinal tract (CST) can remodel spontaneously following iSCI. The first step of this remodeling is the sprouting of newly formed hind-limb CST collaterals, that make synaptic contacts with, among others, long propriospinal neurons (LPSNs) in the cervical cord grey matter. In a second step, the refinement phase, unwanted synapses get removed to refine the circuit. While the regulation of the initial collateral formation has been studied recently in our lab and is mostly understood, the cellular and molecular events guiding the refinement phase are currently unknown.
In previous work, we identified a critical role of neuronal activity during the initial circuit formation phase. Here we sought to understand the role of silencing LPSNs during the subsequent refinement phase. To do so, we used chemogenetics coupled to gene therapy to silence the activity of LPSNs between five to eight weeks following spinal cord injury, a time frame during which synapse refinement occurs. Our preliminary data suggest that LPSN silencing during the refinement phase leads to a decrease in mature synapses of CST collaterals, while the number of immature synapses stays unchanged. These results suggest that proper neuronal activity of LPSNs during refinement is crucial for maintaining mature synapses and with that the newly formed circuit. Since microglia are known for its ability to prune synapses during development of the brain, we expect microglia to also play a vital role in the context of refinement of the detour circuit after iSCI. This is why, as a further step, we want to investigate microglia involvement in the refinement phase during silencing of LPSNs. Also, we plan to analyse whether the decrease in mature CST synapses we found has an impact on behavioral recovery. These results will help us to understand how neuronal activity of a subset of cells within the detour circuit can be manipulated to maintain the circuit and increase functional recovery.

Casein kinase 2 controls functional and structural synaptic plasticity at the Drosophila NMJ

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The precise regulation of synaptic connectivity and plasticity is essential for maintaining neuronal function, and perturbations of synaptic maintenance can result in progressive neurodegenerative disease. Recent studies demonstrated that plasticity mechanisms, such as presynaptic homeostatic potentiation (PHP), can delay symptomatic phases of neurodegenerative disease conditions through compensatory neurotransmitter release. Despite this potential neuroprotective role, the cellular basis for transsynaptic PHP mechanisms at degenerating synapses remains poorly understood. Here, using the neuromuscular junction (NMJ) of Drosophila melanogaster as a model system, we identify the serine/threonine kinase casein kinase 2 (CK2) as a novel regulator of both structural and functional synaptic stability. Consistent with prior work, we demonstrate that presynaptic CK2 is essential to maintain synaptic connectivity. We now show that CK2 is equally important for PHP. By combining genetic, pharmacological, and electrophysiological approaches, we demonstrate that presynaptic CK2 is required for the induction and maintenance of PHP. Interestingly, overexpression of a wild-type copy of CK2 is sufficient to increase synaptic release while expression of a kinase-dead version leads to an impairment in synaptic transmission. These findings highlight the importance of CK2 for the simultaneous control of structural and functional synaptic stability. Future work will focus on the identification of the molecular targets of CK2 impinging on the synaptic release machinery.

Mapping Multisite Network-wide Synaptic Transmission and LTP in the Hippocampal Network

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Learning and memory formation in the hippocampus have widely been accepted as processes depending on changes in synaptic efficiency, allowing sculpting connections between associated neurons . Although various approaches were proposed to identify the mechanisms that underlie this form of activity-dependent synaptic plasticity in the hippocampus, the most commonly used is long-term potentiation (LTP) . Thus, numerous studies have used LTP as the gold standard electrical measure to assess cellular mechanisms of synaptic transmission in physiological, aging, and disease conditions. Particularly, when studied in the context of adult neurogenesis in the dentate gyrus (DG), it may provide a deeper understanding of how the continuous generation of new neurons contributes to the processing and storage of new information in the brain. LTP recordings from brain slices were commonly established using a conventional one-stimulating electrode and another electrode to record evoked field potential upon high-frequency stimulation. Furthermore, low-density microelectrode arrays have also been used to record network responses with low spatial resolution . These conventional methods have hindered the spatial mapping of afferent pathways activation necessary for LTP induction in the entire hippocampus (i.e., recording simultaneously from the hippocampal subfields) and induced inter-experiential variability. To address this significant challenge, we implemented and characterized a large-scale ex vivo electrophysiological setup conceived by an external microelectrode for stimulation and a high-density microelectrode array (HD-MEA) ,for recording simultaneous evoked-synaptic responses and LTP in the hippocampal network. Our platform allows - i) Bidirectionality in stimulating-recording electrically-evoked responses simultaneously from two hippocampal canonical pathways - medial perforant (mPP) to the DG and Schaffer collaterals (SC) in CA3 to CA1; ii) Identifying the cellular layers of evoked-responses matched with their anatomical regions in the hippocampus. The field-excitatory-post-synaptic potential (fEPSP) and population spikes (PS) are identified from a group of neuronal ensembles simultaneously in DG, CA3, and CA1; iii) Studying spatially resolved LTP recordings in the environmental enrichment paradigm to elucidate the prior experience influences on the neural substrates of learning and memory. This is the first report on the large-scale mapping of synaptic activity-dependent synaptic changes modulated by electrical stimulation in the hippocampus.
Prospectively, our approach could instantiate a large-scale model for learning and memory by identifying network LTP-induced signaling cascades and their mechanisms and potentially allowing us to characterize the causes underlying age and disease-related deficits in LTP. Also, it provides a robust platform to assess the pharmacological and toxicological effects of biochemical compounds on synaptic plasticity, which renders a high spatiotemporal screening platform to identify novel therapeutics.

Diurnal varations in the contribution of mGlu5 receptors to hippocampal synaptic plasticity

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The metabotropic glutamate receptor, mGlu5, plays a critical role in the persistency of hippocampal long-term potentiation (LTP), long-term depression (LTD) as well as hippocampus-dependent associative learning and memory (doi: 10.3390/cells11213352). Although well-studied, the majority of investigations as to the role of mGlu5 in these processes in rodents has been done during the light cycle that amounts to the resting phase of rodent species. Sleep-states are regulated by mGlu5 (doi: 10.3390/cells12131761) suggesting that its contribution to hippocampal information processing and storage may differ across the diurnal cycle.
Here, we recorded field potentials from Schaffer collateral (SC)-CA1 synapses of the dorsal hippocampus of freely behaving transgenic (mGlu5-/-) mice and their wildtype(wt) littermates in the daytime and at night (events separated temporally by 12 h) to assess whether the contributions of mGlu5 receptors to LTP vary across the light-dark cycle.
Hippocampal LTP that was induced by high frequency stimulation of SC inputs to CA1 was significantly impaired in mGlu5-/- mice (compared to wt controls) regardless of the phases of the diurnal cycle. Differences in the profile of plasticity changes and in responses to metaplastic priming suggested nonetheless that diurnal variations in the regulation by mGlu5 receptors of LTP may occur.
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Mechanisms for activity dependent adjustments of quantal size at the *Drosophila* NMJ

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Adaptive neural circuit function relies critically on plastic changes in synaptic strength. Decades ago, spontaneous miniature excitatory potentials (mEPSPs) were discovered and attributed to the fusion of single synaptic vesicles (SVs) with the plasma membrane, and quantal size was initially thought to be fixed. However, further investigations revealed that quantal size can be adjusted in an activity dependent manner. At the *Drosophila* NMJ, increased locomotor activity causes an upregulation of neurotransmitter (NT) transporters on the SV membrane, increased SV size and NT content, which in turn increases synaptic transmission amplitude (Daniels et al., 2004; Steinert et al., 2006). However, the underling molecular mechanism has remained unknown.

I found that activity dependent increases in quantal size require calcium influx through voltage gated calcium channels (VGCCs) of the Cav1 family. First, quantal size is increased by reducing the function of the plasma membrane bound calcium ATPase (PMCA). Knock down of PMCA causes reduced calcium extrusion and hyperactivity. Increases in quantal size as induced by reducing PMCA function are fully rescued by concomitant Cav1 knock down, indicating a requirement for Cav1 in activity dependent regulation of quantal size. Neuronal hyper-excitability as induced by the expression of bacterial sodium channels also increases quantal content (QC). Increases in QC are read out immunohistochemically as a significant upregulation of vGlut protein expression in the axon terminal as well as increased mini size in electrophysiological recordings.

Calcium ions act as both charge carriers and second messengers and encodes various physiological processes, including gene transcription. Thus, activity dependent calcium influx through Cav1 channels likely activates calcium as a second messenger to upregulate vGlut expression. Using the vGlut enhancer (OK371) coupled to Gal4 with UAS-tdTomato as a reporter system demonstrates that vGlut is upregulated at the transcriptional level. Thus, I have identified a novel regulatory mechanism in which activity dependent presynaptic calcium influx through Cav1 regulates vGlut transcription. Furthermore, I narrowed down the genomic site responsible for vGlut transcriptional regulation by utilizing different vGlut enhancer fragments. Further bioinformatic and genetic analyses revealed that the NFkB transcription factor (TF) relish is required for the transcriptional upregulation of vGlut.

Typically, *Drosophila* larval motoneurons compensate for increased quantal size through the activation of glutamate gated chloride channels at presynaptic terminals, which drives a reduction in QC and thus presynaptic homeostatic depression (PHD). However, activity and calcium influx dependent global transcriptional upregulation of vGlut expression does not induce PHD, therefore resulting in enhanced synaptic transmission. During physiological challenges, such as sustained crawling, increases in quantal size may enhance transmission and aid crawling speed.

Frequency-dependent synaptic plasticity and NMDAR subunit content are distinct in supra- and infrapyramidal blade of the dentate gyrus in freely behaving animals

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The dentate gyrus serves as an information gateway to the hippocampal formation. It receives sensory information from the entorhinal cortex via the perforant path and can be subdivided into parts including the supra- and an infrapyramidal blade. So far, synaptic plasticity within the DG has typically been examined in the suprapyramidal blade, whereas little is known about the extent to which synaptic plasticity is expressed and maintained in the infrapyramidal blade.
This study compared synaptic plasticity evoked by patterned electrophysiological stimulation of the perforant path in both blades of the dorsal dentate gyrus of behaving rats. Membrane and action potential properties in granule cells of both blades were compared using patch clamp recordings in hippocampal slices. Using immunohistochemistry, N-methyl-D-aspartate receptor (NMDAR) subunit expression was assessed.
In the infrapyramidal blade, long-term depression induced by patterned stimulation of the perforant path at 1 Hz was weaker compared to the suprapyramidal blade. Long-term potentiation (LTP) was induced in the suprapyramidal blade using patterned stimulation at 200 Hz, whereas a higher afferent frequency was necessary to induce LTP in the infrapyramidal blade. Patch clamp recordings revealed differences in specific action potential properties, whereas passive membrane properties were similar in both blades. NMDAR subunit expression was significantly lower in the infrapyramidal compared to the suprapyramidal blade.
These results suggest supra- and infrapyramidal blade of the dentate gyrus can be differentiated based on the frequency-dependency of synaptic plasticity, cellular properties, and NMDAR receptor content, indicating distinct roles of these structures in hippocampal information processing and storage.

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Repetitive magnetic stimulation induced synaptic plasticity relies on cooperative pre- and postsynaptic activation

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Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation technique widely used in clinical practice for both diagnostic and therapeutic purposes. Based on the principle of electromagnetic induction, TMS can stimulate the brain through the intact skin, skull, and meninges. When applied repeatedly (repetitive TMS; rTMS), it induces long-lasting changes in the synaptic strength. Despite its clinical applications, the cellular and molecular mechanisms underlying rTMS-induced plasticity remain poorly understood. In this study, we systematically compared the effects of repetitive magnetic stimulation (rMS) with local electric stimulation of Schaffer collateral-CA1 synapses in entorhino-hippocampal tissue cultures. As previously shown, 10 Hz rMS induces robust long-term potentiation (LTP) of excitatory neurotransmission, while the same stimulation protocol applied locally as electric stimulation results in long-term depression (LTD). Through a series of pharmacogenetic, optogenetic, and calcium imaging experiments, we demonstrate that a cooperative pre- and postsynaptic activation during rMS underlies LTP at low stimulation frequencies. Finally, a computational model that is based on short-term plasticity and spike timing-dependent plasticity successfully reproduces our key findings, providing insights into the frequency-dependent effects of rTMS. These results deepen our understanding of how rTMS induces lasting changes in synaptic transmission and network excitability. They also lay the groundwork for developing of multi-scale computational model to predict and standardize the “biological dose” of rTMS.

Functional and molecular mechanisms underlying plasticity-mediated CNS recovery after spinal cord injury in adulthood and aging

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Although plasticity is limited in the adult mammalian CNS, recent findings suggest that it may be key to functional recovery following injury. One such example is the reorganization of intraspinal circuits after spinal cord injury (SCI), which compensates for the loss of supraspinal input from the motor cortex. The corticospinal tract (CST) is one neural pathway that undergoes injury-induced plasticity. This involves the sprouting of CST axons rostral to the injury site, leading to the formation of new connections with relay neurons unaffected by the lesion, ultimately contributing to the restoration of motor function. While the formation of detour circuits is a well-established model of functional recovery in adulthood, whether and how plasticity occurs during aging remains to be fully understood. This question is particularly important, as the rising geriatric population in recent years has increased the median age of patients experiencing SCI. Our objective is to examine the molecular and cellular changes in plasticity during aging. In this study, we are specifically investigating the responses of neuronal and immune cells in older mice to determine how they influence axonal plasticity and functional recovery following SCI. Our preliminary data suggest that microglia have a dysregulated ability to engulf synapses, which may be linked to poorer functional recovery during aging. Further investigations aim to understand the molecular pathways driving this microglial phenotype in aging, using transcriptomics and proteomics analyses.

Modulation of activity-dependent synaptic plasticity by the AMPAR interacting-protein PRRT2

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In the brain, AMPA receptors interact with a variety of different proteins to form large functional protein complexes, known as AMPA receptor complexes. Mutations in the gene of one of these AMPA receptor interaction partners, the proline-rich transmembrane protein 2 (PRRT2), have been identified as the main cause of a broad spectrum of paroxysmal neurological disorders in humans.
PRRT2 is a synaptic protein, which is found in both the pre- and postsynapse of neurons of many brain regions, including the hippocampus, striatum and cerebellum. Presynaptically, PRRT2 influences neurotransmitter release via interactions with SNAP25 and synaptotagmin; postsynaptically, its role is much less clear.
Here we demonstrate that PRRT2 is a constituent of postsynaptic AMPA receptor complexes. Electrophysiological measurements conducted on dentate gyrus granule cells demonstrate that basal postsynaptic AMPAR-mediated currents (e.g. mEPSCs) remain unaltered or exhibit only minimal changes in PRRT2-deficient neurons. However, the activity-dependent insertion of synaptic AMPA receptors is regulated by PRRT2. Knockout of PRRT2 markedly enhances long-term potentiation (LTP) and heterosynaptic long-term depression (LTD) at perforant path to dentate gyrus granule cell synapses. This suggests a possible involvement of PRRT2 in the activity-dependent insertion of AMPA receptors into the postsynaptic membrane. Based on these findings, we propose an updated model for the role of PRRT2 in synaptic transmission, wherein it exerts dual influences: on the one hand affecting presynaptic transmitter release, and on the other hand modulating postsynaptic activity-dependent AMPAR signalling.

Interaction of actin dynamics and spine geometry as a synaptic tag

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Long-term potentiation (LTP) can have an early phase constituting a transient increase in synaptic strength, and a late phase sustaining this increase. The synaptic tagging and capture hypothesis states that the late phase occurs if the stimulus leads to a transient ‘synaptic tag’ along with plasticity-related protein (PRP) synthesis. If both are present, the post synaptic density (PSD) remodels, giving rise to long-lasting changes in the synaptic weight. With computational modelling, we test the hypothesis that actin dynamics in interaction with spine geometry acts as a synaptic tag.

Actin dynamics is constituted by several actin binding proteins that carry out filament branching, capping, severing and crosslinking which are modulated during LTP. We first study the dynamic (not crosslinked) actin pool coupled with a 3d spine membrane model during LTP and find that while actin activity and spine volume increase after LTP induction, these changes are short-lived, suggesting that dynamic actin alone cannot ‘tag’ the synapse.

We then introduce a stable actin pool (crosslinked) in the model and monitor the LTP-induced changes. We observe that, when the PSD is not remodelled (no available PRPs), LTP-induced perturbations of the stable pool and total spine volume are retained on the time-scale of the synaptic tag, and eventually decay to a state determined by the PSD. Taken together, our model provides proof-of-principle for the interaction of actin dynamics and spine geometry as a potential mechanism for synaptic tagging.

To test for the translation of these structural changes to function, we explore also PSD remodelling by geometry-dependent mechanisms. Availability of PRPs is the trigger for PSD remodelling, and we implement this by opening a time window 30 minutes post-stimulation and measuring the local membrane curvature during this window. When the PSD remodels, it adapts to the perturbed spine geometry, consistent with the capture phase described by the synaptic tagging and capture hypothesis.

Effects of adolescent stress on synaptic transmission and plasticity in the adult mouse dentate gyrus

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Neuropsychiatric disorders are rising globally, notably among adolescents who are particularly vulnerable to stressful life events. Adolescence represents indeed a critical period of late brain maturation marked by cognitive and emotional changes, where excessive stress is a significant risk factor for the development of neuropsychiatric disorders in adulthood, including first and foremost major depression. However, the interplay between environmental stressors and genetic predispositions in driving long-term cognitive and affective impairments remains poorly understood. Here, we asked whether adolescent stress (AS) has a persistent impact on basic features of signal processing in the dentate gyrus (DG). This region of the hippocampal formation is of particular interest as it emerged as a crucial site for the therapeutic action of antidepressant drugs and electroconvulsive therapy alike. With regard to their molecular underpinnings, activin A, a member of the TGF-β family with a pronounced neuromodulatory profile, is increasingly recognized as a significant player in this field.

We used an animal model of AS, in which corticosterone (CORT) was added to the drinking water from postnatal days (PND) 30 - 45. After a CORT-free interval of 45 - 90 days, we recorded excitatory transmission at the medial perforant path - granule cell (mPP-GC) synapse in the DG in *ex vivo* hippocampal slices from CORT-exposed and control wild-type (wt) mice and likewise treated transgenic mice expressing a dominant-negative mutant of activin receptor IB (dnActRIB), which disrupts activin signaling.

As a necessary prelude, we examined first whether endogenous activin modulates the properties of the mPP-GC synapse in adult stress-naïve mice. Whereas the mice did not not exhibit genotype-specific differences in basic synaptic properties and short-term plasticity (STP), long-term potentiation (LTP) was impaired in dnActRIB mice. Lending strong support to the notion that severe stress in early ages leaves a pathogenetic stamp on the brain, we report here that AS led to enhanced synaptic transmission and reduced STP in both groups, with the key difference that wt mice, unlike dnActRIB mice, displayed these effects only after $GABA_A$ receptor-mediated inhibition was blocked with picrotoxin. This finding implicates activin A in re-adjusting the balance between excitation and inhibition after AS exposure. Importantly, LTP-inducing high-frequency stimulation uncovered aberrant hyperexcitability in AS-treated dnActRIB mice, while LTP in adult wt mice remained unaffected by AS.

Western blotting showed that AS causes a reduced expression of stress hormone receptors in the hippocampus of adult wt mice. This apparent down-regulation of CORT signaling may explain why, in preliminary experiments on adult wt mice with AS experience, acute application of CORT (modeling recurrent stress in adulthood) strongly biased synaptic functions.

In conclusion, our study demonstrates that AS has an impact on essential features of synaptic transmission and plasticity that persists into adulthood and is likely associated with alterations in glucocorticoid signaling pathways. Our study also supports the view of activin A as an endogenous antidepressant that is recruited by mood-elevating therapies, since, in our hands, genetic disruption of activin receptor signaling made the adolescent hippocampus much more vulnerable to the detrimental consequences of stress on brain function later in life.

Dendrodendritic inhibition of mitral cells

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In the mammalian olfactory bulb, modulation of synaptic activity and cell interactions are key mechanisms for the processing of odor information. Granule cells form dendrodendritic connections with mitral cells and can regulate their activity through GABAergic pathways. Upon activation, granule cells can release GABA (gamma-aminobutyric acid), which causes a direct inhibitory effect on mitral cells when it binds to their GABA-A receptors. GABA transporters play an important role in this interaction, as they can take up the inhibitory neurotransmitter from the synaptic area and thus decrease the amount of free GABA. However, more research is needed to characterize the precise role of the different GABA transporter subtypes during this process. Here we show that the neuronal GABA transporter 1 and astrocytic GABA transporter 3 (GAT1 and GAT3) are significantly involved in regulating the feedback inhibition of granule to mitral cell dendrodendritic synapses. Using the patch-clamp method for electrophysiological measurements, mitral cell responses to paired pulse stimulations were recorded and their area was analyzed. This way, it can be shown that, in mouse olfactory bulb slices, the simultaneous pharmacological inhibition of GABA transporters 1 and 3 leads to a decreased area of response in mitral cells, while the inhibition of only GAT1 or GAT3 does not show a significant effect on mitral cell activity. Furthermore, GAT2- and BGT1 antagonists have no significant effect on mitral cell excitability. These results demonstrate that both neuronal and glial GABA uptake is involved in GABA clearance from the synaptic cleft and thus are highly important for mitral cell activity and the transmission of information through the olfactory bulb. This study aims to gain more knowledge about the physiological mechanisms and interactions that modulate the processing of information in the main olfactory bulb, aswell as in other brain regions. In addition, it might serve as the basis for further investigations of GABA transporters and feedback interactions in the mammalian brain.

Poster Topic

T9: Glia, Glia-Neuron Interactions

T9-1A Dendritic ATP release mediates cell type-specific neuron-to-astrocyte communication
Jennifer Bostel, Antonia Beiersdorfer, Kristina Losse, Natalie Rotermund, Kristina Schulz, Janina Sophie Popp, Christine E. Gee, Daniela Hirnet, Christian Lohr

T9-2A Chemogenetic activation of Gq in microglia leads to deficits in synaptic plasticity and neuronal communication
Marie-Luise Brehme, Oana M. Constantin, Zhen Yuan, Paul J. Lamothe-Molina, Laura Laprell, Fabio Morellini, Thomas G. Oertner

T9-4A Sex and size specific differences in the extracellular vesicle cargo of oligodendrocyte progenitor cells in response to hyperoxic stress
Donna Elizabeth Sunny, Elke Hammer, Rabea Schlüter, Stephan Michalik, Uwe Völker, Matthias Heckmann

T9-5A Activation of primary somatosensory cortex astrocytes triggers long-term mechanical hyperalgesia
Rangel Leal Silva, Antonio Gonzalez, Ilknur Çoban, Abhirup Dutta, Khaleel Alhalaseh, Alexander Groh, Amit Agarwal

T9-6A The role of N-acetyl aspartate in axo-glial signaling for metabolic support
Lina Sophie Komarek, Julia Edgar, Klaus-Armin Nave

T9-7A Mechanism of impaired cognitive function focusing oligodendrocyte activity
Shiho Kunishima, Daisuke Kato, Bai Xianshu, Jinglei Cheng, Frank Kirchhoff, Hiroaki Wake

T9-8A Life-long myelination can be described by rates of myelin addition and removal
Sebastian Timmler, Claudia Pama, Cagla Kaya, Hendrik Miessner, Rana Eser, Yuting Li, Benjamin Simons, Ragnhildur Thora Karadottir

T9-1B Deletion of Thy-1 induces a distinct partially activated astrocyte phenotype in mice
Juliane Loui, Ute Krügel, Ulrike Winkler, Anja Reinert, Dorit John, Johannes Hirrlinger, Anja Saalbach

T9-2B Interactions of Oligodendrocyte Precursor Cells and Dopaminergic Neurons in the Mouse Substantia Nigra
Ying Sun, Julia C. Fitzgerald, Frederek Reinecke, Elisabeth Bauer, Friederike Pfeiffer

T9-3B Role of BDNF/TrkB and pro-BDNF/p75^{NTR} signaling in modulating the microglia functional state in the aging brain
Yesheng Sun, Alexandra Theurer, Marta Zagrebelsky, Martin Korte

T9-4B Purinergic calcium signaling in astrocytes of the mouse medial prefrontal cortex
Victoria Camel, Jennifer Bostel, Christian Lohr, Antonia Beiersdorfer

T9-5B Impact of two-week repetitive magnetic stimulation on microglia activity and neuronal plasticity
Paolo d`Errico, Christos Galanis, Claudio Elgueta, Dimitrios Kleidonas, Andreas Vlachos

T9-6B Sex-specific molecular and cellular phenotypes of pain resolution in a rat model for neuropathic pain
Felicitas Schlott, Annemarie Sodmann, Beate Hartmannsberger, Alexander Brack, Heike Rittner, Robert Blum

T9-7B Developmental and Neuroinflammatory Changes in Glutamate and Adenosine Receptor-Mediated Ca2+ Signaling in Astrocytes of the Olfactory Bulb
Fatemeh Mohammadpour, Kiana Samad-Yazdtchi, Antonia Beiersdorfer, Charlotte Schubert, Daniela Hirnet, Manuel Friese, Christian Lohr

T9-1C Nav1.2 Expression in Oligodendroglial Cells
Constanza Bravo Rossainz Baez, Mariapia Grassia, Dirk Isbrandt, Friederike Pfeiffer

T9-2C Pharmacological targeting of Smoothened receptor as a promising approach to enhance oligodendrocyte differentiation
Antonella Damiana Recchia, Alessandra Dominicis, Vincenzo Maria D`amore, Tommaso Fabiano, Luciana Marinelli, Francesco Saverio Di Leva, Antonella Ragnini-Wilson

T9-3C The impact of serotonergic signaling on astrocyte function and morphology
Andre Zeug, Markus P. Schirmer, Evgeni G. Ponimaskin, Franziska E. Müller

T9-4C A role of NAD in glial support for axonal integrity
Shuying Mao, Klaus-Armin Nave

T9-5C Effects of LPS-Induced Inflammatory signaling on Intrinsic Calcium Activity of Mouse and Human Astrocytes
Franziska E. Müller, Evgeni G. Ponimaskin, Andre Zeug

T9-6C Cell-cell and cell-matrix interaction of breast tumor cells with brain cells in a 3D hydrogel-based matrix
Esra Türker, Jessica Faber, Mateo S. Andrade Mier, Nicoletta Murenu, Gregor Lang, Silvia Budday, Natascha Schaeffer, Reiner Strick, Pamela Strissel, Carmen Villmann

T9-7C Myelination generates aberrant ultrastructure that is resolved by microglia
Minou Djannatian, Swathi Radha, Ulrich Weikert, Shima Safaiyan, Christoph Wrede, Cassandra Deichsel, Georg Kislinger, Agata Rhomberg, Thorben Ruhwedel, Douglas Campbell, Tjakko van Ham, Bettina Schmid, Jan Hegermann, Wiebke Möbius, Martina Schifferer, Mikael Simons

T9-1D Crosstalk of α_1-noradrenergic Ca^{2+} and cAMP signaling in astrocytes of the murine olfactory bulb
Jessica Sauer, Franz Lennart Schmidt, Antonia Beiersdorfer, Daniela Hirnet, Christine E. Gee, Christian Lohr

T9-2D Developmental profile of oligodendrocyte arrangement, identification and morphology in nuclei of the superior olivary complex
Christina Pätz-Warncke, Alina C. Zacher, Melissa Grabinski, Laura Console-Meyer, Felix Felmy

T9-3D Astrocytic cAMP increases ATP release frequency in hippocampal slices
M. Carolina Pinto, Oana M. Constantin, Thomas G. Oertner, Christine E. Gee

T9-4D Dynamic transcellular molecular exchange: a novel view on extracellular matrix remodeling
Svilen Veselinov Georgiev, Silvio O. Rizzoli

T9-5D Synaptic reorganization and perisynaptic astrocyte plasticity at spines of pyramidal neurons in the motor cortex during a simple motor task
Yoshiyuki Kubota, Mohammed Youssef, Yuri Yanagawa, Shobha Upreti, Jearin Sohn, Estilla Toth, Chris Salmon, Yasuo Kawaguchi

T9-6D Local differences in baseline Na^+ shape astrocytic K^+ uptake by the NKA
Jan Meyer, Sara Eitelmann, Karl W. Kafitz, Christine R. Rose

T9-7D Development of Myelination in Globular Bushy Cells
Laurin Teich, Lars Kunz, Benedikt Grothe

Dendritic ATP release mediates cell type-specific neuron-to-astrocyte communication

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Astrocytes play a crucial role not only in supporting neurons and maintaining tissue homeostasis, but also in surrounding synapses with their microdomains and directly interacting with both pre- and postsynaptic regions. Adenosine triphosphate (ATP), the major messenger molecule in neuron-astrocyte communication, is released at axo-dendritic synapses and activates perisynaptic astrocyte microdomains. Astrocytes themselves also release ATP, affecting nearby neurons and other astrocytes. In this study, we investigated purinergic signaling between dendro-dendritic synapses and astrocytes in the external plexiform layer (EPL) of the olfactory bulb. These synapses consist of glutamatergic release from mitral and tufted cells on the one site and GABAergic release from granule cells on the other site. Electrical stimulation of both mitral/tufted and granule cells led to Ca^{2+} transients in astrocytes expressing GCaMP6s in the EPL, while stimulation of mitral/tufted cells alone did not evoke a response in astrocytes. The Ca^{2+} signals in astrocytes were strongly diminished by $P2Y_1$ (MRS2179) and A_{2A} (ZM) receptor antagonists, whereas glutamatergic and GABAergic antagonists had a minimal effect. These observations were confirmed by stimulation experiments using cell type-specific expression of channelrhodopsin-2 in mitral/tufted or granule cells, respectively. Optogenetic activation of granule cells triggered purinergic Ca^{2+} transients in astrocytes, while activation of mitral/tufted cells did not produce such transients, indicating a cell type-specific pathway for neuron-astrocyte communication. In addition, optogenetic activation of mitral/tufted cells failed to activate astrocytes indirectly by excitation of granule cells by glutamate release. While this dendrodendritic excitation of granule cells failed to evoke Ca^{2+} signals in EPL astrocytes, electrical stimulation of the anterior piriform cortex (APC), another excitatory input into granule cells, led to purinergic Ca^{2+} signaling in astroctyes. Additionally, optogenetic stimulation of astrocytes resulted in Ca^{2+} transients in astrocytes and increased neuronal network activity, whereby glutamate and ATP are the main gliotransmitter in this astrocytes-neuron interaction.

These results suggest that granule cells, but not mitral/tufted cells, release ATP that induces an increase in Ca^{2+} in astrocytes, subsequently enhancing neuronal network activity in the olfactory bulb by gliotransmission.

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Chemogenetic activation of Gq in microglia leads to deficits in synaptic plasticity and neuronal communication

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Activation of microglia by inflammation or chronic disease has profound effects on neurons and synapses. The details of this communication between the immune system and the brain are under active investigation. Common strategies to activate microglia, such as lipopolysaccharide injection or experimental autoimmune encephalomyelitis (EAE) primarily activate the peripheral immune system, triggering a complex and protracted response of the whole organism. In order to activate microglia selectively and with precise timing, we used chemogenetic activation of a Gq-DREADD expressed exclusively in microglia. This approach allowed us to study the effect of microglia on hippocampal synaptic function without the risk of direct neuronal or astrocytic activation. We used single cell electroporation of CA1 neurons and 2-photon microscopy of hippocampal slice cultures to study the impact of Gq-activated microglia on synapses. We detected a sex-specific decrease in the density of excitatory synapses on CA1 pyramidal cell dendrites. Synaptic pruning was prevented by the BDNF scavenger TrkB-FC, suggesting that BDNF signaling is part of the mechanism. In line with these findings, activation of Gq-DREADD in microglia in vivo significantly reduced long-term potentiation (LTP). Interestingly, Gq-DREADD activation did not affect spatial learning; instead, it specifically affects remote memory. Taken together, our findings show that a “phantom CNS inflammation” can be induced by artificial activation of second messengers within microglia, leading to impairments in synaptic plasticity and memory recall.

Sex and size specific differences in the extracellular vesicle cargo of oligodendrocyte progenitor cells in response to hyperoxic stress

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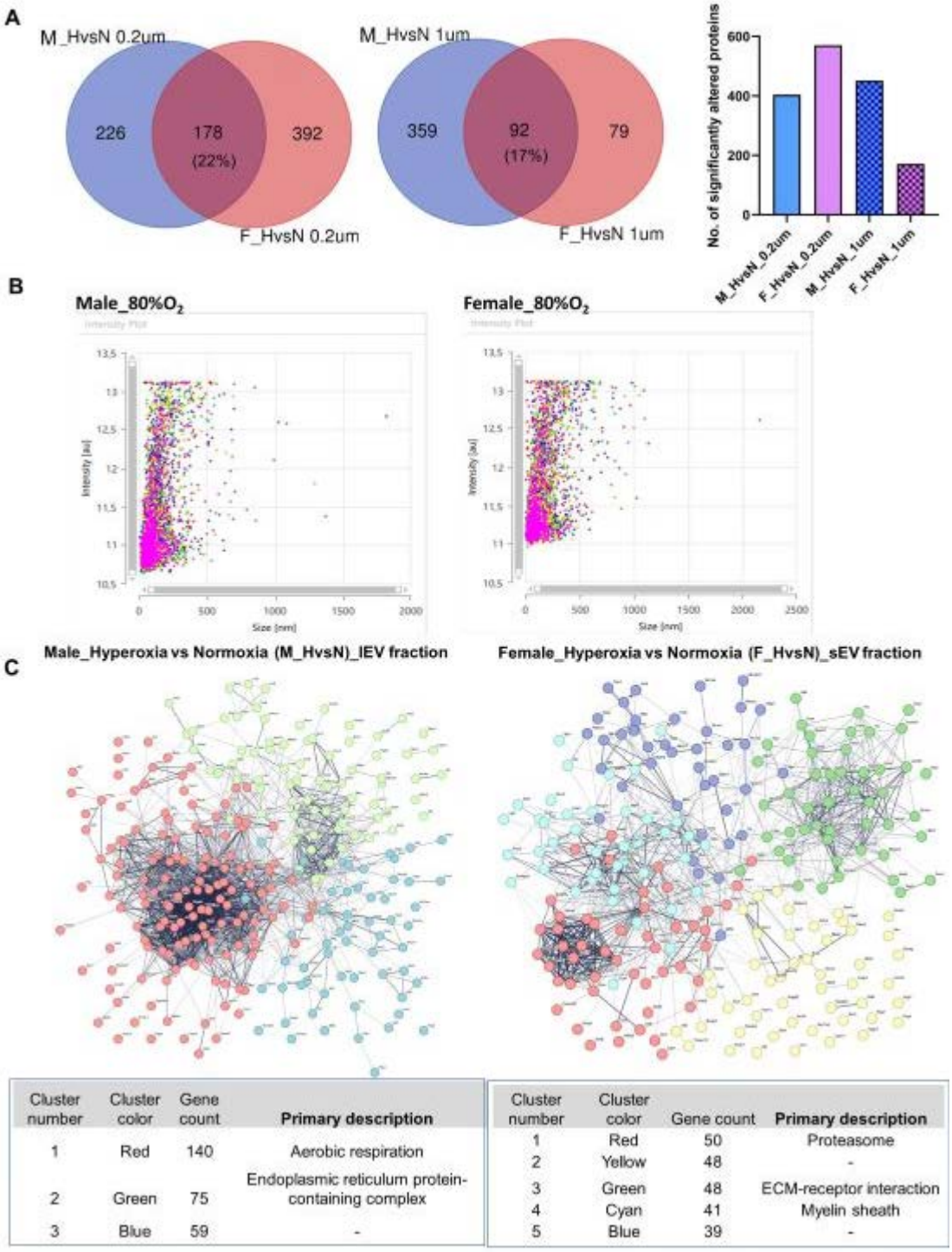
Cerebral oxygenation differences in preterm infants due to exposure to high oxygen levels during intensive care, and sex-specific oxidative stress responses can disrupt oligodendrocyte maturation, affecting neuronal development and function differently in male and female brains. Extracellular vesicles (EVs) are increasingly recognized as important mediators of intercellular communication and stress response in the brain. Here, we used a proteomic approach to identify and characterize the cargo composition of small (<200nm) and large (<1000nm) EVs released by primary, mouse derived oligodendrocyte progenitor cells (OPCs) of both male and female origins. We aim to pinpoint molecular alterations within this cargo that are triggered by hyperoxic conditions in a sex-and size-specific manner.

Primary OPCs, were obtained from neurosphere cultures and were grown as spheroids. Male and female derived OPC spheroids were treated with 80% oxygen for 24h and EVs were isolated using precipitation method. This was followed by size separation using filtration method. The EVs were characterized to meet the Minimal Information for Studies of EVs 2023, including electron microscopy and Western blot analysis. The EVs subsequently underwent an untargeted mass spectrometric analysis on an ESI-LC-MS/MS system followed by initial data analysis using Spectronaut (Biognosys) and subsequent quantification of differences by SpectroPipeR pipeline.

Our analysis revealed distinct sex- and size-specific protein signatures, with only 17% and 22% overlap post hyperoxia between male and female large and small EVs (lEVs and sEVs), respectively (A). We observed that the number of proteins significantly altered after hyperoxia was more than twice as high in male-derived lEVs compared to female-derived lEVs. In contrast, the opposite trend was seen in sEVs, where a greater number of proteins were significantly altered in female-derived EVs upon hyperoxia than in those from males (A). This suggest that female cells might engage sEVs for enhanced stress response signaling, while in male cells, lEVs might play a more significant role. Nanoparticle Tracking Analysis (NTA) showed a slight increase in the intensity of sEVs in female samples post hyperoxia (B). In terms of specific protein cargo, a greater proportion of upregulated proteins were assigned to mitochondrial functions in the lEV fraction from male cells in response to hyperoxic stress, whereas the sEV fraction from female cells showed an abundance of proteasomal complex proteins (C). IPA analysis further supported this, showing enrichment of post-translational protein phosphorylation in female sEVs. In male lEVs, mitochondrial functions, including oxidative phosphorylation and ATP synthesis were affected.

Our results suggest that intercellular stress response signaling is mediated differentially by male and female OPCs via size specific secretion of EVs, whereby large and small EVs carry different protein cargo.



Cluster number	Cluster color	Gene count	Primary description
1	Red	140	Aerobic respiration
2	Green	75	Endoplasmic reticulum protein-containing complex
3	Blue	59	-

Cluster number	Cluster color	Gene count	Primary description
1	Red	50	Proteasome
2	Yellow	48	-
3	Green	48	ECM-receptor interaction
4	Cyan	41	Myelin sheath
5	Blue	39	-

(A) Venn diagram showing overlap between altered proteins in each group and bar graph showing the detected number of significantly altered proteins in each comparison groups. (B) NTA analysis results showing concentration difference between male and female EV size post hyperoxia. (C) STRING k-means cluster analysis of upregulated proteins upon hyperoxia in male large EV and female small EV groups. Cut off p value <0.05. All data are representative of at least 3 bioreplicates.

Figure showing the sex- and size- specific differences in OPC EV cargo in response to hyperoxia.

Activation of primary somatosensory cortex astrocytes triggers long-term mechanical hyperalgesia

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While reactive astrocytes are implicated in the pathogenesis of several central nervous system disorders, including chronic pain, most studies have primarily focused on characterizing this cellular state based on the expression of molecular markers for reactive gliosis. For this study, we chose the spared nerve injury (SNI) model of chronic pain in mice to perform a detailed histological, molecular and functional analysis of astrocytes in the primary somatosensory cortical (S1) of mice during the development of chronic pain. Unexpectedly, we did not observe any alteration in classical molecular markers (e.g., GFAP, glutamate transporters, and potassium channels) or morphological features typically associated with reactive astrocytes. However, by using 2-photon in vivo Ca^{2+} imaging in S1 astrocytes expressing membrane-anchored variant Ca^{2+} sensor mGCaMP3 in GLAST-CreERT2;mGCaMP3 transgenic mice with SNI surgery, we identify that these cells undergo aberrant alterations in Ca^{2+} transient kinetics (functional changes) at late stage of neuropathic pain development (21 days). We did not observe such alterations in baseline Ca^{2+} transients in knockout mice lacking the endoplasmic reticulum Ca^{2+}; channel inositol 1,4,5-trisphosphate receptor type 2 (IP3R2), suggesting the involvement of signaling pathways engaging G-protein-coupled receptor (GPCR) on astrocytes. As a next step, to identify changes in the expression of GPCRs in these astrocytes in response to chronic pain, we performed a detailed gene expression analysis on freshly isolated cortical astrocytes. We found that astrocytes mainly expressed Gi-coupled GABAergic and glutamatergic GPCRs. Remarkably, DREADDs-based chemogenetic activation of S1 astrocytes expressing hM4Di, a designer GPCR coupled to Gi activated by clozapine-N-oxide, induced long-term hyperalgesia in naïve mice without any SNI surgery. Next, our in vivo electrophysiology analysis using silicon probes demonstrated that this effect was associated with local suppression of neuronal activity in layer 5 neurons. Taken together, our results suggest that hyperactivity of Gi-coupled GPRC signaling in astrocytes is sufficient to induce a long-lasting mechanical hyperalgesia in mice and could be a novel mechanism by which astrocytes mediate pathological changes in local neuronal circuit activity and participate in sustaining chronic pain.

The role of N-acetyl aspartate in axo-glial signaling for metabolic support

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Myelin improves signal propagation and provides a route for transport of metabolites to the axon. However, how this metabolic support is regulated is not well understood. Specifically, we are asking the questions whether axons with metabolic stress can signal enhanced energy needs to the myelinating oligodendrocyte. N-Acetyl aspartate (NAA) is an abundant metabolite and potential axon-to-oligodendrocyte signaling molecule, synthesized in neuronal mitochondria but catabolized in oligodendrocytes. We hypothesized that the level of extracellular NAA communicates the axonal energy status to glial cells, with NAA export being a sign of sufficient energy load. To test this, we prepared mixed glial cultures in the presence of 2 mM NAA. Interestingly, when this medium was replaced by an NAA free medium, we observed a significant increase of glycolytic gene expression 48 hours later. Our biochemical assays also revealed an increase in glucose uptake and lactate secretion after NAA withdrawal compared to controls. We suggest a working model in which a decrease in extracellular NAA levels, invariably associated with pyruvate/lactate-starved axons and metabolic stress, causes a compensatory upregulation of glial glycolysis that helps restoring the axonal energy balance.

Mechanism of impaired cognitive function focusing oligodendrocyte activity

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The white matter is composed of myelinated axons, which act as cables connecting different brain regions. Clinically, elderly people and Alzheimer's disease (AD) patients with white matter lesions showed significant impaired cognitive function. Furthermore, abnormalities in molecular expression specific to oligodendrocytes (OLs) and their progenitor cells (OPCs) have been reported in AD pathology, suggesting the pathological association between white matter abnormalities with impaired cognitive function. However, the detailed causality of OLs and myelin impairment with cognitive decline is unknown. Here, we aimed to clarify the dynamic and functional response of OLs/OPCs in aging and AD model mice to elucidate the functional pathological mechanism for OLs/OPCs to show cognitive decline.
We first investigated functional responses of OLs/OPCs in the white matter of 2-month-old wild type mice (2M WT) and 6-month-old AD model mice (6M AD) with in vivo Ca2+ imaging using two-photon microscopy. We found that the intensity and latency of Ca2+ activity was increased in 6M AD mice compared to 2M WT mice. In addition, Ca2+ activity of OLs/OPCs in 6M AD mice increased during the motor learning but not associated with learning process. It is unclear whether these changes in activity are not normal compared to WT, but these results lead us to consider why learning efficiency was declining despite the increase in Ca2+ activity of OLs/OPCs. To answer this question, we observed the morphology of the OLs/OPCs in 2M WT, 6M WT, and 6M AD mice. We quantified the process domains of OPCs and found that were reduced in aging and AD pathology. We then measured the number of OLs/OPCs and changes in expression levels of myelin related protein by immune-histochemistry and protein quantification. The number of OLs decreased, while the number of pre-matured OLs increased in aging and AD pathology. Furthermore, the expression of myelin basic protein was increased due to the increased number of pre-matured OLs, suggesting promotion of the myelin sheath repairment. In fact, the ultrastructure of the myelin sheath analyzed by electron microscope showed that the repair of the myelin sheath was impaired in 6M AD. These results suggest that, in aging and AD, altered Ca2+ activity of OLs/OPCs occurs to induce myelin sheath repairment, resulting in learning deficit. In addition, we plan to do FACS using OPC specific-cre mice to detect the gene and timing associated with the pathology. We anticipate that these experiments will provide new insights into the mechanisms of cognitive decline in aging and AD pathology and identify potential therapeutic targets for the future.

Life-long myelination can be described by rates of myelin addition and removal

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In grey matter, new myelin sheaths can be added until high age. To what extent mature healthy myelin sheaths are removed from axons is still under debate. Both mechanisms offer a rarely-studied pathway to shape and modulate neuronal networks during development and adulthood. Although the mouse is a widely used model system in myelin research, little is known about its region-specific myelin trajectories. Here we introduce a mathematical model to describe region-specific myelination by rates of myelin addition and removal. In a cross-sectional mouse study reflecting human childhood, adolescence, adulthood and aging, we found that the myelination in the mouse brain is regionally diverse and can be ongoing until high age. We found that variability of myelin sheath length increases with age, indicating an age-dependent shift in length of newly added sheaths. Using conditional reporter lines we directly compared sheaths that were added during development (OPALIN-cre) versus sheaths added during adulthood (PDGFRA-cre) and found that later-formed sheaths are shorter than early-formed ones, indicating functional significance beyond maintenance. Our results indicate that local myelination might be regionally suppressed, permitted or promoted at different points in time. This cross-sectional view on myelination serves as an important benchmark for research on regulators of myelin plasticity (e.g., environmental factors, diet, genes or compounds), myelination deficiencies, myelin degeneration and potential treatments.

Deletion of Thy-1 induces a distinct partially activated astrocyte phenotype in mice

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Thy-1 (CD90) is a highly conserved glycosylphosphatidylinositol-anchored cell surface protein with a peculiar expression pattern. In the brain, Thy-1 is expressed exclusively on the surface of mature neurons. Although the Thy-1 promoter is widely used as a neuron-specific promoter for transgenic expression, the exact role of the endogenous Thy-1 protein remains largely unknown. Thy-1 receptors, ITGB1, ITGB5 and Syndecan 4, are expressed on astrocytes suggesting a potential interaction of both cell types. Since the interplay of neurons and astrocytes is crucial for maintaining normal CNS health and function, we investigated the role of Thy-1 in neuron-astrocyte communication using a complete as well as a neuron-specific Thy1-KO mouse model. In both mouse lines, astrocytes exhibited increased expression of a distinct set of activation-associated genes, such as Gfap, Vim, and Tnc. These changes were more prominent in aged mice, indicating a delayed onset of the astrocytes' phenotype. Further, interaction of cultured astrocytes with recombinant Thy-1 in vitro confirmed this phenotype. Functional assays demonstrated that Thy-1 significantly restricts astrocytes growth and inhibits proliferation, while apoptosis remained unaffected. Whole genome expression analysis showed that Thy-1 regulates the expression of neurotransmitter receptors and potassium channels, highlighting its role in synaptic clearance. Taken together, our data demonstrate that Thy-1 controls the activation of astrocytes, resulting in a distinct astrocyte phenotype characterized by reduced expression of a subset of activation-associated genes and reduced proliferation. These findings provide valuable insights into the molecular mechanisms underlying astrocyte activation and suggest potential therapeutic targets for modulating astrocyte function in CNS diseases.

Interactions of Oligodendrocyte Precursor Cells and Dopaminergic Neurons in the Mouse Substantia Nigra

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by the pathological degeneration of dopaminergic neurons in the substantia nigra. Previous studies have implicated a role of oligodendrocyte precursor cells (OPCs) and oligodendrocytes in PD pathology, with alterations in myelin content or structure linked to the disease. However, the precise involvement of OPCs and myelination in the degeneration of dopaminergic neurons in the substantia nigra remains unclear.

Using high-resolution imaging, we investigated the morphological and spatial relationships between dopaminergic neurons and oligodendrocyte precursor cells (OPCs) in the substantia nigra of healthy mice. Our findings reveal a consistently close interaction between OPCs and dopaminergic neurons in the substantia nigra in various age groups. We also conducted quantitative analyses of OPCs and dopaminergic neurons across different age groups. The proportion of OPCs and dopaminergic neurons within the cellular population is comparable, and this ratio remains stable throughout the aging process in mice. Interestingly, while OPCs are uniformly distributed throughout the midbrain, myelin generated by OPC-derived oligodendrocytes is predominantly localized in regions densely populated with dopaminergic neurons, such as the *substantia nigra pars compacta* (SNpc), and is less abundant in areas with sparse dopaminergic neurons, such as the *substantia nigra pars reticulata* (SNpr). Our quantitative analysis of the proportion of OPCs in oligodendrocyte lineage cells in SNpc and SNpr revealed a higher ratio of OPCs in SNpr, suggesting that OPCs in this region may favor proliferation or differentiation cessation, potentially contributing to reduced myelination in SNpr.

Collectively, these findings, based on the physiological conditions of normal mice, highlight the close interaction between oligodendrocyte precursor cells (OPCs) and dopaminergic neurons in the substantia nigra. Our study may provide new insights into the pathophysiological mechanisms underlying Parkinson's disease and offer directions for future research.

Role of BDNF/TrkB and pro-BDNF/p75^{NTR} signaling in modulating the microglia functional state in the aging brain

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Signaling of BDNF and its precursor proBDNF via their receptors TrkB and p75^{NTR} regulates several processes including the development of the neuronal network, its functional and structural plasticity and learning and memory processes. While BDNF-TrkB signaling mediates neuroprotective and plasticity promoting effects, proBDNF-p75^{NTR} is involved in regulating neuronal death. BDNF has been shown to be expressed also in microglia, the resident macrophages in the brain and is suggested to regulate their activation state. Interestingly, while BDNF decreases, proBDNF increases in the brain with age leading to a shift in the BDNF/proBDNF ratio in favor of the pro-form and associated with a progressive alteration in the microglia activation state. However, the mechanisms regulating microglia activation states during aging remain elusive and a possible role of the age-dependent changes in BDNF versus proBDNF signaling in this context is not understood. Since microglia activation is involved in several neurological conditions, including age-related neurodegenerative diseases to analyze how changes in BDNF/proBDNF signaling influence microglia activity and how in turn the activation state of microglia may affect BDNF synthesis and release is especially interesting.

In primary microglia cultures, both wild-type (WT) and bdnfKO^{het} microglia respond in a concentration-dependent manner to activation by Lipopolysaccharide (LPS) with the secretion of the pro-inflammatory cytokines TNF-α and IL-6. The secretion of IL-6 is significantly lower in bdnfKO^{het} than WT cultures. Next, we investigated whether BDNF/proBDNF modulate the LPS-induced microglial activation treating WT primary microglia for 24 hours with increasing concentrations of recombinant human BDNF or uncleavable proBDNF before stimulation with LPS. Interestingly, the lower doses of BDNF and proBDNF suppress the production of IL-6 in a sex-dependent manner. Current experiment analyze the effects of BDNF/proBDNF on the morphological activation of microglia upon LPS stimulation. In addition, upon LPS stimulation p75^{NTR}KO microglia release significantly less IL-1, IL-6 and TNF-α compared to WT. In vivo, p75^{NTR}KO microglia shows a reduced complexity and alteration in process motility. A similar trend was observed in bdnfKO^{het} microglia of 3-month and 9-month-old female mice.

Overall our results so far demonstrate that signaling of BDNF and proBDNF modulate the inflammatory status of activated microglia in a dose- and sex-dependent manner.

Purinergic calcium signaling in astrocytes of the mouse medial prefrontal cortex

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The medial prefrontal cortex (mPFC) is a cortical brain region which plays a major role in cognitive processes, emotion regulation, motivation and sociability. It acts as a control center, therefore integrating information from numerous input structures and then projecting processed information into other structures as efferents. The mPFC contains different cell types such as excitatory pyramidal neurons, inhibitory GABAergic interneurons and astrocytes whose interaction is cruical for the cognitive functions. Both astrocytes and neurons express various neurotransmitter receptors which, when activated, lead to an increase in intracellular calcium (Ca^{2+}). This study aimed to characterize purinergic Ca^{2+} signaling and to identify the receptors involved in astrocytes of the mPFC.

We visualized Ca^{2+} signals in GCaMP6s-expressing astrocytes using confocal microscopy and applied different P2Y receptor agonists and antagonist to analyse the pharmacological profile of purinergic Ca^{2+} signaling. Dose-respond curves of adenosin-5'-(β-thio)diphosphate (ADPßS), uridine 5'- triphosphate (UTP) and uridine 5'-diphosphate (UDP) determined EC50-values of 3,26 µM (ADPßS), 5,16 µM (UTP) and 7,92 µM (UDP).

Both UTP and UDP induced Ca^{2+} signals in astrocytes, with UTP-evoked Ca^{2+} signals being partly reduced by the $P2Y_2$ antagonist AR-C118925XX, suggesting expression of $P2Y_2$, $P2Y_4$ and $P2Y_6$.

Application of the non-hydrolyzable P2Y agonist ADPßS induced Ca^{2+} signals in mPFC astrocytes, which were reduced by the $P2Y_1$ antagonist MRS2179 in some of the cells. The difference in the effect of MRS2179 between cells lead to the assumption of three subpopulation: MRS2179-sensitive cells, partly MRS2179-sensitive cells and a subpopulation of 49% of the astrocytes that is non-sensitive to MRS2179. The expression of the $P2Y_1$ receptor in a subpopulation of astrocytes could be confirmed by immunohistochemical staining of brain slices from floxed mice expressing tdTomato under control of $P2Y_1$-dependent Cre expression. Since 49% of the cells did not show a reduction in ADPßS-evoked intracellular Ca^{2+} release by MRS2179, we aimed to identify the remaining receptors involved in mediating ADPßS-induced Ca^{2+} signaling in mPFC astrocytes. However, the application of the $P2Y_2$ antagonist AR-CXX and the $P2Y_6$ antagonist MRS2578 did not lead to a reduction in ADPßS-evoked intracellular Ca^{2+} signals.

The results indicate that the $P2Y_1$ receptor conveys ADPßS-induced Ca^{2+} signaling in mPFC astrocytes, whereas $P2Y_{2,4,6}$ are expressed, but not involved in ADPßS-induced Ca^{2+} signals.

Impact of two-week repetitive magnetic stimulation on microglia activity and neuronal plasticity

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Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation technique for modulating neocortical excitability in humans. Repetitive magnetic stimulation (rMS) induces functional changes in dendritic spines and enhances excitatory neurotransmission in vitro. In vivo studies have demonstrated rTMS therapeutic potential for psychiatric disorders in both animal models and patients.

Despite its widespread use in research and clinical settings, the molecular mechanisms underlying rTMS effects remain poorly understood. Particularly, the role of microglia, the brain's primary immune cells, in mediating rTMS outcomes has not been thoroughly investigated. This study examined the impact of intermittent theta burst stimulation (iTBS) - 900 daily pulses over ten days - on mouse organotypic brain tissue cultures. Electrophysiological recordings of CA1 pyramidal neurons showed decreased AMPA receptor-mediated spontaneous excitatory postsynaptic current frequencies and reduced spine density following iTBS. These effects were no longer observed after microglia depletion, suggesting their direct involvement. Live imaging experiments further revealed decreased microglial process motility in steady state condition but increased motility after laser-induced lesions. iTBS also reduced markers of reactive microglia, including CD68 expression and released IFN-gamma.

These findings indicate that iTBS can modulate microglial behavior and reactivity, offering therapeutic potential for conditions characterized by neuronal hyperexcitability and microglial hyper-reactivity.

Sex-specific molecular and cellular phenotypes of pain resolution in a rat model for neuropathic pain

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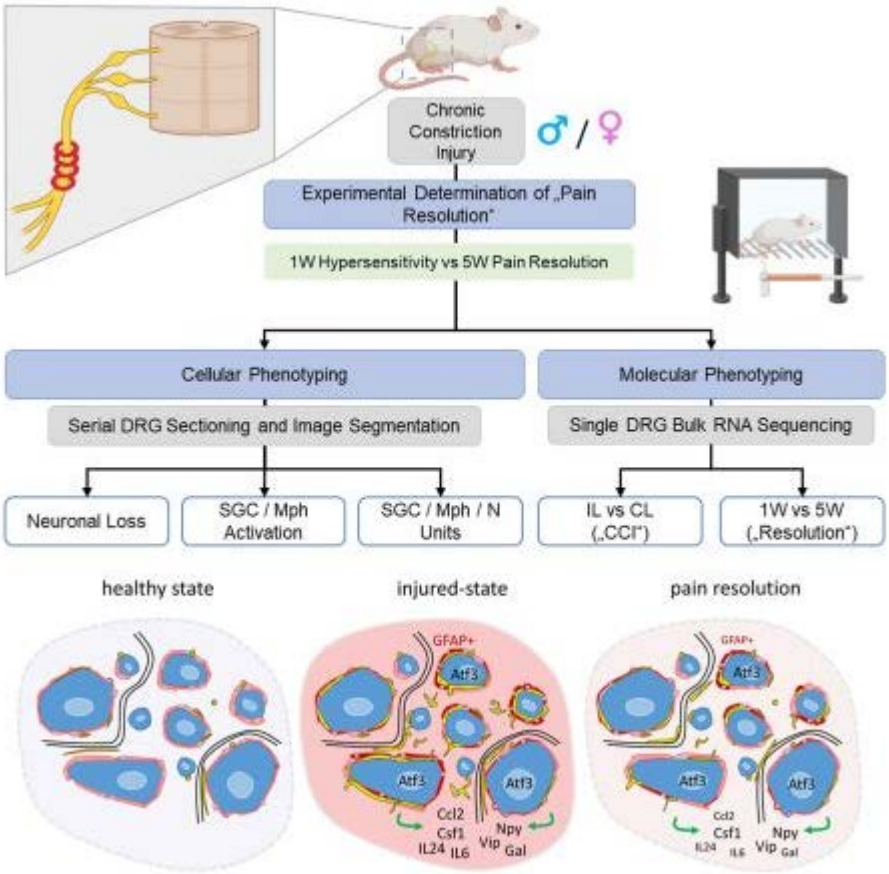
Introduction:
"Neuropathic Pain" is caused by a lesion or disease of the somatosensory system and affects approximately 10% of the general population. As such, it poses a significant socio-economic challenge, with a higher prevalence in women. Neuropathic pain is often accompanied by signs of systemic inflammation. However, recent evidence suggests that approaches that generally suppress inflammation are ineffective and may instead lead to persistence of pain. Thus, new strategies to actively aid the process of pain resolution are needed. However, the mechanisms behind natural pain resolution remain elusive.
Critical for development and persistence of neuropathic pain are multicellular processes in the dorsal root ganglia (DRGs) where the cell bodies of the nociceptive neurons are located. In this project, we aimed to identify molecular and cellular phenotypes and mechanisms of natural pain resolution. We hypothesized that specific phenotypes of sensory neurons, local macrophages and satellite glial cells (SGCs) develop during pain resolution.

Methods:
The chronic constriction injury (CCI) of the sciatic nerve was chosen to model pain resolution, in rats and in both sexes. In the CCI model, a loose ligature is tied around the sciatic nerve. This causes initial pain behavior which resolves naturally within few weeks. We determined ongoing pain resolution experimentally as a 50% improvement of mechanical hypersensitivity.
For analysis of cellular plasticity, whole DRG were sectioned and imaged with confocal and large-scale tile microscopy. Bioimages were analyzed with a deep learning-based approach for image feature segmentation. This strategy has been shown to increase the objectivity and validity of bioimage analysis, and was here applied to thousands (>7500) of multi-color bioimages showing neurons, SGC, and local macrophages. Molecular phenotypes were defined by bulk transcriptome analysis.

Results:
Five weeks after CCI, mechanical hypersensitivity was reduced by 50%. Albeit we see a subtype specific reduction of the non-peptidergic IB4 positive neurons, our data indicate no neuronal loss within five weeks after CCI. Our data show injury- and pain resolution-related phenotypes which are multicellular, multifactorial, and sex-specific: Most striking was the identification of a macrophage subtype, which 'displaces' satellite glial cells (SGC) from the sensory neuron border after injury. While this effect was diminished during pain resolution in males, it persisted in female rats. Conversely, we report a temporary upregulation of GFAP after injury, which persists only in males during pain resolution. Transcriptome changes reflect these multicellular responses of neurons, SGC, and macrophages. A significant downregulation of immune-related GO-terms during pain resolution with distinct sex differences points to macrophages at the neuron-SGC interface. Moreover, the gene profile of pain resolution is not only a reversal of the injury-related expression pattern, but points to resolution-specific processes.

Conclusions:
Our data suggest distinct mechanisms in each sex concerning neuroimmune and glial interactions in the course of pain manifestation and natural pain resolution. The identification of a set of regulated signaling mediators asks for in depth analysis of how the injured DRG signals on the systems level.



Developmental and Neuroinflammatory Changes in Glutamate and Adenosine Receptor-Mediated Ca2+ Signaling in Astrocytes of the Olfactory Bulb

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Astrocytes play a pivotal role in neuronal communication by modulating calcium (Ca^{2+}) signaling in response to neurotransmitters. Although astrocyte function has been widely studied, the developmental changes in their Ca^{2+} signaling dynamics are not fully understood. This study investigates developmental changes in astrocyte Ca^{2+} signaling mediated by group I metabotropic glutamate receptors (mGluRs) and adenosine receptors in the olfactory bulb, and explores the influence of neuroinflammation on the astrocyte Ca^{2+} signaling. Using confocal Ca^{2+} imaging in acute brain slices from C57BL/6 mice aged between postnatal day 7 and 26, we evaluated changes in astrocyte Ca^{2+} responses in the glomerular layer following stimulation with (S)-3,5-dihydroxyphenylglycine (DHPG), a group I mGluR agonist, and adenosine. Our findings revealed a significant decrease in DHPG-induced Ca^{2+} signaling during development, predominantly mediated by mGluR5, as evidenced by substantial reduction with the mGluR5 antagonist MTEP. Similarly, adenosine-induced Ca^{2+} responses decreased with age and were largely inhibited by an A2A receptor antagonist ZM241385, highlighting the critical role of A2A receptors in developmental Ca^{2+} signaling. To study the impact of neuroinflammation, we induced experimental autoimmune encephalomyelitis (EAE), a widely used animal model of multiple sclerosis, in a cohort of mice. As a result, EAE-induced neuroinflammation did not significantly alter DHPG-evoked and adenosine-evoked Ca^{2+} responses in olfactory bulb astrocytes compared to controls. In conclusion, our study provides insights into the developmental regulation of astrocyte physiology in the olfactory bulb. The observed decrease in mGluR5 and A2A receptor-mediated Ca^{2+} signaling with age underscores the dynamic nature of astrocyte-neuron interactions during brain maturation. Understanding these developmental changes enhances our insights into astrocytic contributions to synaptic physiology and may help to develop strategies for modulating neuronal communication in neurodevelopmental and neuroinflammatory disorders.

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Nav1.2 Expression in Oligodendroglial Cells

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SCN2A encodes the sodium channel $Na_V1.2$, which plays an important role in neuronal action potential generation, especially in the early stages of brain development. Loss of function of the *SCN2A* gene has been associated with autism spectrum disorder and intellectual disability, while a gain of function has been associated with infantile-onset seizures and encephalopathy.
It has been shown that *Scn2a* is also expressed in oligodendroglial cells. Oligodendrocyte progenitor cells (OPCs) reach the highest density of Na_V channels as they begin to differentiate into oligodendrocytes (OL) and myelinate. The density of Na_V channels declines once developmental myelination is completed [3] $Na_V1.2$ has been proposed to be important for axon-glial communication and maturation of oligodendrocytes.[1]
In primary cell cultures from *Scn2a* mutant and wild-type mice, we examined $Na_V1.2$ expression across different stages of oligodendrocyte development. Findings from these cultures revealed that the *SCN2A* mutation led to altered $Na_V1.2$ expression in proliferating and pre-myelinating OPCs, with the mutation significantly reducing $Na_V1.2$ expression in proliferating cells. This suggests that the mutation may impair early OPC development. These results are consistent with the observed mild changes in OPC and oligodendroglia cell numbers in the tissue analysis.
It is known that neuronal activity increases OPC proliferation and differentiation, depending on the stimulation paradigm. Therefore, mutations in $Na_V1.2$will not only influence the activity of neurons but will also impact the behavior of OPCs.
We investigated the effect of an *SCN2A* mutation, causing an increase in neuronal excitability, on the development of oligodendroglia cells and myelination. We used brain tissue from heterozygous mutant and control mice to analyze the impact of the *Scn2a*p.A263V gain-of-function [2] mutation on oligodendroglia cell number and myelination in different age groups.

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Pharmacological targeting of Smoothened receptor as a promising approach to enhance oligodendrocyte differentiation

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Remyelination is a natural repair process of the central nervous system (CNS), that restores axonal insulation, promoting neuroprotection and functional recovery after myelin damage. Several demyelination pathologies could benefit from improved CNS remyelination, especially during ageing. Despite this, to date, no remyelination agent arrived at the clinic. There is an urgent need for innovative pharmacological strategies to enhance remyelination and improve the effectiveness of current therapeutic molecules. Recent phenotypic screening studies have highlighted the promyelinating properties of some glucocorticoids (GCs) in multiple sclerosis animal models. This specific class of GCs interacts, not only with the Glucocorticoid Receptor (GR), but also with the Smoothened (Smo) receptor of the Hedgehog pathway. However, how their binding to Smo influences oligodendrocyte precursor cells (OPCs) remains unclear (Al Jaf et al., 2024). Additionally, the individual contributions of each receptor to the observed promyelinating effects are yet to be fully understood. Gaining deeper insights into how these ligands modulate Smo receptor activity could provide critical information for structure-based drug design, paving the way for more precise and effective remyelination therapies.
Building on this knowledge, we focused on studying two molecules that bind Smo: the GSA-10, a synthetic molecule derived from the pharmacophore of Smoothened agonist SAG, and the Budesonide, a GC that binds to the cysteine-rich domain (CRD) of Smo. Both these drugs prevent Smo activation in fibroblasts. Our latest study employed a combination of cellular, biochemical, and molecular dynamics approaches to demonstrate that budesonide treatment promotes myelination in oligodendroglia cells by facilitating synthetic axon ensheathment. Moreover, Budesonide reduces the conformational flexibility of the Smo CRD, thereby inhibiting canonical Smo-mediated signaling. At the same time, Budesonide activates the Liver Kinase B1 (LKB1)/AMP-activated protein kinase (AMPK) pathway, which leads to the upregulation of Myelin Basic Protein (MBP) expression (Recchia et al., 2024). These data reinforce previous evidence that Smo plays a key role in remyelination via activation of the LKB/AMPK pathway. How activation of this pathway can regulate MBP gene transcription represents the next crucial step in understanding a basic mechanism of OPCs differentiation in myelinating oligodendrocytes. Together, these findings lay a solid foundation for pharmacologically targeting the Smo as one of the strategies to enhance OPC differentiation and promote remyelination, opening up new avenues for therapeutic intervention in demyelinating diseases.

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The impact of serotonergic signaling on astrocyte function and morphology

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Astrocytes express various serotonin receptors, with region-specific pattern of expression that mediate distinct effects on their morphology and functions. We have previously shown that the serotonin receptor 4 (5-HT4R) shapes hippocampal astrocyte morphology and function (Müller, Schade, et al., 2021). Astrocyte function is reflected by complex patterns of intracellular Ca^{2+} activity, for which we have previously developed a multi-threshold event detection (MTED) approach to capture and analyze these Ca^{2+} signals in great detail (Müller, Cherkas, et al., 2021). Here, we combined Ca^{2+} imaging with morphological analysis and pharmacological activation to study how astrocyte morphology shapes Ca^{2+} dynamics.

We examined the effects of 5 HT4R and 5 HT7R signaling on the Ca^{2+} activity in hippocampal and prefrontal cortex astrocytes in vitro and in situ, and further analysed corresponding knockout models. Elevated basal Ca^{2+} levels were observed in hippocampal versus cortical astrocytes, and selective serotonin receptor activation resulted in distinct Ca^{2+} responses. Our findings suggest that serotonergic signaling in astrocytes modulates their morphology, neuronal interactions, and their Ca^{2+}-mediated communication.

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A role of NAD in glial support for axonal integrity

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Nicotinamide adenine dinucleotide (NAD^+) is essential to maintain axonal integrity. The loss of NMNAT2, a rate-limiting enzyme in the NAD^+ synthesis pathway, activates sterile alpha and TIR motif containing 1 (SARM1) after axonal injury, further depleting NAD^+ levels and triggering active axonal degeneration (Gerdts et al., 2015). How NAD^+ and its precursors are taken up by axons is not well understood, especially in the white matter where axons are wrapped by myelinating oligodendrocytes. In addition to the well-known role of saltatory conduction, oligodendrocytes have been proposed to metabolically support axonal compartments (Fünfschilling et al., 2012). This raises the question of whether oligodendrocytes participate in the NAD^+ metabolism of the axonal compartment and help maintain the axonal integrity. To test this, we cultured myelinated optic nerve *ex vivo* and monitored the axonal degeneration by time-lapse imaging under low glucose over the next 10 hours. We found that NAD^+ supplementation delays the formation of axonal blebs as a sign of ongoing degeneration, suggesting that this requires the transfer of NAD^+ through the myelin compartment in the white matter tract. We also found NMNAT2 expression in purified myelin using western blot analysis, indicative of local NAD^+ synthesis in the oligodendrocyte myelin compartment. We suggest a working model in which oligodendrocytes import circulating NAD^+ or its precursors and serve as a local source for the NAD^+ pool of myelinated axons.

Effects of LPS-Induced Inflammatory signaling on Intrinsic Calcium Activity of Mouse and Human Astrocytes

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Mouse and human astrocytes exhibit notable species-specific differences in their response to inflammatory stimuli, yet the impact of lipopolysaccharide (LPS)-induced inflammation on their endogenous calcium (Ca^{2+}) activity remains underexplored.

In this study, we investigated how LPS-induced inflammation affects Ca^{2+} activity in astrocytes derived from the mouse hippocampus and prefrontal cortex (PFC), as well as human induced pluripotent stem cell (iPSC)-derived astrocytes. Cultured astrocytes were exposed to LPS to induce an inflammatory response, and both morphological changes and Ca^{2+} activity were analyzed using our previously developed multi-threshold event detection (MTED) approach (Müller, Cherkas, et al., 2021). This method enabled a comprehensive assessment of Ca^{2+} activity patterns and their relation to cell morphology, revealing significant alterations in response to LPS treatment, and further between mouse and human astrocytes. Our findings highlight the unique sensitivity of human astrocytes to inflammatory signals and explore Ca^{2+} activity dysfunction in neuroinflammatory conditions. Understanding these species-specific responses is crucial for translating preclinical findings to human models and advancing therapeutic strategies targeting neuroinflammation and Ca^{2+} dysregulation in neurological diseases.

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Cell-cell and cell-matrix interaction of breast tumor cells with brain cells in a 3D hydrogel-based matrix

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3D cellular disease models better remodel the native surroundings than 2D models. Here, we study breast cancer as one of the most widespread diseases seen in women able to metastasize to the brain depending on its subtype. The triple-negative breast cancer (TNBC) is one of the malignant subtypes that has the highest probability of metastasizing to distant organs such as the brain, bone, liver and lung. This type of tumor is associated with a poor prognosis due to limited therapeutic options. The metastasis of the cancer cells is not a random process. Before tumor cells (seed) metastasize, the host microenvironment (soil) is modified to support tumor growth and proliferation. The non-cellular three-dimensional (3D) host microenvironment called extracellular matrix (ECM) provides biomechanical and biochemical signaling that is necessary for tissue morphogenesis, differentiation and homeostasis. The proliferative cancer cells remodel the microenvironment by secreting excess fiber-forming matrix proteins which render the ECM stiffer. The mechanical and compositional changes in the matrix interfere with the interaction between the microenvironment and healthy cells but also with cell-cell interactions and cell polarity.

The ECM of breast tumor is characterized by fiber-forming collagen types I, III, and V which play an important role in invasion and migration while collagen IV is decreased due to the degradation of the basement membrane. Hyaluronic acid is another important matrix component in the breast and is expressed at elevated levels and used mainly as a biomarker in breast cancer. In the brain, hyaluronic acid is also highly expressed and thus presents a main component of brain ECM. Hence, a microenvironment harbouring hyaluronic acid, as well as collagen IV, seems to represent a suitable surrounding for breast tumor growth.

In this project, one of our aims is to establish a 3D cellular disease model of breast cancer in a brain-like ECM to better understand how neuronal signaling promotes breast cancer cell growth and proliferation. To do so, we used a thiolated hyaluronic acid (HA-SH) hydrogel reinforced by melt electrowritten (MEW) scaffolds and supplemented with ECM protein e.g. laminin, collagen subtypes and fibronectin. Breast tumor cells usually form spheroids in an HA-SH surrounding with high viability regardless of hydrogel stiffness. Following, ECM supplementation of the HA-SH tumor cells changed their morphology towards a spread-like appearance. Moreover, neurons and astrocytes have been shown to functionally interact with breast cancer cells promoting breast cancer growth. The breast cancer cells form pseudo-tripartite synapses with pre- and postsynaptic neurons to take advantage of neuronal glutamate release that activates AMPA/NMDA receptors expressed in the plasma membrane of tumor cells. Indeed, the presence of neurons and astrocytes in a 3D network also promoted breast tumor cell spreading and cell-cell interactions. The characterization of those cell-cell contacts is currently under investigation. The functionality of the tumor cell-primary brain cell interactions will also be assessed by calcium imaging analysis. The structural and functional degree of maturation is a measure for the suitability of the 3D model to further continue with drug testing strategies.

Myelination generates aberrant ultrastructure that is resolved by microglia

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To enable rapid propagation of action potentials, axons are ensheathed by myelin, a multilayered insulating membrane formed by oligodendrocytes. Most of the myelin is generated early in development, resulting in the generation of long-lasting stable membrane structures. Here, we explored structural and dynamic changes in central nervous system myelin during development. To achieve this, we performed an ultrastructural analysis of mouse optic nerves by serial block face scanning electron microscopy (SBF-SEM) and confocal time-lapse imaging in the zebrafish spinal cord. We found that myelin undergoes extensive ultrastructural changes during early postnatal development. Myelin degeneration profiles were engulfed and phagocytosed by microglia using exposed phosphatidylserine as one “eat me” signal. In contrast, retractions of entire myelin sheaths occurred independently of microglia and involved uptake of myelin by the oligodendrocyte itself. Our findings show that the generation of myelin early in development is an inaccurate process associated with aberrant ultrastructural features that require substantial refinement.

Crosstalk of α_1-noradrenergic Ca^{2+} and cAMP signaling in astrocytes of the murine olfactory bulb

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cAMP is a ubiquitous second messenger involved in regulating gene expression and synaptic transmission. While calcium imaging is a well-established technique utilizing a variety of chemical and genetically encoded sensors, cAMP sensors have been lacking until recently; hence, cAMP signaling has not been well studied in astrocytes so far. In this study, we investigated noradrenergic cAMP signaling in astrocytes of the main olfactory bulb using Flamindo2 and confocal imaging in acute brain slices. Flamindo2, developed by Odaka et al. (PLOS One 9:6, 2014) is a genetically encoded cAMP sensor whose fluorescence intensity decreases in response to increased cAMP levels.

To specifically target astrocytes, we performed a retroorbital injection of the AAV-packed Flamindo2 construct controlled by the astrocyte-specific promoter GFAP. The olfactory bulb and its glomerular layer, innervated by noradrenergic projections from the locus coeruleus, play a key role in olfactory processing, with norepinephrine (NE) modulating neuronal plasticity. NE induces cAMP signals in astrocytes in the glomerular layer of the olfactory bulb via α_1-, α_2- and β-adrenergic receptors. Although the α_1-receptor is known to be coupled to G_q, the application of the α_1-receptor agonist phenylephrine (PE) leads to dose-dependent cAMP signals in astrocytes, suggesting activation of adenylyl cyclases (ACs).

Experiments conducted in a Ca^{2+} -free environment showed that PE-induced cAMP signals were Ca^{2+}-dependent. Blocking IP_3 receptors confirmed the role of Ca^{2+} release from the endoplasmic reticulum (ER) in this signaling. Furthermore, blockage of Ca^{2+}-sensitive AC subtypes AC1 and AC3 suppressed PE-evoked cAMP signals, indicating that PE-evoked increases in Ca^{2+} led to activation of AC subtypes AC1 and AC3 and subsequent production of cAMP. The presence of AC1 and AC3 in astrocytes of the glomerular layer was confirmed via immunohistochemical staining. Moreover, experiments conducted in the presence of subtype-specific α_{1A}- and α_{1D} receptor antagonists demonstrated that both receptor subtypes are involved in the PE-induced cAMP signal.

In summary, our findings indicate that PE-induced cAMP signals in astrocytes of the glomerular layer are mediated by α_{1A}- and α_{1D}- receptor subtypes, with Ca^{2+} release from the ER activating AC1 and AC3. This crosstalk pathway may have significant implications for understanding astrocyte function in olfactory processing.

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Developmental profile of oligodendrocyte arrangement, identification and morphology in nuclei of the superior olivary complex

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Oligodendrocytes provide myelination and metabolic support for neurons, and are crucial for the establishment and remodeling of neural circuits. Their essential role in fast neuronal communication has been shown in the auditory superior olivary complex (SOC), where they facilitate precise timing of sound signals. While the early postnatal development of oligodendrocytes in the SOC has been documented, an in-depth analysis of nucleus-specific differences, their identification and single cell morphology throughout postnatal development is lacking. We used immunofluorescence labeling and single-cell electroporations to quantify oligodendrocyte distribution, density, identification and morphology in the SOC of postnatal day (P) 5 to P59 gerbils. Oligodendrocytes showed developmentally regulated, region-specific accumulations and redistributions, e.g. an increase in density in the low frequency region of the medial nucleus of the trapezoid body to potentially fine-tune myelination in neurons requiring microsecond precision. Oligodendrocyte density developed in a nucleus-dependent manner and varied between gerbil and Etruscan shrew, indicating a region- and species-specific necessity for the presence of oligodendrocytes. The identification of oligodendrocytes by Olig2 and SOX10 varied with age, while S100 labeling consistently detected myelinating cells regardless of age revealing that oligodendrocytes that remain at synapses are mostly non-myelinating cells. Quantification of single-cell morphology showed a decrease in the number of myelinating processes and an increase of process length, diameter and coverage area per cell during postnatal development. These findings suggest that oligodendrocytes mature and myelinate in a region-specific manner during synaptic and overall circuit refinement, implying roles beyond axon myelination. Moreover, the temporal overlap of alterations in oligodendrocyte arrangement, density, and morphology with key developmental stages, highlights their adaptability to circuit-specific needs.

Astrocytic cAMP increases ATP release frequency in hippocampal slices

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Cyclic AMP is a ubiquitous second messenger that initiates complex intracellular signalling cascades in response to extracellular signals. The role of cAMP in glial cells is still not clear, here we focus on astrocytes. These cells are responsible for the uptake and secretion of soluble factors like glutamate, ATP, D-serine, pro- and anti-inflammatory cytokines. While the relationship between astrocytic calcium and astrocytic function has been extensively investigated, much less is known about cAMP signaling in astrocytes and its impact on brain function. In the hippocampus, many studies have shown that forskolin stimulation of endogenous adenylyl cyclases increases the strength of synaptic connections (“chemical LTP”). However, such manipulation affects all cells in the tissue, making it impossible to distinguish between neuronal pre or postsynaptic cAMP effects or to evaluate the possible effects of increased cAMP in specific cells. Here, we expressed the photoactivatable adenylyl cyclase PACmn (Yang et. al, BMC Biology 2021) specifically in astrocytes to control cAMP signaling with blue light.

One of the main gliotransmitters released from astrocytes is ATP. We use GRAB-ATP, a genetically encoded G protein-coupled receptor activation-based (GRAB) sensor for extracellular ATP (Wu et. al, 2022). When expressed on the surface of neurons or astrocytes, we observed that in the absence of stimulation, spontaneous ATP release events occur in hippocampal slice cultures at a frequency of about 0.2 per minute. When cAMP was optogenetically elevated in astrocytes, the frequency of events doubled and returned towards baseline after the light was switched off. Interestingly, ATP release events do not overlay with PACmn-expressing astrocytes, suggesting that either non-expressing astrocytes or a different type of cell is responsible for the ATP release.

Increasing astrocytic cAMP rapidly and transiently increased the frequency of miniature excitatory postsynaptic currents (mEPSCs), but not their amplitude, suggesting a modulation of synaptic transmission. We observed no long-term effects of increased astrocytic cAMP on synaptic transmission and no change in neuronal excitability. In summary, astrocytic cAMP affects the frequency of spontaneous ATP release events via a yet unidentified mechanism and acutely affects glutamate release at excitatory synapses.

Dynamic transcellular molecular exchange: a novel view on extracellular matrix remodeling

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The brain extracellular matrix (ECM) is a lattice-like structure that occupies the intercellular space in the central nervous system (CNS). The ECM constituents are extremely long-lived and are widely believed to stabilize neurons and synapses in the brain. They are thought to be renewed only rarely, through the activation of matrix metalloproteinases (MMPs) that cleave the existing ECM components, followed by de novo ECM synthesis. However, various studies have demonstrated that synapses change their structural organization on a time scale of minutes to hours, calling for additional mechanisms for ECM remodelling. Using advanced microscopy techniques, molecular biology tools and biochemical assays, we report here novel insights on the ECM dynamics. The proteoglycan neurocan (Ncan) frequently shifts between the neuronal surface ECM and astrocytic intracellular compartments. Ncan is secreted initially by glia cells, and predominantly resides on their surfaces for at least one week *in vitro*. It is later transferred to neurons, and continues to shuttle between these cells throughout later developmental stages. Inhibiting this process with specific drugs or mutations results in Ncan accumulation in glia. Our findings challenge the prevailing view of ECM remodelling, highlighting a previously unknown mechanism of dynamic transcellular molecular exchanges within the CNS.

Synaptic reorganization and perisynaptic astrocyte plasticity at spines of pyramidal neurons in the motor cortex during a simple motor task

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Motor learning is correlated with a significant increase in the formation of new spines on the apical tufts of layer 5 pyramidal cells in the primary motor cortex (M1) (Xu T et al. Nature 2009) indicating that spine plasticity during motor learning is associated with motor skill acquisition. Dendritic spine plasticity is thought to be the cellular basis of learning and memory and involves a dynamic orchestration of existing spines and the formation as well as elimination of new spines. We showed that two presynaptic neural circuits supervise distinct programs of spine dynamics to execute a simple motor learning (Sohn J et al. Sci Adv 2022). We first imaged spine dynamics in M1 during learning and then performed post-hoc identification of their afferent presynaptic neurons. New spines that appeared during learning initially received small synaptic contacts from corticocortical (CC) neurons but these contacts were subsequently eliminated on skill acquisition. In contrast, spines receiving from axons from thalamo-cortical (TC) neurons were newly formed, persisted, and enlarged. These results suggest that pyramidal cell dendrites in M1 show a neural circuit-dependent division-of-labor during skill learning, with the initial dynamic instructive? contacts from top-down intracortical axons followed by synaptic memory formation driven by thalamic axons. Dual spine supervision may govern diverse skill learning in the neocortex (Sohn J Sci Adv 2022).

Perisynaptic astrocyte processes (PAPs) in excitatory tripartite synapses are crucial for glutamate clearance, synapse isolation, and ion homeostasis. Therefore, we sought to examine any role of PAPs in the synaptic dynamics observed during the motor learning. The functions of PAPs can be modulated by their structural dynamics, which in turn affect synaptic activity. We analyzed the level of PAP access to the synaptic cleft at each synapse, and compared this index between newly formed and stable spines during the motor skill learning task. Serial sections of Automated Tape-Collecting Ultramicrotome Scanning Electron Microscopy (ATUM-SEM) micrographs of the M1 were 3D-reconstructed, and the spatial pattern of PAPs approaching the synaptic cleft at newly formed and stable tripartite synapses of trained mice was investigated. For this analysis, we developed a method to quantify the synapse-approaching pattern of PAPs, using fractional coverage volume, surface area, and mitochondria distribution. Our results revealed that the positions of PAPs relative to the synaptic cleft significantly varied depending on the timing of synapse formation, maturity, and training period during motor skill learning.

Local differences in baseline Na^+ shape astrocytic K^+ uptake by the NKA

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The inwardly directed Na+ gradient is the main driving force for a plethora of transport processes across the astrocyte plasma membrane. This Na+ gradient is maintained by the Na+/K+-ATPase (NKA), which at the same time, is a major mechanism for the reuptake of K+ from the extracellular space, thereby controlling neuronal excitability. As NKA activity not only depends on the extracellular K+ levels, but is also regulated by the intracellular Na+ concentration, knowledge on baseline Na+ and activity-related changes in astrocytic Na+ is required to understand this essential function. However, whereas recent studies using intensity-based fluorescence imaging enabled the quantitative measurement of Na+ in cell bodies, Na+ concentrations in processes of astrocytes were not determined so far.
To address this question, we employed rapid fluorescence lifetime imaging microscopy (rapidFLIM) with the fluorescent indicator dye ION-NaTRIUM Green-2 in astrocytes of acute mouse hippocampal tissue slices. Experiments were performed in bolus-loaded (AM-loaded) slices as well as in astrocytes dye-loaded via patch clamp. Neuronal activity was mimicked by increasing the extracellular K+ concentration to about 7.5 mM for two minutes. RapidFLIM data obtained from bolus-loaded slices indicated that the baseline Na+ in astrocyte somata is approximately 10-15 mM, confirming earlier results obtained using intensity-based imaging. Surprisingly, we found that Na+ in primary and secondary astrocyte processes was significantly higher, ranging from 15 to 20 mM. This result was confirmed in cells filled individually through a patch pipette. Brief elevation of extracellular K+ resulted in a transient decrease of Na+ in astrocyte somata and processes, indicating activation of the NKA. The amplitude of this decrease strongly correlated with the baseline Na+ in the respective cellular compartment.
In summary, the spatiotemporal visualization and quantification of Na+ using rapidFLIM revealed an unexpected difference in the baseline Na+ between somata and individual processes of astrocytes. Moreover, our data demonstrates that this results in a different degree of activation of the NKA in response to increases in extracellular K+. We therefore conclude that differences in baseline Na+ shape astrocytic K+ uptake by the NKA, enabling astrocytes to locally adapt their uptake of K+ to the strength of neuronal activity.

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Development of Myelination in Globular Bushy Cells

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Globular bushy cells in the cochlear nucleus of the auditory brainstem circuit are responsible for the fast relay of signals to the superior olivary complex. Their axons possess a low internode length/axon diameter ratio (L/D) of ~60 that allow action potentials to travel along their axons with exceptional speed and precision. We investigate how the unusual myelination pattern develops. Recently, we showed that the internode length is determined early in development followed – after hearing onset - by an increase in axon diameter, which causes the L/D ratio to decrease. Additionally, we found that the unmyelinated regions of the axon, the Nodes of Ranvier, develop sequentially along the axon [1]. However, it remains unclear whether patterned spontaneous neuronal activity is crucial for this process. This patterned network activity is present well before hearing onset and has been reported to influence other properties of the circuit's development [2]. Therefore, the aim of this study is to determine how spontaneous neuronal activity before hearing onset affects myelination. We therefore established organotypic slice cultures of the gerbil auditory brainstem. Cultures could be maintained over a period of two weeks, allowing to study the development of myelin ex vivo. Additionally, we are using a viral vector encoding a light sensitive ion channel to manipulate the bushy cell activity at early developmental timepoints.

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Poster Topic

T10: Aging and Developmental Disorders

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T10-2C Impaired auditory maturation and its involvement in audiogenic seizure susceptibility in a mouse model of Fragile X Syndrome
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T10-3C Therapeutic efficacy is significantly improved with bilateral vs. unilateral intracerebroventricular drug application in a rodent model of absence epilepsy
Rosa Beatriz Rojas, Anna-Sophia Buschhoff, Elke Edelmann, Peer Wulff

T10-4C Novel therapeutic options for *KCNA2*-related epilepsy
Elisabeth Marianne Mechtild Brand, Peter Müller-Wöhrstein, Thomas Ott, Holger Lerche, Ulrike B. S. Hedrich

T10-1D Pathophysiological mechanisms of epileptogenesis in a mouse model of Dravet syndrome.
Albina Farkhutdinova, Nikolas Layer, Edueni Erharhaghen, Peter Müller-Wöhrstein, Friederike Pfeiffer, Ulrike Hedrich-Klimosch, Holger Lerche, Thomas Wuttke

T10-2D Fragile X mice show context-dependent deficits in vocal behaviour during opposite sex interaction
Ursula Koch, Julia Freitag, Thorsten Michael Becker, Virginia Baatz, Daniel Breslav, Leon Marquardt

T10-3D A sandwich of glioblastoma cells and a brain tissue slice: an in vitro model to explore interactions of tumour cells with neural tissue
Maurice Meseke, Benjamin Schwindenhammer, Igor Jakovzwski, Ramon Rebstock, Firat Acur, Marie-Luise Kümmel, Eckart Förster

T10-4D Transcriptomic insights into epileptogenesis in a *Kcna2* loss-of-function mouse
Peter Müller-Wöhrstein, Hayri Calap, Elisabeth Marianne Mechtild Brand, Nikolas Layer, Thomas Ott, Holger Lerche, Thomas Wuttke, Ulrike B. S. Hedrich

Mechanisms of the Mast1-associated Mega-Corpus Callosum Syndrome

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Mutations in the Microtubule-associated Serine/Threonine Kinase 1 (MAST1), a member of the MAST kinase family, have been implicated in a spectrum of neurodevelopmental disorders, including Mega-Corpus Callosum Syndrome (MCCS).
This study elucidates the cellular and molecular underpinnings of the MAST1-associated MCCS. Using electron microscopy, we present a detailed timeline of corpus callosum development in mice from birth to adulthood and unveil key developmental differences between healthy and MAST1-mutant mice. Furthermore, we establish a MCCS patient-derived stem cell line to model the development of the MCCS in-vitro. Finally, we explore the molecular underpinnings of this disorder and present a putative disease mechanism.

The role of MAST2 in neurodevelopment and disease

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Constructing a human brain—from progenitor cells to transhemispherically connected mature neurons—relies on a complex interplay of molecular and cellular processes. Disturbances caused by genetic mutations affecting neuronal birth, migration, or differentiation can lead to severe neurological disorders. Notably, recent studies have implicated the MAST family of microtubule-associated kinases in neurodevelopmental conditions such as mega-corpus-callosum syndrome and developmental and epileptic encephalopathy; however, the underlying mechanisms of these diseases remain unknown.
In this work, we present the clinical profile of patients with MAST2 mutations, who exhibit hallmark features of neurodevelopmental disorders, including early-onset epilepsy, autism spectrum disorder, and intellectual disability. To investigate the pathological impact of these mutations, we developed two complementary mouse models: a MAST2 knockout line and a model carrying a patient-specific mutation. Through behavioral, anatomical, and molecular analyses of these mice, we aim to uncover how MAST2 deficiency alters brain development. Lastly, to deepen our understanding of MAST2's role in human neurodevelopment, we are establishing a patient-derived cerebral organoid model. Taken together, this study will aid in the molecular diagnosis of neurodevelopmental disorders and shed light on the pathophysiological mechanisms associated with MAST2 mutations.

Exploring Cognitive-Motor Dual-Tasking: Neuroimaging Insights into Behavioral Variability Between Young and Older Adults

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Background: Dual-task performance, which involves the simultaneous execution of cognitive and motor tasks, becomes increasingly challenging with age. It significantly impacts the daily lives of older adults. Understanding the behavioral differences and neural mechanisms of dual-tasking between young and old adults can provide insights into strategies to enhance dual-task ability and improve quality of life in aging populations.

Methods: We developed a novel walking-like pedal device compatible with magnetic resonance imaging (MRI) to accurately measure foot movement during a cognitive-motor dual-task paradigm. Twenty healthy young adults (aged 20-39) and forty-three older adults (aged 50-80) participated. The study included two single tasks—a Go/NoGO (cognitive) and a motor task—and a dual-task combining both. Participants responded to Go/NoGo stimuli using their fingers while simultaneously pedaling in response to motor stimuli with their feet. Motor reaction times were recorded to assess response times mimicking walking-like movements. Dual-task cost (DTC), defined as the relative difference of motor reaction time between the dual-task and single-task conditions, was calculated for both age groups.

Results: In the single motor task, no significant difference in motor reaction time was observed between the young and old groups ($p = 0.8428$). However, during the dual-task, older adults exhibited significantly slower reaction times (mean = 0.4899 s) compared to younger adults (mean = 0.4265 s, $p = 0.0437$). Furthermore, older adults showed a much higher dual-task cost (DTC = 52.89%) in reaction time variability compared to younger adults (DTC = 8.36%), with a highly significant difference ($p = 0.0012$).
Group-level brain activation analysis in the older group revealed significant activations in multiple regions comparing the dual-task condition to the two single tasks condition. These areas included:
• The superior frontal gyrus, associated with high-order motor functions and cognitive control.
• The primary motor cortex, responsible for executing voluntary motor movement.
• The premotor cortex and supplementary motor area (SMA), critical for motor planning and coordination.
• Subcortical structures such as the basal ganglia, as well as the left cerebellum (VI), right cerebellum (V), and occipital regions.
These results indicate that the dual-task condition elicited stronger brain activations in both cortical and subcortical areas compared to performing two single tasks separately in older adults. In contrast, the young group showed no significant differences in brain activation between the conditions, suggesting that younger adults are more efficient in managing cognitive and motor tasks simultaneously, requiring fewer additional neural resources.

Conclusion: We found that older adults exhibited slower reaction times and higher dual-task cost in reaction time variability during dual-tasking, which is concomitant with increased brain activity in regions associated with motor planning and coordination and cognitive control. These findings suggest that older adults rely more heavily on additional neural resources to maintain performance in complex cognitive and motor dual-task situations.

Can early postnatal environment rescue impaired auditory processing and sensorimotor gating in a genetic rat model for autism spectrum disorder?

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BACKGROUND AND AIM: Autism spectrum disorder (ASD) is a neurodevelopmental condition affecting one in 160 children worldwide. The homozygous Cntnap2-knockout (Cntnap2-/-) rat is a preclinical genetic model for studying ASD-related phenotypes. Previous work has demonstrated that there are greater ASD-like deficits in the Cntnap2-/- rat when bred and reared by a Cntnap2-/- compared to a heterozygous (Cntnap2+/-) dam. Considering that these Cntnap2-/- offspring have the same genetic mutation, it suggests that environmental factors are influencing their development. This confirmatory project investigated if these environmental effects occur pre- or postnatally.
METHODS: We conducted a cross-fostering paradigm in which Cntnap2-/- offspring were bred from a Cntnap2-/- dam and transferred to be reared by a Cntnap2+/- dam. These cross-fostered animals were compared to Cntnap2-/- animals bred and reared by a Cntnap2-/- dam as well as Cntnap2-/- and wildtype animals bred and reared by a Cntnap2+/- dam. All animal groups contained both sexes and met adequate sample sizes. Throughout development, we examined ASD-like deficits in auditory processing and sensorimotor gating.
RESULTS: All Cntnap2-/- regardless of parental genotype and cross-fostering showed impaired neural responsiveness in the auditory brainstem response (the neural activity in the brainstem in response to auditory input), the acoustic startle response (the whole-body contraction reflex elicited by the sudden presentation of a loud auditory stimulus), and prepulse inhibition (the reduction in the startle response if the startling stimulus is preceded by a low-intensity prepulse). However, cross-fostering restored a deficit in the maturation of hearing sensitivity for Cntnap2-/- rats bred from a Cntnap2-/- dam.
CONCLUSIONS: Together, this research provides evidence that some ASD characteristics observed in the Cntnap2-/- are not fixed by genetic predisposition but can be malleable by early postnatal environmental conditions. Furthermore, the results have implications for how all researchers conduct breeding when using genetic animal models to study neurodevelopmental conditions. This research was funded by a CIHR and NSERC-USRA.

Oligodendrocyte mechanotransduction channel Tmem63a fine-tunes myelin sheath thickness in the central nervous system

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Mechanotransduction channels (MTCs) play a crucial role in the process of peripheral myelination by Schwann cells. Although it has been proposed that mechanical forces can regulate myelination in the central nervous system (CNS), very little is known about the expression of MTCs and their role in oligodendrocytes (OLs) development and myelination. To systematically identify the expression of all mechanotransduction ion channels in OLs, we performed single-cell Split-Seq RNA sequencing analysis on Magnetic-activated cell-sorted O4+ OLs from the adult cerebral cortex. We found that several MTCs are expressed by OLs and discovered Tmem63a (also called OCaR1) as one of the most abundant MTCs in OLs. To characterize the expression of Tmem63a in the CNS, we used our newly generated knock-in transgenic mouse lines expressing endogenous levels of Tmem63a protein tagged with enhanced yellow fluorescent protein (Tmem63a-eYFP). An extensive immunohistochemical analysis showed that Tmem63a is mainly expressed by myelinating as well as mature OLs in both grey- and white matter but not in oligodendrocyte precursor cells (OPCs). Our electrophysiological and Ca2+ imaging analysis on OL derived from control and Tmem63a knock-out (Tmem63a-/-) mice revealed that mechanical membrane stretches resulted in Tmem63a-dependent influx of positive cations and induced robust Ca2+ signals in the OL. A systematic histological analysis of the brains from control and Tmem63a-/- mice showed a severe developmental hypomyelination at postnatal days (P) 11, which persisted at the juvenile stage (P21) but was resolved while mice reached sexual maturity and adulthood around 5-6 weeks of age (P35). Although the gross developmental myelination was recovered by P35, a detailed electron microscopic analysis of axonal myelination in the motor cortex and corpus callosum revealed aberrant myelination of very small caliber axons (<150nm) and hypomyelination of large caliber axons, indicating that Tmem63a plays a decisive role in tuning myelin sheath thickness on individual axons. In Tmem63a-/- mice and OL-specific conditional knock-out mice (Mogi-Cre; Tmem63a fl/fl), we observed that OLs layered shorter myelin internodes with diffused and elongated nodes of Ranvier. Next, we performed a systematic motor behavior analysis on 7-8 weeks-old Tmem63a -/- mice to test whether such radial and longitudinal ultrastructural deficits in myelin sheath could result in motor dysfunction. While Tmem63a-/- mice had no significant movement dysfunction and ataxias, mutants exhibited deficits in fine motor functions such as motor coordination and gait. To gain mechanistic insights into intracellular signaling regulated by Tmem63a in OL, we performed bulk-RNA sequencing on enriched OLs isolated from the cortex of Tmem63a -/- mice and littermate controls at P11 and P35. Analysis of differentially expressed genes between the P11 and P35 stages indicates that Tmem63a-mediate Ca^{2+}signaling might regulate vesicular transport and endocytotic secretory pathways. In summary, we identified Tmem63a as a key MCT in OLs, which links mechanical forces sensed by OLs during active myelination with the Ca^{2+}dependent transport and secretory pathways required for fine-tuning myelin sheath thickness on axons.

Altered topography and ensemble activity in auditory cortex of *FMR1* knockout mice

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Autism spectrum disorder (ASD) is often associated with social communication impairments and specific sound processing deficits, such as impaired auditory filtering and problems in following speech in noisy environments. Loss-of-function mutations in the *Fragile X Messenger Ribonucleoprotein 1* (*FMR1*) gene, leading to Fragile-X-Syndrome, are the most frequent monogenetic cause of ASD. We here studied sound-evoked activity patterns within the auditory cortex (AC) of *FMR1* knockout (KO) mice and littermate controls to explore possible causes of auditory deficits in ASD, using two-photon imaging of AC layer 2/3 neurons in awake animals. Topographic representation of frequency (tonotopy) was less well defined in KO mice when observing activity within primary AC and the anterior auditory field, yet unchanged for secondary AC. Subfield-specific differences were also found when analyzing ensemble activity in response to both pure tones and complex sounds. Ensemble correlations were lower in primary AC of KO mice in general, but higher in secondary AC in response to complex sounds. Furthermore, sound specificity of ensemble activity was decreased in the anterior auditory field of KO mice. To investigate network stability and representational drift, experiments were repeated one week later. However, no major differences between genotypes were found. Nevertheless, subfield- and genotype-specific changes in ensemble correlation values between the two experimental days hint at alterations in network stability in KO mice. Our study contributes to the understanding of impaired sound processing in Fragile-X-Syndrome and ASD in general.

Investigating Mitochondrial Abnormalities in a Mouse Model of Rett Syndrome

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Rett syndrome is a neurodevelopmental disorder and one of the leading causes of cognitive impairment in females. Affected individuals appear to develop normally until 6-18 months of age, after which they experience developmental stagnation and regression of acquired skills such as speech and fine motor control. In addition to cognitive impairments, patients with Rett syndrome typically develop seizures, social deficits, breathing abnormalities, and metabolic changes, all of which contribute to the complex clinical condition. Moreover, recent evidence suggests that mitochondrial alterations play a crucial role, though the precise mechanisms by which these organelles contribute to the various symptoms remain unclear.

We therefore aimed to investigate mitochondrial function in the brains of MeCP2-deficient mice, with a focus on mitochondrial metabolism, by analysing symptomatic male and pre-symptomatic female MeCP2-deficient mice on postnatal day P50 to identify any abnormalities. Moreover, as a therapeutic approach, we over-expressed a mitochondrial catalase, a key scavenger of reactive oxygen species, to potentially counteract the systemic oxidative stress linked to Rett syndrome.

Specifically, we quantified across various brain regions citrate synthase activity, a marker of mitochondrial density, as well as ATP content, which reflects the energetic homeostasis of the tissue. Additionally, we measured cortical oxygen consumption linked to the mitochondrial electron transfer system using high resolution respirometry for the different mitochondrial complexes and various respiratory states, to evaluate mitochondrial respiratory efficiency in detail. Lastly, we examined the redox status of pyramidal neurons in the CA1 and cortex of acute brain slices in correlation with MeCP2 expression, using correlative ratiometric 2-photon redox imaging. Disruptions in redox balance may culminate in oxidative stress, which can lead to neuronal damage and is hypothesized to play a role in Rett syndrome.

Our results demonstrate significant differences in mitochondrial density and ATP content across different brain regions, most of which are not genotype-related. In MeCP2-deficient mice a decreased mitochondrial respiratory function is evident. Over-expression of mitochondrial catalase extends the life-span of male Mecp2-deficient mice. Ongoing investigations into mitochondrial morphology and neuronal redox status are expected to provide further insights into the cellular consequences of mitochondrial dysfunction. This research contributes to further define the involvement of mitochondrial alterations in Rett syndrome pathology, suggesting a mechanistic contribution and underscoring the need for mitochondria-targeted therapies.

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DNMT1-Mediated Regulation of Inhibitory Interneuron Migration Affects Cortical Architecture and Function

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The fine-tuned establishment of neuronal circuits during the formation of the cerebral cortex is pivotal for its functionality. Developmental abnormalities affecting the composition of cortical circuits, which consist of excitatory neurons and inhibitory interneurons, are linked to a spectrum of neuropsychiatric disorders. Excitatory neurons originate in cortical proliferative zones, while inhibitory interneurons migrate from discrete domains of the basal telencephalon into the cortex. This migration is intricately governed by extrinsic cues, intrinsic genetic programs, and various epigenetic mechanisms. Among these, the most prominent ones are histone modifications, non- coding RNAs and DNA methylation.
Our current study reveals that DNA methyltransferase 1 (DNMT1)-meditaed DNA methylation controls the expression of key genes implicated in mouse cortical interneuron development. The deletion of Dnmt1 in postmitotic somatostatin (SST)-expressing interneurons significantly alters both DNA methylation signatures and the expression of genes implicated in guiding the migration of these interneurons within the developing cortex. We found that this dysregulation causes SST+ interneurons to exit prematurely from the superficial migratory stream.
In addition to the perturbed migration patterns and the corresponding gene expression changes due to Dnmt1 deletion in SST+ interneurons, our study also identified a discernible non-cell autonomous effect on the cortical progenitors. Cell cell interaction analyses extracted from single cell RNA sequencing datasets revealed altered signalling between cortical interneurons and cortical progenitors, due to the changed expression of genes coding for cell surface signalling molecules in Dnmt1 deficient SST+ interneurons. Thus, the altered migration pattern of SST interneurons in combination with changed expression of genes coding for signalling factors elicit changes in cortical progenitor pool and timed neurogenesis that resulted in nuanced alterations in layer thicknesses within the adult cortex. This functionally manifests in altered network activity and behavioural abnormalities. In sum, these findings underscore the crucial role DNMT1 has in governing cortical interneuron migration, and emphasise the instructive role played by cortical interneurons, importing external signals that modulate cortical layer formation. These results highlight how disruptions in epigenetic regulation can lead to complex changes in brain structure and potentially contribute to neuropsychiatric conditions.

Investigating TGFβ signalling in choroidal endothelial cells using immortalized primary cell cultures

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Introduction: In age-dependent macular degeneration choroidal endothelial cells break through the Bruch membrane and retinal pigment epithelium (RPE) - the outer blood-retinal barrier – and build up neovascularisations within the retina. A conditional knockout of TGFβRII in endothelial cells leads to a higher degree of neovascularisations in a laser-induced murine AMD model. In this study two questions should be answered: which TGFβ signalling pathway is active in choroidal endothelial cells, and which effects does TGFβ signalling convey to these cells, that might in the end inhibit neovascularization. In this context proliferation, migration and matrix degeneration were addressed.

Results: Two lines of immortalized choroidal endothelial cells were used throughout this study. qPCR analysis revealed a ten times higher transcription of Alk1 compared to Alk5 in choroidal endothelial cells. In agreement, the Alk1 target Smad1/5 was highly phosphorylated after TGFβ2 stimulation, while the Alk5 Target Smad2/3 only showed week phosphorylation. In a next step the effects of TGFβ2 signalling on choroidal endothelial cells were addressed. TGFβ2 leads to an increase in matrix metalloproteinase 2 (MMP2) transcription and also activity as shown in zymografie gels. In addition, it decreases cell proliferation in one of the two cell lines, and it has no obvious effect on cell migration in a scratch assay.

Conclusion: TGFβ signalling in choroidal endothelial cells in mainly conveyed via the Alk1 Smad1/5 pathway. It increases MMP2 expression and activity in these cells and might have an effect on proliferation. Increase in MMP2 expression seems rather contradictory to the previous result. But MMP regulation as well as TGFβ signalling are highly complex and this is just a very first approach to access this issue.

Outlook: Several differences between the two immortalized cell lines under investigation indicate the necessity to reproduce these findings with a higher number of independent cell lines to get reliable and reproducible results. In addition, a more complex in vivo matrix degeneration assay will be used to further understand the effect of TGFβ on cell-matrix interaction in living cells.

Extraembryonic source of Serotonin involved in Neurodevelopment

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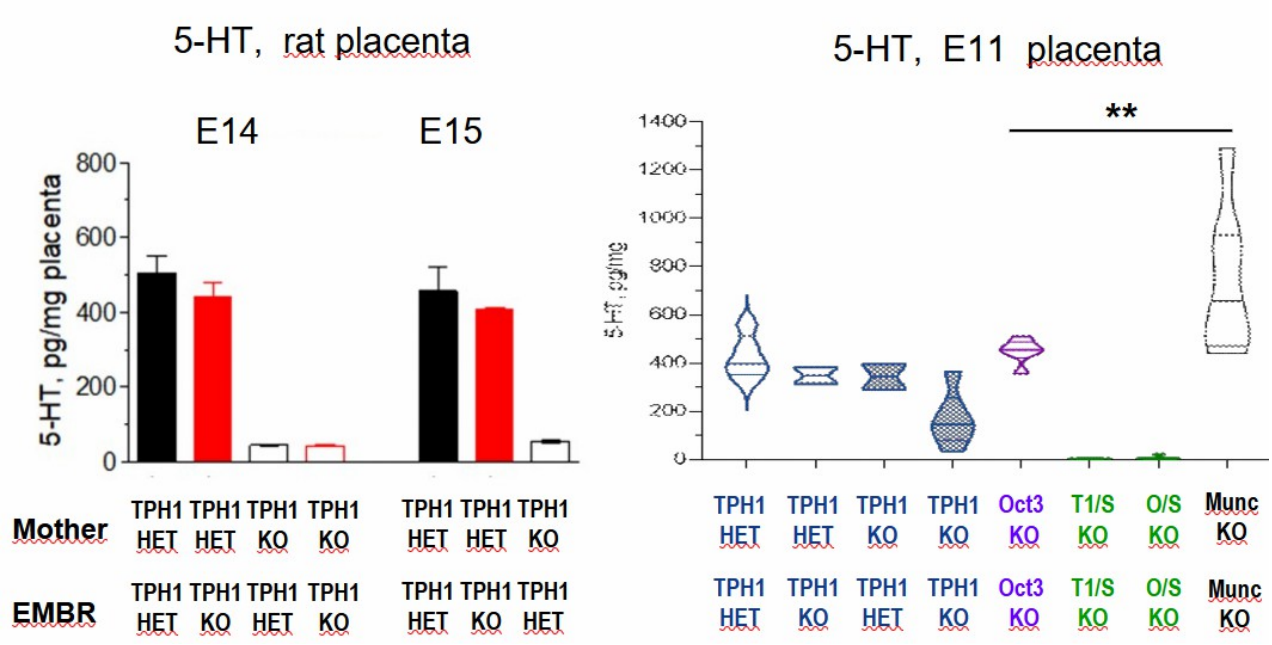
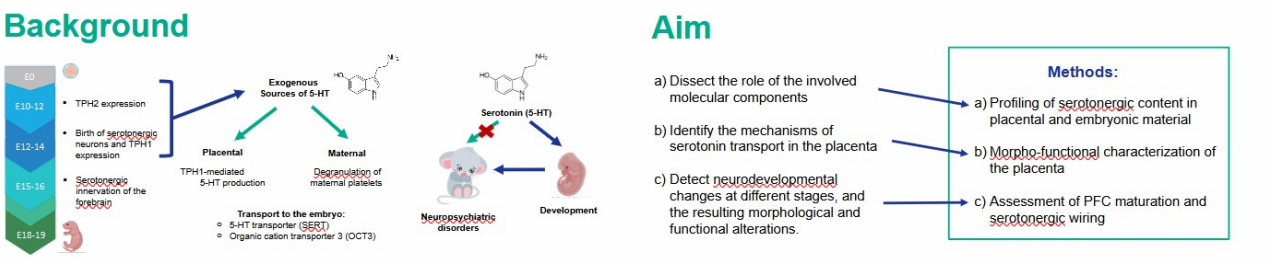
Studies in recent years have suggested that maternal and extraembryonic sources of serotonin, such as placenta, play pivotal roles in embryonic brain development. However, the identity of serotonergic system components and cell types expressing serotonergic genes during development, as well as mechanisms of serotonin transport to the embryo remain controversial (Bonnin et al., Nature. 2011; 472(7343):347-50; Kliman et al., Endocrinology. 2018;159(4):1609-1629).
The aim of this project is to evaluate the contribution of extraembryonic sources of serotonin to PFC development and to dissect the involved cellular and molecular components. Since such an approach is not possible in humans, we use mouse models deficient in genes encoding the serotonin synthesizing enzymes, TPH1 (Walther et al. Science 2003;299:76) and TPH2 (Alenina et al. Proc Natl Acad Sci USA 2009;106:10332-7), and the monoamine transporters, SERT and OCT3, to clarify if these proteins contribute to the supplementation of the fetus with serotonin in the absence of own serotonin production and what is their role in brain development. We investigate the effect of maternal and placental SERT, OCT3, TPH1 and TPH2 depletion on the serotonin levels in placenta and different parts of the embryonic brain before the onset of Tph2 expression at embryonic day (E) 10-11; after the birth of serotonergic neurons (E12-14) and upon serotonergic innervation of the forebrain (E15-16) and on serotonergic-innervation pattern at later stages of embryogenesis.
For this purpose, this project takes advantage of available animal models, including double and triple knockouts for genes involved in serotonin synthesis and transport. We use breeding strategies and embryo transfer technology to create mothers and fetuses with different genotypes. Furthermore, we use tetraploid aggregation (Popova et al. Hum Reprod 2011;26:662-70) to segregate the effects of serotonin production from extraembryonic tissues and the embryo itself. The serotonin content in the embryonic fore- and hind-brain will be measured by HPLC-MS. The PFC maturation in different mutants will be assessed in embryonic development using immunohistochemistry and ECi 3D recostruction. The morpho-functional structure and serotonergic transport mechanisms of the placenta will be assessed using multifluorescent labeling of placental tissues. Additionally, any abnormalities in the placentas of KO animals will also be investigated.
This research is part of “The Serotonin & Beyond project” and has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No. 953327.



5-HT content in placentas of early stage KO rodens model (E11)



Project Structure and Aim

Impaired auditory maturation and its involvement in audiogenic seizure susceptibility in a mouse model of Fragile X Syndrome

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Background: Auditory hypersensitivity is a prominent symptom in Fragile X Syndrome (FXS), the most prevalent monogenic cause of autism and intellectual disability. FXS arises through the loss of the protein encoded by the *FMR1* (*Fragile X Messenger Ribonucleoprotein 1*) gene, FMRP, required for normal neuronal circuit excitability. In the brainstem, FMRP is necessary for normal development of acoustic reactivity, and its loss has been implicated in audiogenic seizures (AGS) in *Fmr1* knock-out (KO) mice, modelling auditory hypersensitivity and seizures in FXS patients.
The present study investigated the correlation between auditory brainstem function of *Fmr1* KO mice and behavioral expression of AGS during early (postnatal day P19, infancy) and late (P33, adolescence) auditory development.

Methods: We tested responsiveness of select auditory pathway elements through Auditory Brainstem Response amplitudes; and neural synchronization to amplitude envelopes of modulated acoustic stimuli through Auditory Steady State Responses. AGS was scored for severity during 5-minute exposure to loud sound. Sound-induced increase in neuronal activity was further assessed by quantifying the expression of immediate early gene cFos in brain slices.

Results: During infancy, higher AGS susceptibility in *Fmr1* KO mice was accompanied by increased responsiveness to acoustic stimuli and stronger neural synchronicity in the lower auditory brainstem. Preliminary cell counting analysis showed more cFos positive neurons in sound exposed *Fmr1* KO mice than in wild-type controls at this early auditory developmental stage. With age, both AGS susceptibility and exaggerated acoustic stimulus-evoked activity in the lower auditory brainstem subsided.

Conclusion: Our findings support evidence that AGS activity relies upon hyperexcitability in the auditory system, particularly in the lower brainstem, possibly due to delayed maturation. A better understanding of FXS-related circuit and behavioral symptoms of auditory processing across development provides the potential to identify therapeutic strategies to achieve auditory function recovery in FXS.

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Therapeutic efficacy is significantly improved with bilateral vs. unilateral intracerebroventricular drug application in a rodent model of absence epilepsy

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Epilepsy is a common neurological disorder affecting approximately 50 million people worldwide. Although many of those affected by the disease can be successfully treated with systemic drug therapy, about 30% of patients show insufficient seizure control with systemic antiseizure drugs (ASDs). One great challenge in systemic pharmacotherapy is the blood-brain-barrier (BBB). The BBB restricts the distribution of drugs into the central nervous system, decreasing their therapeutic efficacy and leading to potential peripheral side effects.

Intracerebroventricular (ICV) drug application is an interesting option to bypass the BBB, as it has been shown to be well tolerated and effective in humans and animals. However, drug distribution following ICV injection is not well understood. Using Strasbourg Genetic Absence Epilepsy Rats (GAERS) we have recently shown that unilateral application of ASDs is highly effective in suppressing seizure activity but may suffer from limited distribution to the contralateral hemisphere even after several hours.

We here explored whether bilateral ICV application would indeed prove more efficient in seizure suppression than unilateral application. GAERS underwent sessions of unilateral or bilateral ICV injections of valproic acid (VPA) and Ringer's solution (control). The doses of VPA administered were 0.6 mg/12 µL and 1 mg/20 µL per animal. To gauge therapeutic efficacy we measured seizure activity, detected by a classifier from amplitude, frequency, and waveform morphology of surface EEG-recordings.

We found that acute bilateral VPA treatment is superior to unilateral treatment in seizure suppression supporting the hypothesis that ICV application may produce mostly ipsilateral pharmacologic effects.

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Novel therapeutic options for *KCNA2*-related epilepsy

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KCNA2 encephalopathy is a rare, early-onset neurological disorder associated with severe epilepsy and developmental delay, often resulting in intellectual disability and movement disorders. In recent years, *de novo* variants have been identified in the *KCNA2* gene, which encodes the voltage-gated α-subunit $K_V1.2$. These variants can cause a gain-of-function (GOF) effect, leading to an increased channel activity and premature opening. However, the exact pathological mechanisms by which *KCNA2*-GOF variants contribute to neural network hyperexcitability in epilepsy patients remain unclear.
To gain deeper insight into the disease mechanism, we generated a *Kcna2* knock-in mouse model carrying the *Kcna2*-GOF variant p.Arg297Gln (R297Q). These mice showed a severe seizure phenotype, which we analyzed through video/EEG-recordings. In PhenoMaster experiments, we additionally observed reduced activity in the mice, which was restored to normal levels, comparable to healthy controls, after treatment with the potassium channel blocker 4-aminopyridine (4-AP).
However, 4-AP does not specifically target *Kcna2*, highlighting the growing need for personalized therapeutic approaches tailored to the genetic cause of *KCNA2* encephalopathy. To address this, we aim to develop antisense oligonucleotides (ASOs) that reduce the expression of the $K_V1.2$ channel subunit and counteract the functional effects of GOF variants. We have designed and optimized ASOs targeting *Kcna2*, which demonstrated selective, dose-dependent downregulation of *Kcna2* in primary cortical neurons isolated from wildtype mice. In addition, ASOs suppressed *Kcna2* expression in human induced pluripotent stem cell (iPSC)-derived neuronal networks. The next step is to evaluate the tolerability, safety and efficacy of ASO treatment *in vivo* using RNA and protein analysis and single-cell recordings. In *Kcna2* knock-in animals, the impact of the ASO treatment on the epileptic phenotype using video/EEG-recordings and the behavior including PhenoMaster experiments will be investigated.

In summary, we developed a *Kcna2* knock-in mouse model that mimics the phenotype observed in affected patients, providing valuable insights into *Kcna2*-GOF encephalopathy. This model will help pave the way for the development of an effective, targeted treatment using *Kcna2*-selective ASOs.
Furthermore, we aim to investigate the underlying mechanism of epileptogenesis in the *Kcna2* knock-in mouse model. To this end, single-cell recordings will be conducted in acute brain slices to comprehensively assess the altered neuronal activity induced by the *Kcna2*-GOF variant.

Pathophysiological mechanisms of epileptogenesis in a mouse model of Dravet syndrome.

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Dravet syndrome (DS) is one of the classic forms of developmental epileptic encephalopathies, which is characterized by severe pharmacoresistant epileptic seizures and other neurological comorbidities, altogether posing a severe clinical burden with impaired patients' quality of life. The disease progresses through three stages: pre-seizure, severe (emergence of epileptic seizures), and compensatory (reduction of seizure frequency while comorbidities persist). DS is mainly caused by *de novo* loss-of-function variants in the voltage-gated sodium ion channel $Na_V1.1$, encoded by the *SCN1A* gene, and is linked to dysfunctional action potential initiation of fast-spiking interneurons (FS INs). Previously it was demonstrated that the recurrent human Dravet missense variant p.A1783V leads to a biophysical loss of $Na_V1.1$ function, confirming reduced action potential firing in these interneurons. Notably, in a Dravet mouse model, this firing deficit emerges only at the severe disease stage, while impaired GABAergic synaptic transmission is detectable in CA1 pyramidal neurons during the pre-seizure phase. In contrast, no significant differences in firing properties of FS INs were detected in the cortex at the severe stage.
To further investigate early mechanisms of epileptogenesis, single-nucleus RNA sequencing of cortical and hippocampal regions was conducted at both the pre-seizure and severe stages. Interestingly, several pathways are found to be dysregulated, including voltage-gated ion channels known to be critically involved in the generation of high-frequency action potential firing of FS INs. Further analysis revealed evidence of both ultrastructural and functional abnormalities, specifically affecting the axons and synapses of FS INs. These findings suggest that early disruptions in synaptic transmission, beyond $Na_V1.1$ dysfunction, may contribute to the onset and progression of DS.

Fragile X mice show context-dependent deficits in vocal behaviour during opposite sex interaction

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Fragile X syndrome (FXS) is the most common genetic cause of intellectual disability and autism spectrum disorder. Among other symptoms, people with FXS suffer from auditory hypersensitivity and communication deficits. The vocal behaviour of Fmr1 KO mice, a common mouse model of FXS, has been extensively studied. These studies have found various, but small differences in vocal behaviour during opposite-sex and same-sex interactions. Many of them were inconsistent between studies.
We were interested whether presenting a more complex type of interaction between opposite-sex mice could better recapitulate the vocalization deficits in FXS, similar to human patients. To do this, we recorded the vocalizations of male-female pairs of Fmr1 KO ((FVB.129P2-Pde6b+Tyrc-ch Fmr1tm1Cgr/J)) or their wildtype littermates (WT), which were separated by a translucent wall. The lower part of the wall contained a series of holes that allowed snout but probably no whisker interactions. Vocalizations of Fmr1 WT and KO mice were also recorded from pairs that were allowed to interact freely. Vocalizations for both settings were automatically detected and analysed using DeepSqueak.
Wall-separated pairs of WT mice, most likely only the males, produced a large number of traditional ultrasonic vocalizations (USVs) similarly to direct interacting pairs. In contrast, Fmr1 KO mice produced only few traditional USVs during separation, whereas the number of USVs was similar to that of WT mice when they were allowed to interact freely. During separation the Fmr1 KO mice produced mostly vocalizations in the lower frequency ranges (10-40kHz) as previously described for wall-separated same-sex pairs. These vocalizations were also shorter and showed narrower bandwidth.
This shows that Fmr1 KO mice display profound deficits in vocal behaviour in specific behavioural contexts such as wall separation, whereas differences during direct interaction are small. It also suggests that the behavioural context plays a greater role in the analysis of communication disorders than previously thought.

A sandwich of glioblastoma cells and a brain tissue slice: an in vitro model to explore interactions of tumour cells with neural tissue

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Glioblastoma (GBM) is a frequent, treatment resistant adult brain tumour which is classified as a grade IV glioma (Louis et al., 2021). To date treatment options for glioblastoma brain tumours are limited and no reliable markers are available to distinguish migratory active from resting glioblastoma cells. Here, we investigated different glioblastoma cell lines like rat C6, human U87 and freshly dissected glioblastoma from human patients and their invasive behaviour on living brain tissue, represented by rat hippocampal slice cultures. We established a model system where organotypic slice cultures are overlayed with fluorescently labelled glioblastoma cells. Our aim is to explore local interactions of glioblastoma cells with neural tissue with focus on interactions that promote glioblastoma cell migratory behaviour. In the long term, we want to use our model system to explore the behaviour of different human GBM cells with various genotypes in rodent brain tissue environment, similar as recently reported (Mann et al., 2023). In our model system we can observe the single cell migration mode and infiltrative tumour growth of GBM, cell behaviours that are characteristic for GBM. For instance, effects of the extracellular matrix protein reelin on C6 glioma cell behaviour were described. We are therefore interested in the response of GBM cells to reelin enriched cell layers, such as the outer molecular layer of the dentate gyrus in hippocampal organotypic slice cultures (Förster et al. 2006). We expect to acquire new insights into local interactions of glioblastoma cells with their local tissue environment.

Transcriptomic insights into epileptogenesis in a *Kcna2* loss-of-function mouse

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Rare developmental and epileptic encephalopathies have been linked to *de novo* variants in *KCNA2* encoding $K_V1.2$, a voltage-gated channel subunit conducting D-type potassium current. Patients with the recurrent variant p.Pro405Leu (P405L) exhibit focal seizures and under heterologous expression the variant displayed dominant negative loss-of-function phenotype. To investigate how this loss-of-function impacts neuronal development, while distinguishing between compensatory and epileptogenic mechanisms, we generated a *Kcna2*$^{+/P405L}$ knock-in mouse model.

We combined metabolic and behavioral phenotyping, intracranial video EEG monitoring, immunohistochemistry, Golgi-stainings, whole-cell patch-clamp recordings of excitatory neurons in acute slices from the primary sensory and entorhinal cortex as well as transcriptomic analysis using single-nucleus RNA sequencing of cortical and hippocampal-formation tissue at two developmental time points to characterize this model.

Heterozygous *Kcna2*$^{+/P405L}$ mice exhibited focal and bilateral tonic-clonic seizures, with premature death occurring within the first few months of life, aggrevated by genetic background. In addition, *Kcna2*$^{+/P405L}$ mice were hyperactive, and males showed signs of being underweight.

In c-Fos stainings, we observed an increase in active neurons in somatosensory cortex and the hippocampus of mutant animals. The number of $K_V1.2$ patches per node of Ranvier was comparable in $K_V1.2$/Caspr stainings from the corpus callosum. Golgi stainings revealed alterations in spine-type composition, predominantly affecting thin spines.

Surprisingly, at developmental stages P12-15, P16-P20 and P30-35, the firing frequency of pyramidal cells remained unchanged compared to to wildtype mice. However, we did observe an increase in afterhyperpolarization amplitude and broadening of the action potential.

Using gene set enrichment analysis on our RNA-Seq data, we could identify that axonal and synaptic pathways were upregulated in *Kcna2*$^{+/P405L}$ mice, revealing developmental, regional and background-specific preferences. These patterns were observed in both excitatory and inhibitory neurons, suggesting that the epilepsy in these animals is not driven by a single cell type, but rather pointing to dysregulation of cortical circuits.

In summary, we developed a new mouse model for a *KCNA2* loss-of-function variant. These mice mice exhibit premature death and mimic the human phenotype with focal seizures. The origin of these seizures may lie in the cortex, as seizures frequently evolved from motor to bilateral tonic-clonic. However, seizure generation does not appear to be caused by increased excitability of pyramidal neurons, as we only observed broader action potentials and increased afterhyperpolarization in single-cell recordings. RNA-Seq revealed changes in axonal and synaptic pathways of cortical neurons, consistend with altered spine composition, while the strucutral organization of the axon remained unchanged.

Poster Topic

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

T11-1A Constitutive activity of serotonin receptor 5-HT4R in the context of neurodegenerative diseases
Lukas Eilers, Alina Brüge, Cara Grün, Frauke Bahr, Saskia Borsdorf, Josephine Labus, Andre Zeug, Evgeni G. Ponimaskin

T11-2A Examining accumulation rate of neuromelanin in the locus coeruleus as a critical factor for neurodegeneration
Csilla Novák, Andrés Jaramillo Flautero, Cristian Ariel González-Cabrera, Ernesto Durán, Miquel Vila, Matthias Prigge

T11-3A Common cellular responses to rotenone and Helicobacter pylori affecting alpha-synuclein
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T11-4A Glutamatergic Neurotoxicity in MCOPS12: A Disease Caused by Mutations in Vitamin A Receptor
Hanna Semaan, Jean-Marc Strub, Christine Schaeffer-Reiss, Wojciech Krezel

T11-5A Suppressing of large-vessel signal to improve voxel-wise analysis of Quantitative Susceptibility Mapping (QSM) MR images
Björn Hendrik Schott, Renat Yakupov, Joram Soch, Anni Richter

T11-6A NI^{2}N - Network for Interdisciplinarity and Innovation in Neurodegeneration Research: A Networking Project to Promote Progressive Multidimensional Research Concepts
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T11-7A Network mechanisms of epileptogenesis in a mouse model of HCN1 developmental and epileptic encephalopathies
Viktoriia Shumkova, Jacquelin Kasemir, Dirk Isbrandt

T11-8A Memory processing in the mammillary body in a mouse model of Alzheimer’s disease
Melika Kashizenuzi, Marla Yasmin Witt, Lara Mariel Chirich Barreira, Gina Marie Krause, Anja M. Oelschlegel, Katarzyna M. Grochowska, Michael R. Kreutz, Anne Albrecht, Anne Petzold, Oliver Barnstedt

T11-9A Assessing gastrointestinal alterations in Parkinson`s disease through stool analysis: Indications of increased inflammation
Martin Weidenfeller, Verena Schmitt, Sophie Korkisch, Alexandra Cosma-Grigorov, Franz Marxreiter, Mario Zeiss, Patrick Süß, Martin Regensburger, Stefan Wirtz, Wei Xiang, Jürgen Winkler

T11-10A Predicting Future Cognitive Decline Using Novel fMRI-Based Biomarkers in Preclinical Alzheimer's Disease
Laura Bertram, Joram Soch, Anni Richter, Jasmin Kizilirmak, Hartmut Schütze, Frederic Brosseron, Luca Kleineidam, Christoph Laske, Oliver Peters, Josef Priller, Anja Schneider, Alfredo Ramirez, Stefan Teipel, Jens Wiltfang, Frank Jessen, Miranka Wirth, Michael Wagner, Emrah Düzel, Björn Hendrik Schott

T11-1B CA3 hippocampal region drives epileptogenesis in an SCN2A (p.A263V) mouse model
Daniil Kirianov, Yana Reva, Birgit Engeland, Michela Barboni, Tony Kelly, Heinz Beck, Stephan Marguet, Dirk Isbrandt

T11-2B The network-wide impact of pallidal deep brain stimulation in generalized dystonia
Denise Franz, Fabiana Santana-Kragelund, Stefanie Perl, Malin Kotyra, Henning Bathel, Marco Heerdegen, Angelika Richter, Jens Starke, Konstantinos Spiliotis, Rüdiger Köhling

T11-3B Spreading depolarizations exhaust neuronal ATP in a model of cerebral ischemia
Karl Schoknecht, Felipe Baeza-Lehnert, Johannes Hirrlinger, Jens P. Dreier, Jens Eilers

T11-4B Synaptic dysfunction and p53 activation cause cerebellar circuit pathology in spinal muscular atrophy
Florian Gerstner, Sandra Wittig, Christian Menedo, Sayan Ruwald, Leonie Sowoidnich, Gerardo Martin Lopez, Chloe Grzyb, Livio Pellizzoni, Charlotte Jane Sumner, Christian Marc Simon

T11-5B Presynaptic APP proteolysis: A Double-Edged Sword of Excitotoxicity and Compensatory Responses
Akshay Bhupendra Kapadia, Ezgi Daskin, Anne-Sophie Hafner

T11-6B Analysis of presynaptic active zone disassembly in different models of neurodegeneration
Maximilian Goy, Marlene Barth, Jan Pielage

T11-7B *In vivo* imaging of mitochondrial transport across neuronal cell types reveals tau-mediated dysfunction in the locus coeruleus
Theresa Niedermeier, Paul Feyen, Lars Paeger, Jochen Herms

T11-8B Dysfunction of proprioceptive sensory synapses is a pathogenic event and therapeutic target in mice and humans with spinal muscular atrophy
Leonie Sowoidnich, Christian Marc Simon, N. Delestree, J. Montes, Florian Gerstner, E. Carranza, Jannik Maximillian Buettner, John G. Pagiazitis, G. Prat-Ortega, S. Ensel, S. Donadio, J. L. Garcia, P. Kratimenos, W. K. Chung, Charlotte Jane Sumner, L. H. Weimer, E. Pirondini, M. Capogrosso, Livio Pellizzoni, D. C. De Vivo, George Z. Mentis

T11-9B Transcutaneous Vagus Nerve Stimulation (tVNS) as a therapeutic approach towards the functional deterioration of the Locus Coeruleus – noradrenergic system
Aleksandra Gritskova, Kaushik More, Cristian Ariel González-Cabrera, Andres Jaramillo Flautero, Matthew Betts, Matthias Prigge

T11-10B Effects of optogenetic inhibition of parvalbuminergic striatal interneurons on extracellular levels of neurotransmitters in DYT1 knock-in mice
Jakob Marx, Susen Becker, Lisa Höfert, Angelika Richter, Anja Schulz

T11-1C Resident macrophage-like cells are activated in brain barrier structures of APP/PS1 male mice
Annarita Patrizi, Valentina Scarpetta, Marco Sassoè-Pognetto, Elena Marcello

T11-2C Hippocampal low frequency stimulation alleviates focal seizures, memory impairments and synaptic pathology in epileptic mice
Piret Kleis, Enya Paschen, Andrea Djie-Maletz, Andreas Vlachos, Carola A. Haas, Ute Häussler

T11-3C Comparative Analysis of Navigation Ability and Short-Term Memory Binding as Potential Early Diagnostic Markers for Alzheimer's Disease: An Evolutionary Perspective
Eva Christine Gellert, Younes Adam Tabi, Katharina Helzel, Dorothee Neufeldt, Thorsten Bartsch

T11-4C How does autophagy cope with specific synaptic needs? Consequences in brain health and disease
Sandra Fausia Soukup

T11-5C The Role of TDP-43 as a Co-Proteinopathy in Alzheimer's Disease: Associations with Tau Pathology and Disease Progression
Amrei Purwien, Nike von Borcke, Yvonne Bouter

T11-6C Cellular mechanisms underlying progressive neurodegeneration: Insights from the Drosophila neuromuscular junction
Marlene Barth, Maximilian Goy, Jan Pielage

T11-7C Expression of endocannabinoid receptor 1 is reduced in the brain of Alzheimer’s disease patients
Nike von Borcke, Amrei Purwien, Henrike Hasecke, Yvonne Bouter

T11-8C Evaluation of CA3 place cell remapping in the APP/PS1 model mouse of Alzheimer’s Disease
Eva Maria Robles Hernandez, Solene Escoffier, Maxi Blei, Jill Dorozalla, Rina Patel, Matthias Haberl, Silvia Viana Da Silva

T11-9C Measuring and manipulating neuron excitability in a TDP-43 based model of Amyotrophic Lateral Sclerosis
Freya Thurn, Jonas Peper, Silvan Hürkey, Lena Lörsch, Axel Methner, Marion Silies

T11-10C Motor neuron pathology drives spinal circuit defects and phenotype of a mouse model for spinal muscular atrophy with respiratory distress type 1
Christian Marc Simon, Katharina Sophie Apel, Margarita Koehler-Sanchez, Florian Gerstner, Aaron Lorenzo Norman, Leonie Sowoidnich, Nathanael Otte, Marie Luise Stephan, Sibylle Jablonka

T11-11C Breaking Social Bonds: How LC Degeneration could impact Social Behavior in Parkinson's Progression
Anbarasi Pugazandhi, Diana Municchi, Cristian Ariel González-Cabrera, Matthias Prigge

T11-1D Effects of long-term thiethylperazine treatment on Alzheimer's pathology in Tg4-42 mice
Lisa Katharina Ruoff, Irina Wanda Helene Bänfer, Thomas Bayer, Jens Wiltfang, Yvonne Bouter

T11-2D Effects of Low Dose Δ9-tetrahydrocannabinol (THC) on Alzheimer's Disease Pathology in 5XFAD Mice
Marzieh Enayati, Jannek Moritz Wagner, Yvonne Bouter

T11-3D Neuronal excitability in entorhinal cortex layer II pyramidal neurons regulates tau propagation in early stage of Alzheimer's disease
Seiko Ikezu, Arun Reddy Ravula, Stephanie Radhakishun, Justice Ellison, Nibedita Basu Ray, Brendan Gibbs, Tsuneya Ikezu

T11-4D Early disease-modifying treatment in a mouse model of Parkinson's disease: Exercise demonstrates its potential
Leonie Susan Baldauf, Malte Feja, Milos Stanojlovic, Julia Hankel, Christian Visscher, Eva Schäffer, Daniela Berg and Franziska Richter

T11-5D Neuroprotective Effects of Lycopene: Modulation of Oxidative Stress, Neuroinflammation, and Tryptophan Pathway Metabolites in In Vitro and In Vivo Models
Shital Panchal, Pallav Gandhi

T11-6D Molecular imaging of alpha-synuclein as a path towards Parkinson's disease diagnosis
Donatus Krah

T11-7D Vascular pathology induced by alpha-synuclein overexpression renders the brain tissue more vulnerable to bacterial endotoxins
Kristina Lau, Anna-Sophia Hartke, Christopher Käufer, Franziska Richter

T11-8D Investigations on proteinopathies along the gut-brain axis in dogs
Diana Voitsekhovych, Kristina Lau, Ivo Wiesweg, Nina Meyerhoff, Georg Byethien, Andreas Beineke, Holger Volk, Franziska Richter

T11-9D Brain region-specific and systemic transcriptomic dysregulation in a human alpha-synuclein overexpressing rat model
Vivien Hoof, Olaf Riess, Nicolas Casadei, Julia Schulze-Hentrich, Thomas Hentrich

T11-10D Electrophysiological and Neurochemical Effects of the Kynurenic Acid Analogue SZR104 in Physiological Conditions and Cerebral Ischaemia: Insights from In vitro Models
Evelin Fehér, Nóra Gödör, Tamás Farkas

Constitutive activity of serotonin receptor 5-HT4R in the context of neurodegenerative diseases

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The serotonergic system plays a pivotal role in modulating several neurophysiological processes, including mood regulation, cognition and memory. However, alterations in serotonin receptor signaling pathways are linked to neurodegenerative diseases characterized by Tauopathies, such as Alzheimer's disease (AD) and frontotemporal dementia (FTD). These tauopathies are defined by abnormal hyperphoshorylation and aggregation of the Tau protein, which disrupts microtubule dynamics. Despite advances in understanding Tau pathology, the role of the 5-HT4R remains widely unexplored, raising important questions about its involvement in neurodegenerative diseases.

In this study, we aim to explore the role of the 5-HT4R constitutive activity in modulating Tau expression and phosphorylation. Our results will highlight how 5-HT4R signaling affects Tau levels and aggregation. In addition, we will demonstrate how the modulation of this pathway by an inverse agonist can alter Tau accumulation. This suggests that 5-HT4R signaling plays a significant role in the development of tauopathies and emphasizes therapeutic potential of targeting the serotonergic system to modulate these neurodegenerative processes.

The study aims to elucidate the impact of 5-HT4R constitutive activity on Tau pathology, thereby establishing a foundation for novel therapeutic strategies targeting neurodegenerative diseases characterized by tauopathies through the modulation of 5-HT4R signaling.

Founding: This study is supported by German Research Foundation (DFG) and Alzheimer Research Initiative e.V. (AFI).

Examining accumulation rate of neuromelanin in the locus coeruleus as a critical factor for neurodegeneration

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Locus coeruleus (LC) neurons, especially those with high content of the neuromelanin (NM) pigment, usually succumb early during the course of Parkinson's disease. To dissect the behavioral and cellular effects of NM accumulation, we developed viral tools that allow the selective expression of human tyrosinase in the LC of DbH-Cre mice.

Our results show that tyrosinase expression induces progressive NM accumulation in the LC, and high levels of NM lead to neurodegeneration. The accumulation rate is dependent on the concentration of the injected virus. Higher titer injections cause almost complete LC degeneration by 10 weeks post-injection. Nevertheless, mice injected with low titers of the same virus have intact, albeit melanized LCs at 7 months post-injection, and levels of neurodegeneration remain moderate even after 14 months. This model resembles the natural age-dependent NM accumulation seen in human catecholaminergic cells.

Despite the marked presence of NM in the LC, effects on behavior remain minor. In our low-titer model, we see slightly increased levels of anxiety-like behaviors 7 months post-injection, which drop below baseline by 11 months. In our high-titer model, we see a strong and consistent increase in locomotor activity, however, our control experiments suggest that this effect is partially due to the inherent toxicity of the high-titer virus.

In conclusion, while a high-titer injection leads to quick NM accumulation and cell death, its effects are confounded by viral toxicity. Low-titer injections induce a slow-paced accumulation without any viral toxicity, which is similar to healthy NM accumulation in humans.

Common cellular responses to rotenone and Helicobacter pylori affecting alpha-synuclein

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Intense inflammation of the infected gastric mucosa is a hallmark of infections with Helicobacter pylori (Hpy), the major pathogen of the human stomach [1]. The gastric mucosa is connected to the brain by the vagus nerve at sites where neurodegeneration is thought to be initiated [2]. Indeed, Parkinson's disease (PD) has been associated with gastrointestinal dysfunction [1, 3], and Hpy is reported to be more common in PD patients than in healthy subjects [4-6]. In addition, several human studies have shown that eradication of Hpy with antibiotics significantly improves the health status of PD patients [4].
One of the main reasons for the so-called "PD pandemic" over the past two decades is believed to be exposure to environmental toxic pesticides such as rotenone. Rotenone easily crosses the blood-brain barrier (BBB) and affects the dopaminergic neurons (DN) in the brain [7, 8]. On the one hand, rotenone induces a huge oxidative stress response and phosphorylation of alpha-syn at serine 129, leading to the formation of alpha-syn aggregates [9]. On the other hand, rotenone appears to decrease autophagy, a cellular mechanism required for the degradation of alpha-syn aggregates. Therefore, through these two mechanisms, rotenone leads to the accumulation of alpha-syn aggregates, which ultimately results in the death of DN [9].
The aim of our work was to delineate the initial triggers and cellular signaling pathways leading to alpha-syn phosphorylation, specifically at serine 129. To this end, we used sophisticated tools for signaling pathway analysis as well as functional kinome and transcriptome analysis. To our surprise, we identified a central kinase for both stimuli that is triggered by environmental cues and is responsible for alpha-syn phosphorylation. Inhibition of this kinase led not only to a loss of alpha-syn phosphorylation but also to transcriptional changes in alpha-syn synthesis. We also identified additional downstream factors whose inhibition reduced alpha-syn modification. Overall, our data corroborate the notion that alpha-syn phosphorylation and the accumulation of alpha-syn aggregates can be caused by entirely different microbial and chemical agents via related pathways driving oxidative stress and a drop-down of autophagy.

Glutamatergic Neurotoxicity in MCOPS12: A Disease Caused by Mutations in Vitamin A Receptor

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Syndromic-microphthalmia 12 (MCOPS12) is a rare neurodevelopmental disorder (57 patients worldwide) characterized by a range of clinical features, including intellectual disabilities, craniofacial abnormalities, and motor impairments. It is caused by de novo point mutations in the Retinoic Acid Receptor Beta (RARβ) gene, a key regulator in Vitamin A signaling, essential for brain development and neuronal function. Importantly, mice carrying null mutation of RARβ (RARβKO-/-, model of MCOPS-12) displays selective dysfunction and loss of a subpopulation of medium spiny neurons expressing dopamine D2 receptor. The mechanism of such vulnerability is not known, however, in vitro studies suggested that it may involve enhanced sensitivity to glutamatergic neurotoxicity. To address this point, we have studied striatal synaptosome in RARβKO-/- mice using proteomics-based approach. We identified significant changes of glutamatergic pre- and post-synaptic proteins related to glutamatergic synapse of cortico-striatal projection neurons and cholinergic interneurons, as well as proteins related to dopaminergic signaling, indicating enhanced activity of these two circuits in the striatum. Molecular mechanisms of such enrichment will be discussed, in addition to their functional relevance for behavioral consequences of RARβ deletion. Such findings open new avenues for therapeutic interventions to mitigate these behavioral changes that include hyperactivity and motor coordination deficits. Therefore, tested pharmacological agents will also be discussed with their behavioral outcomes aiming at the end to improve the quality of life for individuals affected by MCOPS12.

Suppressing of large-vessel signal to improve voxel-wise analysis of Quantitative Susceptibility Mapping (QSM) MR images

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Background: Quantitative Susceptibility Mapping (QSM) is a powerful MRI technique used to measure magnetic susceptibility variations in the brain, most prominently reflecting iron accumulation. Recent studies have demonstrated the utility of QSM in investigating changes in tissue composition related to aging and pathology. However, application of QSM in voxel-based analyses and multimodal imaging is thus far limited by the high magnetic susceptibility of venous blood, resulting in a strong QSM signal from large blood vessels. Here we explored the potential utility of Macro-Vessel-Suppressed Susceptibility Mapping (MVSSM) to suppress vascular QSM signal and to enable voxel-based analyses.

Methods: Single-echo phase and magnitude susceptibility-weighted images were acquired on two Siemens 3T MR tomographs from two cohorts of 50 young and 70 older adults each (TR = 28 ms, TE = 20 ms, 32 channels, separate images were stored for each channel). Image reconstruction was performed using multi-scale dipole inversion (MSDI), using two different regularization parameters (λ_1 = 749, λ_2 = 39). Calculation of MVSSM images was performed by masking the QSM images reconstructed with the group-optimized λ_1 with a mask obtained from the inverted, smoothed, and binarized high-pass images reconstructed with λ_2, the latter reflecting predominantly susceptibility signals from large veins. The resulting MVSSM images were co-registered with high-resolution T1-weighted MPRAGE images, normalized into the MNI reference space, and smoothed, to allow for voxel-based statistics.

Results and Conclusion: The MVSSM approach allowed for successful generation of high-quality QSM images, with a significantly reduced impact of large blood vessels. Preliminary evaluations demonstrate suitability of MVSSM for SPM-based voxel-wise analyses. Age-related voxel-wise group differences in magnetic susceptibility and preliminary associations with the novelty-related fMRI signal will be shown.

NI^{2}N - Network for Interdisciplinarity and Innovation in Neurodegeneration Research: A Networking Project to Promote Progressive Multidimensional Research Concepts

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Today's aging societies face a myriad of health challenges; neurodegenerative diseases such as Alzheimer's and Parkinson's disease range among the top of the list, contributing significantly to cognitive decline, disability, and mortality. Devastating to the affected individual and their family and caregivers, the prevalence of cases also poses a serious socioeconomic burden. While diagnostic methods driven by more and more advanced techniques have vastly improved over the last decade, the main challenge remains the lack of viable treatment options that can prevent, or at least significantly decrease pathological decline or improve symptoms. Here, the pitfall lies in the multifactorial causality and complexity of most neurodegenerative disorders and the fact that their exact individual pathogenesis is still far from understood, rendering prevention as well as therapy difficult. Advancements in research have also sparked a debate about disease definition, i.e. whether complex neurodegenerative diseases such as Alzheimer's disease (AD) or Parkinson's disease should rather be considered a syndrome than a single disease entity. Many AD cases for example carry a genetic component, while many more display aging and lifestyle as the main risk factor, showcasing the complex disease nature and limited treatment options. These facts pose an urgent need for more interdisciplinary communication, collaboration, and innovative research concepts.

NI^{2}N, the *Network for Interdisciplinarity and Innovation in Neurodegeneration Research* (ni2n.org), is a Think Tank Project funded by the Dr. Eberhardt Strebel-Stiftung of the Stifterverband für die Deutsche Wissenschaft that aims to help reshape the necessary research landscape around AD and related neuropathologies by "expanding the box". Through offering a networking platform beyond the mainstream, NI^{2}N strives to facilitate innovative global collaborations, enabling established as well as young scientists to share novel insights, challenge and expand existing hypotheses, and propose progressive concepts to target neurodegeneration. Moreover, this project aims to pinpoint existing knowledge gaps to stimulate new research directions. The network's further objective is to promote early-career researchers through seed funding for innovative research ideas and by offering collaborative opportunities. Through this initiative, NI^{2}N encourages a progressive multidimensional approach to understanding, diagnosing, and treating neurodegenerative disorders.

Network mechanisms of epileptogenesis in a mouse model of HCN1 developmental and epileptic encephalopathies

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Developmental and Epileptic Encephalopathies (DEE) are disorders in which cognitive functions are affected by abnormal brain development, seizures, and interictal epileptiform activity. A growing number of DEEs have been linked to de novo mutations in voltage- and ligand-gated channels, which frequently do not respond to classic antiseizure medications. As shown previously, knock-in mouse models replicating de novo sequence variations in the human HCN1 voltage-gated channel gene, p.G391D and p.M153I (*Hcn1*$^{G380D/+}$ and *Hcn1*$^{M142I/+}$ in mouse), associated with severe drug-resistant neonatal- and childhood-onset epilepsy, respectively. Heterozygous mice from both lines displayed spontaneous generalized tonic-clonic seizures. Animals replicating the p.G391D variant had an overall more severe phenotype with locomotor hyperactivity, deficits in motor coordination and cognitive impairment. In agreement with clinical data from patients with pathogenic HCN1 sequence variants, administration of the antiepileptic sodium channel antagonists lamotrigine and phenytoin resulted in paradoxical induction of seizures, suggesting impaired function of inhibitory neurons.

To investigate the network mechanisms underlying epileptogenesis in a mouse model of HCN1-related DEEs, we performed multichannel depth recordings in somatosensory cortex and hippocampus of mice in *Hcn1*$^{p.G380D}$ mice (GD) *in vivo*. Neonatal mice (10 weeks) were recorded in the Mobile HomeCage (MHC) using high-density Neuropixels electrodes. Recording in MHC was combined with parallel video acquisition to monitor pupil diameter/behavior.

Recordings in neonatal mice (P6–P14) showed electrographic seizures in the hippocampus and/or cortex, indicating early postnatal onset epileptogenesis. Preliminary results in adult GD mice show changes in hippocampal local field potentials, especially a reduction in ripple frequency and power during awake immobility. These results are compatible with an interneuronopathy in *Hcn1*$^{p.G380D}$ animals.

To investigate the role of interneurons, we sought to remove the GD allele from GABAergic interneurons. To this end, we generated a new mouse line in which the GD variant is flanked by loxP sites. This mouse line, which exhibits a similar epilepsy phenotype to GD mice, was then crossbred with Gad2-cre animals, resulting in a line, in which the GD variant is expressed in all neurons except Gad+/GABAergic interneurons. Preliminary electrocorticogram (ECoG) recordings from adult mice of this line indicate that they do not exhibit spontaneous seizures or sensitivity to sodium channel blockers but still show changes in ECoG properties and locomotor hyperactivity also present in GD mice.

These findings support the hypothesis of impaired interneuron function underlying the epilepsy phenotype in mice with the p.G380D variant in HCN1.

Memory processing in the mammillary body in a mouse model of Alzheimer's disease

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Abstract:
The ability to form and recall episodic memories is a vital determinant for leading a meaningful and self-determined life. Memory performance typically declines with age, and this decline is exacerbated by dementias such as Alzheimer's disease (AD). The growing ageing population is expected to lead to a tripling of dementia cases by 2050, without new interventions or treatments that prevent or at least slow disease progression. Therefore, core brain areas involved in memory processing, particularly the hippocampal formation, have been extensively studied in the last decades. The main output structure of the hippocampal formation is the subiculum (SUB). In AD animal models, progressive neurodegeneration typically starts in this region. One of the main projection targets of the SUB is the mammillary body (MB), a small hypothalamic nucleus located at the base of the brain, receiving almost exclusive inputs from the subiculum. Positioned ventrally to the supramammillary nucleus (SUM), it consists mainly of the lateral MB (LMB) and medial MB (MMB), the latter of which also has a medial part and a lateral part. Lesion studies in human patients and rodents over the past decades have revealed a critical role of the MB in episodic and spatial memory performance, but the cellular mechanisms for such a role, which subnuclei may be involved specifically, and how this may be affected by AD remain largely unknown.

Here, we studied memory processing in the MB and SUM using cFos as a neuronal marker of elevated neuronal activity and plasticity. Adult mice underwent contextual fear conditioning in which they received electric shocks in a context to which they were later re-exposed while freezing levels were measured. Shortly after fear context retrieval, mouse brains were extracted and stained for the activity marker cFos. We identified strong cFos labelling in the SUM, very low levels in the LMB, and found many cFos-positive neurons in the MMB. Importantly, we found a medial MMB-specific significant positive correlation of cFos-positive neurons and freezing levels, suggesting that the levels of neuronal activity in the medial MMB relate to the animal's memory retrieval performance. To understand how this MB-specific memory processing is affected by AD, we have used 5xFAD transgenic mice expressing five AD-linked mutations that lead to rapid amyloidosis. Histological analysis of 5xFAD brains reveals mild structural pathological changes in the MB at 6 months and more severe changes at the age of 12 months.

Overall, these findings suggest an active role of the medial MMB in memory retrieval processes that may be disrupted by progressive amyloidosis in this region. Future studies will identify functional changes in MB neurons during amyloidosis.

Assessing gastrointestinal alterations in Parkinson`s disease through stool analysis: Indications of increased inflammation

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Objectives:
Parkinson`s disease (PD) is the most common neurodegenerative movement disorder, hallmarked by motor symptoms resulting from the dysfunction and loss of dopaminergic projections in the midbrain. Neuropathologically, PD is characterized by alpha-synuclein (aSyn) aggregation and neuroinflammation. Emerging evidence increasingly links the presence of aSyn aggregates in the gastrointestinal (GI) tract to early gastrointestinal symptoms frequently reported in PD patients. This association, along with systemic inflammation, supports the hypothesis of a gut-to-brain spread of PD-related pathology. Analyzing stool samples from patients offers a non-invasive approach to identify PD-associated alterations in the GI tract. Hence, this study aimed to identify cellular and molecular alterations in PD-derived stool samples, focusing on: i) the abundance of eukaryotic and prokaryotic cells, ii) calprotectin levels as a marker of GI inflammation, iii) acetylation of proteins, which may trigger the production of anti-acetylated protein autoantibodies (AAPA), a process known to play an important role in autoimmune responses and systemic inflammation; and iv) microbiota composition. Furthermore, we explored whether gut contents influence the brain by examining astrocyte responses to stool extracts from PD patients and healthy controls.

Methods:
The cellular composition of stool samples was assessed by quantifying prokaryotic and eukaryotic cells using RT-PCR for 16S and 18S rRNA genes. Calprotectin and protein acetylation (PA) levels were determined via immunoblotting, while microbiome analysis was conducted via 16S rRNA sequencing. To evaluate astrocytic responses, primary mouse astrocytes were exposed to stool extracts, and the astrocytic response was measured by RT-PCR for key genes, including glutamate transporter 1 (GLT1) and glial fibrillary acidic protein (GFAP).

Results:
Compared to stool samples from the controls, PD stool samples exhibited a marked increase in prokaryotic cells but a significant decrease in eukaryotic cells. Elevated calprotectin levels were observed in PD patients, indicating heightened gastrointestinal (GI) inflammation. Interestingly, protein acetylation (PA) levels were significantly reduced in the PD stool samples, suggesting that acetylated proteins may be preferentially absorbed by the intestine, likely due to compromised intestinal barrier integrity. Additionally, AAPA levels were elevated in the serum of 58% of the PD patients, indicating that AAPA production may be associated with specific subtypes of PD, potentially reflecting distinct immune or inflammatory processes. Alterations in the gut microbiota were noted with a consistent decrease in Faecalibacterium species in PD stool samples. Furthermore, astrocytes exposed to PD stool extracts

showed significantly reduced GLT1 and increased GFAP expression compared to controls, suggesting altered astrocytic responses.

Conclusions:
We identified significant alterations in cell composition, calprotectin, PA levels, and microbiota in stool samples derived from PD patients. Distinct astrocytic responses to PD- and control-derived stool extracts further confirm the altered stool composition in PD. Although stool composition may not fully reflect all GI changes, our data highlight the potential of stool analysis in identifying diagnostic markers suitable for stratifying different stages and subtypes of PD.

Predicting Future Cognitive Decline Using Novel fMRI-Based Biomarkers in Preclinical Alzheimer's Disease

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Despite significant advances in biomarker-based detection of preclinical Alzheimer's disease (AD), the prediction of future cognitive decline in at-risk individuals still remains challenging. We have recently demonstrated the diagnostic potential of fMRI-based single-value scores— Functional Activity Deviation during Encoding (FADE) and Similarity of Activations during Memory Encoding (SAME)—which reflect deviations from or similarities to prototypical activation patterns observed in young, healthy adults (Soch et al., Brain, 2024). These scores were associated with disease stage (healthy controls [HC], subjective cognitive decline [SCD], mild cognitive impairment [MCI], early AD dementia) and biochemical risk factors (CSF Aβ ratio, ApoE genotype).

In the present study, we have explored the prognostic utility of FADE and SAME scores by examining their relationship with neuropsychological test performance over three years. Using data from the DZNE's multi-center DELCODE study, encompassing individuals across the AD spectrum, we assessed the association of these scores at baseline with the change in the PACC5, a composite measure of cognitive performance, from baseline to follow-up visits 1, 2, and 3. Preliminary canonical correlation analysis identified SAME_memory and FADE_novelty as the strongest predictors of cognitive performance during follow-up. Regression analyses using contrast coding for the timepoints baseline to follow ups revealed that SAME_memory scores were linked to cognitive decline three years after baseline, particularly in individuals with MCI and indicated that this relationship varied as a function of plasma Aβ ratio. FADE_novelty scores showed a similar, though weaker, pattern.

Our findings suggest that fMRI-based single-value scores could serve as valuable prognostic markers for predicting disease progression in individuals at risk for AD. The association between baseline scores and cognitive decline up to three years later indicates that an individual's similarity to memory-related brain activity in healthy young adults may reflect neurocognitive reserve in older adults.

CA3 hippocampal region drives epileptogenesis in an SCN2A (p.A263V) mouse model

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The voltage-gated sodium channel Nav1.2, encoded by SCN2A, plays a crucial role in the initiation, propagation, and backpropagation of action potentials during early brain development. Nav1.2 predominates in the axon initial segment (AIS) of human and mouse pyramidal cells during the first postnatal years and weeks, respectively, before being replaced by Nav1.6 as neurons mature. Pathogenic variants in the SCN2A gene have been strongly associated with a broad spectrum of neurodevelopmental disorders, ranging from autism spectrum disorder to epilepsy and schizophrenia. This study focuses on the p.A263V gain-of-function variant, which is linked to neonatal/infantile-onset seizures in patients, with varying outcomes. Intriguingly, some patients carrying the p.A263V variant experience a benign epilepsy phenotype, with seizures disappearing during brain development. It has been hypothesized that the developmental switch from Nav1.2 to Nav1.6 expression may contribute to this seizure remission.

To investigate the disease mechanisms associated with SCN2A p.A263V, we generated a mouse model carrying this pathogenic variant in Scn2a. Hippocampal seizures were observed in heterozygous and homozygous pups from postnatal day 2-3 (P2-P3) until P20, corresponding to the known period of Nav1.2 to Nav1.6 expression switch in the AIS of mouse hippocampal pyramidal cells. Quantification of local field potential depth profiles and dynamics of early Sharp Waves (eSPWs) in the hippocampal CA1 region revealed alterations in the rate and amplitude of one type of eSPWs (“inverted” eSPWs -- with a sink in stratum oriens), likely originating from the CA3 region. Notably, seizure epochs were often followed by an increase in the rate of these inverted eSPWs.

We next conducted in vivo multichannel extracellular recordings from CA3 and CA1 hippocampal regions of head-fixed mice at various ages using Neuropixels 2.0. Single-unit firing activity and connectivity were quantified and compared with results from ex vivo patch-clamp analyses of intrinsic neuronal properties in acute brain slices. Our findings suggest a dominant role of abnormal CA3 region activity in driving early epileptogenesis in the Scn2a p.A263V mouse model.

The network-wide impact of pallidal deep brain stimulation in generalized dystonia

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Introduction
The therapy of deep brain stimulation (DBS) of the globus pallidus internus (GPi) is an effective treatment of generalized or cervical dystonia but with previously unknown mechanisms. It is thought that DBS leads to an increase in GPi activity, thereby counteracting the hyperkinesia of dystonia. Our working hypothesis, therefore, involves increased inhibition of motor thalamic neurons as a result of increased GPi activity after DBS of that target structure. Instead of altered inhibitory input to motor thalamic neurons, we found an increase in the excitatory synaptic spike frequency. That brought us to further investigations within the motor circuit and the excitatory projections from the motor cortex and cerebellum to the motor thalamus.

Methods
We investigated synaptic transmission and network activities of motor thalamic neurons, motor cortical neurons of layer VI, and cerebellar cortex with patch-clamp and high-density microelectrode array in vitro measurements in an animal model of paroxysmal generalized dystonia treated with pallidal DBS. We implanted bipolar stimulation electrodes bilaterally into the dtsz mutant hamster's globus pallidus internus and the STELLA stimulation system in the hamster's flank for continuous long-term DBS (130 Hz, 50 µA) over 11 days.

Results
Our results indicated unexpected effects of pallidal DBS on the motor thalamic neurons by upregulating the excitatory tone rather than direct inhibitory projections. That may be an effect of altered intracortical inhibition. The cortical motor neurons of the DBS group indicated decreased spike amplitudes, which may relate to inhibitory projections from interneurons. We also found that GPi DBS significantly changed the interspike intervals of the synaptic spikes. We confirmed an enhanced neuronal network activity within the cerebellar layers after GPi DBS that was in the range of healthy control. Moreover, with the help of a mathematical model, dtsz hamsters were characterized by a reduced synaptic activity that was increased to the level of healthy controls after DBS.

Conclusions
Our measurements on the synaptic and network activities of motor thalamic neurons, motor cortical neurons, and the cerebellar cortex pointed to a modulatory effect of DBS on the global network rather than a local impact on the stimulation side.

Spreading depolarizations exhaust neuronal ATP in a model of cerebral ischemia

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Spreading depolarizations (SDs) have been identified in various brain pathologies. SDs increase the cerebral energy demand and, concomitantly, oxygen consumption, which indicates enhanced synthesis of adenosine triphosphate (ATP) by oxidative phosphorylation. Therefore, SDs are considered particularly detrimental during reduced supply of oxygen and glucose. However, measurements of intracellular neuronal ATP ([ATP]i), ultimately reporting the balance of ATP synthesis and consumption during SDs, have not yet been conducted.
In this study, we investigated neuronal ATP homeostasis during SDs using 2-photon imaging in acute brain slices from adult mice, expressing the ATP sensor ATeam1.03YEMK in neurons. SDs were induced by application of potassium chloride or by oxygen and glucose deprivation (OGD) and detected by recording the local field potential, extracellular potassium, as well as the intrinsic optical signal.
We found that, in the presence of oxygen and glucose, SDs were accompanied by a substantial but transient drop in neuronal [ATP]i. OGD, which prior to SD was accompanied by a slight reduction in [ATP]i only, led to an even larger, terminal drop in [ATP]i during SDs. Subsequently, we investigated whether neurons could still regenerate ATP if oxygen and glucose were promptly resupplied following SD detection. The data show that ATP depletion was essentially reversible in most cells.
Our findings indicate that SDs are accompanied by a substantial increase in ATP consumption beyond production. This, under conditions that mimic reduced blood supply, leads to a breakdown of [ATP]i. Therefore, our findings support therapeutic strategies targeting SDs after cerebral ischemia.

Synaptic dysfunction and p53 activation cause cerebellar circuit pathology in spinal muscular atrophy

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Spinal muscular atrophy (SMA) is a motor neuron disease, primarily characterized by the degeneration of spinal motor circuits resulting in impaired voluntary movement and muscle atrophy. The contribution of motor circuits in the brain to the SMA pathology is largely unknown. The motor circuits in the cerebellum are critical for motor learning and voluntary movements by processing proprioceptive input and modulating motor output, both of which are both affected in SMA. A few studies reported alteration of Purkinje cells (PC) - the sole functional output of the cerebellar cortex - in SMA patients, implicating a cerebellar contribution to the disease pathology. In this study, we investigated the extent and mechanisms of cerebellar pathology in a SMA mouse model as well as human post mortem tissue. We first performed immunohistochemistry and confocal analysis on the sagittal vermis section of end-stage mutants and control cerebelli. Our results showed that the severe SMA SMNΔ7 mouse model exhibits selective affected lobules with vast PC death. Subsequent analysis indicates smaller PC dendritic trees and a reduction of excitatory synaptic inputs onto PC in SMNΔ7 mutant mice. In agreement, whole-cell patch-clamp recordings revealed reduced functional output from PCs, as well as altered synaptic transmission from granule cells innervating PCs. To gain insight into the pathomechanism of PC death, we investigated the p53 pathway which has been shown to induce motor neuron death in SMA. Importantly, vulnerable PC exhibited a robust p53 upregulation prior to their death. Furthermore, p53-knockdown experiments restored PC number to almost wild-type level, suggesting p53-dependent neurodegeneration in the cerebellum. Our results demonstrate a cerebellar circuit pathology comprising of selective PC death and reduced excitatory synaptic input in a SMA mouse model and in human patients. This demonstrates a similar pathology in the cerebellum as currently reported in the spinal cord and suggests the cerebellum as a contributor to SMA pathology.

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Presynaptic APP proteolysis: A Double-Edged Sword of Excitotoxicity and Compensatory Responses

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Aim
This study investigates the molecular mechanisms behind Alzheimer's disease (AD) onset, focusing on presynaptic processing of amyloid precursor protein (APP) and its implications for synaptic pathology. While synaptic dysfunctions are known to drive the onset of AD, it remains unclear what triggers the observed hyperexcitability. Accumulating evidence points to the role of soluble forms of amyloid-β (Aβ) but also to a contribution of APP-Carboxyl Terminal Fragments (APP-CTFs) and particularly APP-CTFβ (C99). We aim at dissecting to respective contributions of those two products of APP proteolysis in initiating synaptic dysfunctions.

Methods
Using transgenic vGLUT1-GFP mice, we isolated and sorted synapses for biochemical analysis. Wild-type primary cortical neurons were treated with γ-secretase inhibitors to increase APP-CTF accumulation or enhance amyloidogenic APP processing. We assessed mitochondrial function, local protein synthesis, and monitored neuronal calcium dynamics and synaptic vesicle activity.

Results
Our findings show that full-length APP, APP-CTFs, and their processing enzymes localize primarily to excitatory presynaptic compartments. Inhibition of γ-secretase caused significant accumulation of APP-CTFs, leading to disrupted calcium dynamics and impaired synaptic vesicle release. This accumulation also resulted in mitochondrial dysfunction and dysregulated local protein synthesis, independent of Aβ. While increasing Aβ concentrations could initially rescue cell-autonomous defects in synaptic excitatory/inhibitory function (hyperexcitability), this compensatory response ultimately fails, further contributing to synaptic loss and neurodegeneration.

Conclusions
In conclusion, presynaptic APP-CTF accumulation, particularly C99, is critical in early synaptic dysfunction associated with AD, disrupting calcium dynamics, mitochondrial function, and local protein synthesis. While Aβ peptides may provide temporary compensatory responses, prolonged exposure leads to synaptic failure. Our study highlights the need to target APP-CTF accumulation and modulate Aβ interactions as potential strategies to delay or prevent AD onset. Understanding these mechanisms is vital for developing effective therapeutic approaches to address early synaptic impairments in AD.

Analysis of presynaptic active zone disassembly in different models of neurodegeneration

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Neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS), which is characterized by the selective degeneration of motoneurons pose a major challenge to modern medicine due to their lack of effective treatments. While the precise molecular mechanisms underlying ALS remain unclear, the dysregulation of cytoskeletal dynamics has been identified as a key factor. Altered expression and function of the microtubule-destabilizing protein stathmin, which plays a crucial role in cytoskeletal organization and neuronal integrity, have been associated with neurodegeneration in ALS pathology. This contrasts with Wallerian degeneration, a process of injury-induced axonal degradation, that is independent of disease pathology but still offers a valuable model for studying neurodegenerative mechanisms. Understanding the distinct pathways underlying different neurodegeneration models, including disease-driven and injury-induced degeneration, is critical for identifying novel therapeutic targets.

Here, we use the larval neuromuscular junction (NMJ) of *Drosophila melanogaster* to characterize differences between neurodegeneration associated with ALS and Wallerian degeneration. One major difference lies in the timeframe of synapse loss. In the fruit fly, motoneuron degeneration in an ALS-like pathology happens relatively slow in a retraction-like manner. However, following axonal injury, Wallerian Degeneration occurs in a rapid timeframe and leads to the fragmentation of the entire NMJ.

Here we use genetic models of ALS-like and Wallerian Degeneration to examine the structural consequences of both degeneration types at the presynaptic NMJ on a nanoscale level using confocal and super-resolution microscopy. Using molecular markers for structural components at the presynaptic terminal, we aim to identify key differences in the disassembly of synaptic active zones. By distinguishing between these neurodegenerative pathways, we aim to identify and understand the specific molecular targets that may enable the development of therapeutic approaches alleviating neurodegenerative disease progression.

In vivo imaging of mitochondrial transport across neuronal cell types reveals tau-mediated dysfunction in the locus coeruleus

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The Locus Coeruleus (LC) is a brainstem nucleus of special interest in the context of neurodegenerative diseases such as Alzheimer's disease (AD), where it is the first region to show hyperphosphorylated 'pre-tangle' tau. Due to the bioenergetic needs of the tonically active LC neurons with their extensive unmyelinated axonal projections throughout the entire forebrain, tau-dependent impairment of mitochondria has been suggested to underlie early LC axon loss.
The study of mitochondria is often restricted to immunohisto- or cytochemical analysis, limiting conclusions about dynamics and progression over time. Reports on the fraction of motile mitochondria show discrepancies between *in vitro* and *in vivo* models. To expand on available *in vivo* studies we utilized mice expressing GFP in the outer mitochondrial membrane in a Cre-dependent manner. We applied *in vivo* acousto-optic two-photon imaging to visualize mitochondrial transport cell type specifically.
The presence of a significant number of moving mitochondria in adult mammals has been a matter of controversy. We reveal for the first time *in vivo* in LC, Parvalbumin (PV) and CamKIIα neurons alike a high fraction (15-20%) of motile mitochondria. Intriguingly, long unmyelinated LC axons showed drastically increased velocities as compared to PV and CamKIIα neurons.
Custom build AAVs were utilized to express human tau (P301S) Cre-dependently in all three cell types. Dbh-Cre animals revealed a significant reduction in mitochondrial velocity in LC axons *in vivo*, which progressively increased over the course of three months. Critically, decreased mitochondrial velocity correlated with a progressive loss of axonal projections in the cortex. The effect on mitochondrial motility was exclusive to LC mitochondria across the studied neuronal types, highlighting the cell type specific vulnerability of the LC during tauopathy.
To further explore this vulnerability under disease conditions, Dbh-Cre animals were transfected with a custom Cre dependent AAV to model α-synucleinopathy (A53T). Mitochondrial velocity was unaltered, showcasing the differences between neurodegenerative diseases. Extending the imaging paradigm to control mice aged over 12 months revealed significantly slower mitochondria in LC axons, which, however, did not reach the same extent as during tauopathy.
Collectively, we show not only an abundance of mitochondrial axonal transport past neonatal stages across cell types, but also the importance of further investigations of mitochondria in tauopathies and other diseases *in vivo*. For the first time we correlate mitochondrial dysfunction with the "dying-back" hypothesis in the LC *in vivo*.

Dysfunction of proprioceptive sensory synapses is a pathogenic event and therapeutic target in mice and humans with spinal muscular atrophy

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Introduction. Spinal muscular atrophy is a childhood-onset monogenetic disorder caused by homozygous inactivation of the survival motor neuron 1 gene (SMN1) gene. Motor impairments in patients and mouse models of spinal muscular atrophy (SMA) are largely caused by dysfunction of sensory-motor circuits. Hallmarks of motor circuit pathology are degeneration of motor neurons (MNs), as well as dysfunction and loss of sensory and neuromuscular junction synapses. Sensory (Ia proprioceptive) synaptic loss occurs pre-symptomatically prior to motor neuron death, making it the earliest and most conserved disease feature across different mouse models of SMA. Importantly, restoration of proprioceptive synaptic function improves motor function in both mouse and fly models of SMA, underlying the significance of sensory synapses on motor neuron dysfunction in SMA. One clinically well-established measure for the Ia proprioceptive-motor circuit is the Hoffman reflex (H-reflex). Whether proprioceptive dysfunction occurs in SMA patients, similar to SMA animal models, has not been established. It is also unknown whether defects in proprioceptive transmission extend from proximal to distal motor circuits in SMA mice during disease progression.

Methods. Proprioceptive neurotransmission onto individual 5th lumbar segment (L5) MNs in late-stage severe SMA SMNΔ7 mouse model were investigated by whole-cell patch-clamp recordings following stimulation of proprioceptive axons. In addition, we used an ex vivo spinal cord-hind limb preparation and assessed the sensory-motor circuit innervating the tibialis anterior muscle to quantify the muscle-response (M-response) and proprioceptive-mediated-response (H-reflex) in control, untreated and SMN-C3 compound (similar to SMN restoring FDA-approved drug Risdiplam) treated SMNΔ7 mice. We also measured the M-response and H-reflex in control and ambulatory SMA patients, either untreated or treated with FDA-approved SMN restoring drug Nusinersen. Finally, immunohistochemistry and confocal imaging were used to quantify proprioceptive synapses in both murine and human post-mortem spinal

cord tissue.
Results. We found that impairment of proprioceptive neurotransmission occurred at late stages of disease in motor circuits innervating distal limbs, in the absence of significant loss of proprioceptive synapses and MN numbers in SMNΔ7 mice, suggesting that impaired function of proprioceptive synapses might be a sensitive measure for disease progression. Recordings from the ex vivo spinal cord-hind limb assay revealed amplitude reductions of 50% in the M-response and 75% in the H-reflex of SMA mice. Daily intraperitoneal injections of SMN-C3, improved both M-response and H-reflex in SMA mice. To investigate whether proprioceptive degeneration also occurs in humans, post-mortem tissue from 4 controls and 6 SMA patients revealed an almost complete lack of proprioceptive synapses from MNs in SMA patients. To assess proprioceptive function, muscle recordings of 6 control, 3 naive SMA patients and 4 Nusinersen-treated SMA patients revealed that M-response was reduced by approximately 70%, the H-reflex was nearly absent, consistent with severe loss of proprioceptive synapses in SMA patients. Strikingly, SMN-restoring treatment with Nusinersen did not improve the M-response, but had a strong beneficial effect on the H-reflex in treated patients.
Conclusion. Our findings suggest that proprioceptive dysfunction is a sensitive measure of motor circuit and behavioral impairments in both SMA mice and ambulatory patients. The ease of access of the Hoffman reflex may therefore serve as a reliable biomarker for clinical assessment of disease progression and efficacy of SMN-restoring therapies in SMA patients.
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Transcutaneous Vagus Nerve Stimulation (tVNS) as a therapeutic approach towards the functional deterioration of the Locus Coeruleus – noradrenergic system

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The Locus coeruleus (LC), the main noradrenaline-producing nucleus in the brain, is affected in a wide range of neurological conditions such as Parkinson's disease and Alzheimer's disease. Notably, the locus coeruleus-noradrenergic (LC-NA) system can be a therapeutic target through Vagus Nerve Stimulation. Transcutaneous auricular Vagus Nerve Stimulation (taVNS), an alternative technique that stimulates the auricular branch of the vagus nerve, is being studied as it’s less invasive, yet the nuanced neurocircuitry linking this branch of the vagus and LC remains unelucidated, as does the optimal stimulation parameters for LC-NA system activation. We conducted taVNS experiments in anesthetized mice using techniques like immunohistochemistry, viral tracing, and electrophysiology to engage the LC-NA system and study stimulation parameters that can maximize activation in the LC.

We measured the cFOS in the LC and peri-LC region in two groups of animals, that were either stimulated with low current or with high current amplitude in experimental vs sham conditions. An increased cFOS signal was observed in the LC in the group with a higher current regime. Next, we used the juxtacellular recording and labeling technique to record from single neurons in the LC during taVNS while observing the firing pattern of the neuron for a higher temporal and spatial resolution than the cFOS readout. We observed increased firing activity in the neuron during the stimulation bouts and an increased effectiveness of stimulation after several repeated bouts. Furthermore, using pupillometry, we detected significant pupillary responses to taVNS, providing additional evidence of LC activation.

Effects of optogenetic inhibition of parvalbuminergic striatal interneurons on extracellular levels of neurotransmitters in DYT1 knock-in mice

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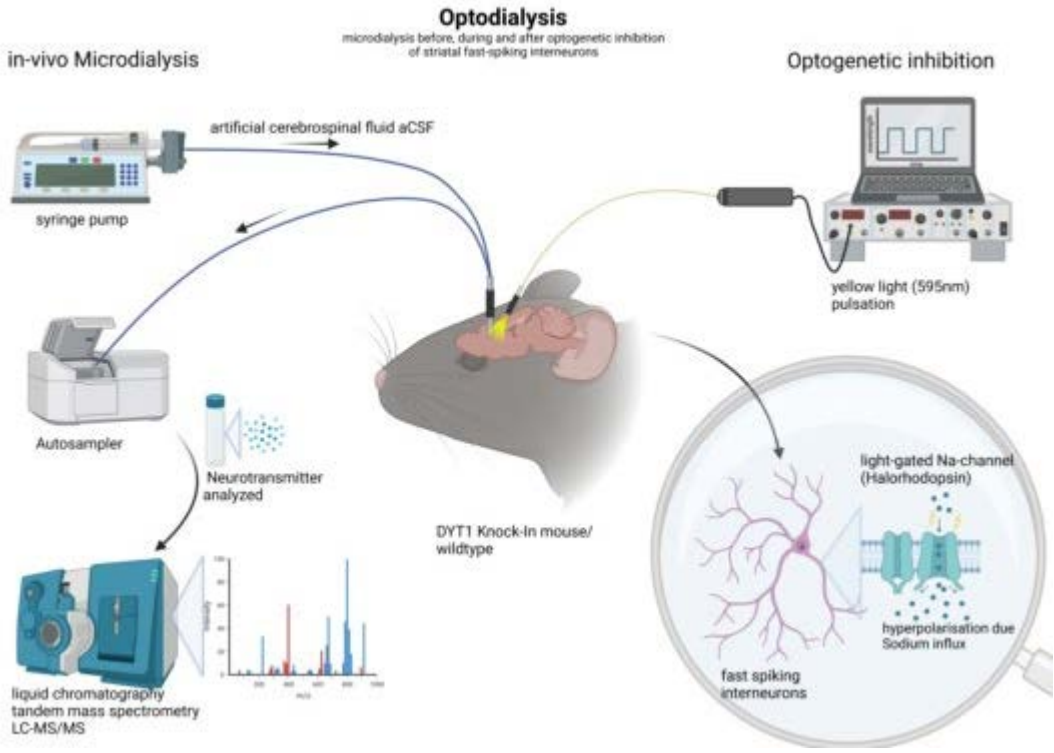
Introduction: Neurochemical imbalances in the striatum have been suggested to play a crucial role in the pathophysiology of DYT1 dystonia (TOR1A), an early-onset generalized movement disorder. Loss of inhibition, linked to altered activity of striatal GABAergic interneurons, is discussed in dystonia. Parvalbuminergic GABAergic fast-spiking interneurons (FSI PV+) exert a powerful inhibitory control within the striatal microcircuitry and optogenetic inhibition of FSI PV+ has been shown to elicit genotype-specific alterations in neuronal activity in a mouse model of DYT1 dystonia

Study Aim:To elucidate the impact of FSI PV+ on striatal neurotransmission and their role in the pathophysiology in DYT1 knock-in mice, we used a combination of optogenetic inhibition of FSI PV+ and in-vivo microdialysis techniques (optodialysis) in the present study.

Material and Methods: Groups of 6-month-old DYT1 knock-in mice (DYT1 KI) and wildtype (wt) littermates on a C57BL/6J background expressing halorhodopsin (eNpHR3.0) in FSI PV+ were used. Microdialysis guide cannulas were stereotactically implanted into the left striatum as well as ipsilateral optogenetic fibres. After a post-surgery recovery period, optodialysis experiments were conducted. Samples were taken during baseline (light off), stimulation (light on, 595 nm, 500 ms light pulse at 1 s intervals) and post stimulation (light off) phases. The dialysates were analysed using LC-MS/MS. Localisation of the implants was confirmed in Nissl-stained brain slices and eNpHR3.0 expression in FSI PV+ by immunohistochemistry.

Results: LC-MS/MS analysis showed striatal basal GABA levels of 10.25 ± 5.90 ng/ml (wt, n = 8) and 6.57 ± 2.73 ng/ml (DYT1 KI, n = 9). During the stimulation, i.e. inhibition of FSI PV+, GABA was significantly ($p < 0.05$) lower in wt (7.64 ± 3.62 ng/ml) and DYT1 KI (5.82 ± 2.31 ng/ml) and remained reduced during the post stimulation phase (wt: 7.98 ± 3.81, DYT1 KI: 6.01 ± 2.94 ng/ml) in comparison to basal concentrations. Differences among the genotypes remained above the level of significance.

Conclusion and Outlook: As expected, the data demonstrate that optogenetic inhibition of FSI PV+ reduces striatal extracellular GABA levels, but no significant genotypic differences in GABA levels were observed. Ongoing simultaneous measurements of other neurotransmitters and neuromodulators associated with dystonia, such as dopamine and adenosine, will show if reduced striatal GABAergic inhibitory control leads to neurochemical imbalances in DYT1 KI mice after FSI PV+ inhibition.



Graphical abstract. In-vivo microdialysis with simultaneous optogenetic inhibition of Parv+ FSI in DYT1 knock-in mice. Analysis of neurotransmitter level using liquid chromatography tandem mass spectrometry (LC-MS/MS). Created with BioRender.com

Resident macrophage-like cells are activated in brain barrier structures of APP/PS1 male mice

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The choroid plexus (ChP), an intraventricular structure composed primarily of specialized epithelial cells in contact with fenestrated capillaries, establishes the blood-cerebrospinal fluid (CSF) barrier. The ChP is well known for actively producing CSF and transporting solutes to and from the brain, thus playing a crucial role in maintaining cerebral homeostasis. In healthy aging, the ChP undergoes structural and functional modifications, also exhibiting higher levels of inflammatory markers and increased infiltration of immune cells. Interestingly, some of these architectural and functional disturbances are thought to accelerate brain aging and contribute to the progression of neurodegenerative disorders, such as Alzheimer's disease (AD). However, the involvement of the ChP in AD onset and progression, the timing of its disruption and the specific cell types involved in the process are still poorly understood. Here, we characterized the impact of AD in the ChP of APP/PS1 mice at different stages of the disease. We detected neither major structural modifications nor amyloid plaque formation in epithelial cells of male and female APP/PS1 mice. However, we found that the density of resident and ChP-specific macrophage-like cells was significantly increased in APP/PS1 mice compared to littermate controls. Both cell types displayed a clear phagocytic activated state, suggesting an active inflammatory response. Notably, this phenotype was present only in APP/PS1 male mice, indicating a sex-specific ChP response in AD mice. These observations suggest a potential role of ChP inflammatory responses in AD pathogenesis, challenging the traditional neuron-centric view.

Hippocampal low frequency stimulation alleviates focal seizures, memory impairments and synaptic pathology in epileptic mice

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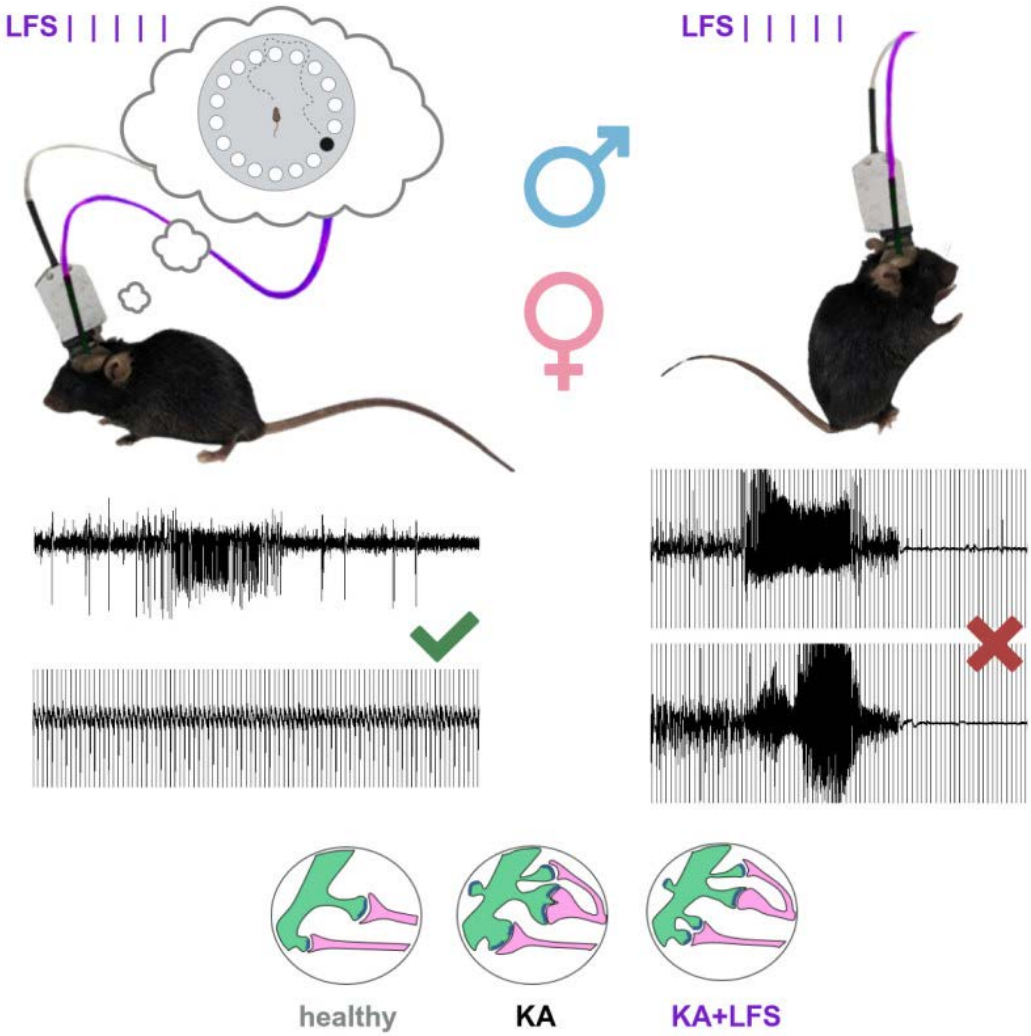
Mesial temporal lobe epilepsy (MTLE) is a prevalent form of focal epilepsy characterized by seizures originating from the hippocampus and adjacent regions. Approximately one-third of MTLE patients suffer from drug-resistant seizures, necessitating invasive interventions like epilepsy surgery or neurostimulation. Conventionally, neurostimulation for seizure control is delivered at high frequency to the anterior nucleus of the thalamus or directly to the seizure focus. However, the efficacy of high-frequency stimulation in MTLE varies among patients. As an alternative, hippocampal low-frequency stimulation (LFS) has shown promising antiepileptic effects in animal studies and small clinical trials. Yet, the long-term effects of LFS on specific seizure types, hippocampal function, and synaptic plasticity remain insufficiently understood. Additionally, previous studies have been conducted exclusively in males, overlooking potential sex differences.

In this study, we used the intrahippocampal kainate model in both male and female mice to replicate the main features of MTLE, including chronic spontaneous seizures, hippocampal sclerosis, and memory deficits. Like MTLE patients, kainate-injected mice exhibit generalized convulsive seizures infrequently, requiring extended stimulation protocols and recordings to evaluate LFS effects on this specific seizure type. We applied 1 Hz electrical LFS in the sclerotic hippocampus six hours a day, four times a week for five weeks. We examined its effect on epileptiform activity, behavior, and kainate-induced pathological features at the cellular and synaptic levels. We integrated findings from electrophysiological recordings, histology, electron microscopy, and behavioral assessments, including the Barnes maze, open-field, and light-dark box tests.

Our findings reveal that long-term hippocampal LFS consistently diminished focal seizures in chronically epileptic male and female mice, with seizure reduction extending beyond the stimulation period. LFS also improved spatial memory in the Barnes maze test and reversed pathological long-term potentiation-like changes at perforant path-dentate granule cell synapses. However, LFS had no significant effect on generalized convulsive seizures, locomotion, anxiety-like behavior, neurogenesis, hippocampal sclerosis, excitatory synapse marker expression, or the presynaptic vesicle pool in perforant path fibers. These findings provide clinically relevant insights into the seizure type-specific effects of hippocampal LFS, which, alongside synaptic and behavioral improvements, could contribute to enhanced seizure control and quality of life in MTLE patients.



Graphical abstract. Hippocampal LFS suppresses focal but not convulsive generalized seizures. Repetitive LFS improves spatial memory deficits and reverses pathological synaptic plasticity at the perforant path-dentate granule cell synapse.

Comparative Analysis of Navigation Ability and Short-Term Memory Binding as Potential Early Diagnostic Markers for Alzheimer's Disease: An Evolutionary Perspective

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Alzheimer's disease, with its origins in the hippocampus—a hub for spatial orientation and short-term memory binding—poses a unique challenge in its early diagnosis. Here we shed light on the potential for more effective early detection methods and explored the extent to which evolutionary history influences disease progression as well as whether this insight could provide indications for optimizing early diagnosis.

Given its hippocampal origin, cognitive functions originating here are among the first to be affected in the disease's progression. In the present study, navigation ability and binding in feature memory were compared as potential early diagnostic markers. Navigation ability was tested using a virtual navigation task, while short-term memory binding was tested using a “what was where” task. 36 Alzheimer's patients were evaluated alongside 41 controls aged 30 years and younger and 35 controls over the age of 30.

Alzheimer's patients demonstrated significantly lower performance in both assessments, showing deficits in navigational memory as well as impaired binding in short-term memory, compared to healthy control subjects. Regarding misbinding, a strikingly large performance difference was observed between healthy subjects and individuals with Alzheimer’s disease, whereas this difference was notably less pronounced in the virtual navigation task. These findings suggest that feature memory is more severely impaired in Alzheimer's patients compared to navigation memory.

Given that navigational memory emerged in early vertebrates, while feature memory was first described in anthropoid primates, we hypothesized that the evolutionary development of memory systems may also influence the trajectory of neurodegeneration. Our findings support the hypothesis that memory functions that have undergone prolonged evolutionary pressure, such as navigational memory, are preserved longer in humans experiencing neurodegeneration compared to those with more recent evolutionary origins, like feature memory.

How does autophagy cope with specific synaptic needs? Consequences in brain health and disease

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Synapses are the communication center of the neuron and to secure their metabolic demand and to avoid accumulation of toxic components during intense neuronal communication and development, synapses locally recycle proteins, lipids and even organelles. Neurons are highly polarized cells with most of the protein synthesis and degradation occurring at the soma. This complex morphology implies challenges to transport metabolites, proteins, and lipids to and from the synapses, that are often far away from the cell body. Autophagy is an evolutionary conserved “self-eating” mechanism critical to maintain cellular proteostasis within the brain. At the synapse, the regulation of this process has compartment-specific mechanisms to degrade and recycle cellular components to support synaptic function and deregulation of synaptic autophagy can impair synaptic homeostasis and jeopardize neuronal survival. Defects in autophagy, together with aberrant protein and lipid accumulation, are present during aging and in neurodegenerative diseases, but how defects in synaptic autophagy are mechanistically linked to synaptic dysfunction, decay and neuronal loss are not fully understood. A deeper understanding of these mechanisms will be necessary to safely exploit autophagy as a therapeutic target to treat brain diseases.
Our work shows that autophagy is locally regulated at presynaptic terminals by a set of synaptic proteins. We also started to decipher that besides aminoacid starvation, a common activator of autophagy in most body cells, synaptic activity is crucial to activate autophagy at the presynaptic terminal. Unfortunately, most of the studies focus on how amino acid deprivation activates autophagy. I will not only present some unique characteristics of synaptic autophagy but also data that demonstrate the existence of molecular distinct autophagy pathways at the synapse. Furthermore, I will show that under physiological condictiones different autophagy pathways execute different functions to support the neuron. Moreover, our work revels that these different autophagy pathways have different implication under pathological conditions. This is particularly relevant in the context of Frontotemporal dementia since we found a specific autophagy pathway that functions in the degradation of pathological Tau.

The Role of TDP-43 as a Co-Proteinopathy in Alzheimer's Disease: Associations with Tau Pathology and Disease Progression

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Transactive response DNA binding protein of 43 kDa (TDP-43) is an intranuclear protein frequently associated with various neurodegenerative diseases. This study aims to explore the role of TDP-43 as a co-proteinopathy in Alzheimer's disease (AD), focusing on its relationship with established AD markers, particularly tau and amyloid-beta (Aβ), in the cortex and hippocampus of patients diagnosed with AD.
The objective is to gain a clearer understanding of the regional distribution and effects of pathological TDP-43 in AD.

Tissue samples from the Netherlands Brain Bank (NBB) of the cortex (n= 140) and hippocampus (n= 30) were analyzed using diaminobenzidine (DAB) staining to identify the presence of TDP-43, Aβ, and tau proteins. Semi-quantitative assessments were performed to stage the severity of proteinopathies. The data was then compared with clinical and neuropathological parameters to evaluate the association between TDP-43 co-pathology and traditional AD markers. Additionally, fluorescence immunostaining will be used to examine the co-localization of these proteins.

In the cortex, patients showed significantly higher levels of pathological cytosolic TDP-43 compared to control subjects. A correlation was also found between TDP-43 pathology and Alzheimer's severity, as measured by the Reisberg score.
In the hippocampus, TDP-43 showed a correlation with both tau and Aβ proteinopathies, but no significant difference in cytosolic TDP-43 levels was observed between patients and controls, likely due to the small sample size from the hippocampus. Moreover, no correlations were found between TDP-43 and other AD markers such as ApoE, gender or age in either region.

The results suggest that TDP-43 could plays a role in Alzheimer's disease progression, The elevated presence of cytosolic TDP-43 in AD patients supports its contribution to the disease and underscores its potential as a therapeutic target in addressing Alzheimer's pathology.

Cellular mechanisms underlying progressive neurodegeneration: Insights from the Drosophila neuromuscular junction

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Progressive neurodegeneration represents the gradual loss of the structural and functional integrity of neuronal cells which can cause sensory disorders, motoric problems, and memory loss. Despite the high prevalence and importance of progressive neurodegenerative diseases, the cellular and molecular mechanisms underlying neurodegeneration in different diseases including Alzheimer´s Disease, Parkinson´s Disease and different forms of motor neuron disease remain unclear. Here we use the Drosophila neuromuscular junction (NMJ) as a model system to identify both common and disease-specific mechanisms of degeneration. To analyze their molecular basis, we compare genetically induced progressive disease conditions to injury-induced Wallerian degeneration (WD). WD is described as an active degeneration mechanism leading to the rapid removal of the separated cellular part. It has also been associated with nerve damage caused by ischemia and chemotherapy. As genetic models we use different degeneration inducing knock-down and overexpression lines that represent common aspects of neurodegenerative diseases such as ALS and Parkinson´s Disease. We characterize these models using immunohistochemical methods, live imaging and functional assays. Analysis of synaptic, cytoskeletal and membrane marker proteins enable visualization of differences in the degeneration sequence. Additionally, live imaging of subcellular compartments provides insight into cellular processes including autophagy. First characterization of WD at subcellular resolution at the larval NMJ revealed clustering and removal of synaptic proteins, accompanied by disassembly of cytoskeletal markers, and finally followed by membrane fragmentation and degeneration. We now utilize these markers and models to identify neurodegeneration modifying factors to determine molecular signaling hubs that have the potential to either enhance or ameliorate disease progression.

Expression of endocannabinoid receptor 1 is reduced in the brain of Alzheimer's disease patients

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Objectives

The endocannabinoid system has gained increasing recognition as a potential therapeutic target for treating neurodegenerative diseases, including Alzheimer's disease (AD). However, the relationship between changes of endocannabinoid receptor 1 (CB1) and the progression of AD neuropathology remains unclear and often contradictory. In this study the potential connection between CB1 and the progression of AD was investigated.

Methods

Cortex and hippocampus post-mortem brain tissue was provided from the Netherlands Brain Bank (n=143). Next to AD patients and controls, Down syndrome patients were analyzed. In addition to the human tissue, AD mice 5xFAD and Tg4-42 were examined (n=20). After cutting the paraffin-embedded brain samples into 5 µm sections, DAB staining for CB1 was done on human and mice samples.

Results

In both cortex and hippocampus CB1 was significantly less expressed in the brain of AD patients compared to the controls. The Reisberg score and Braak stage had a significant negative correlation with CB1. Concerning age, gender and apolipoprotein-E-genotype the AD patients showed no significant difference to the control group. Likewise to the human results, the AD mice 5xFAD and Tg4-42 showed a significant lower expression of CB1 in the cortex and hippocampus in comparison to wild-type mice.

Conclusions

CB1 demonstrates potential as a marker for AD. The observed changes in the endocannabinoid system indicate that CB1 could be a promising target for the treatment of AD.

Evaluation of CA3 place cell remapping in the APP/PS1 model mouse of Alzheimer's Disease

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Spatial navigation impairments are among the earliest clinical manifestations of Alzheimer's Disease (AD). Pyramidal cells of the hippocampus fire selectively when the animal is in a specific location in the environment, leading to the theory that the hippocampus plays a crucial role in forming a cognitive map of the environment. The phenomenon of "remapping", where specific cells exhibit selective firing in distinct environments, is thought to support the formation of different memories. By performing in-vivo electrophysiological recordings in freely moving mice while they navigate through different environments, we characterized 1) if the remapping of different hippocampal place cells (CA1, CA3, DG) is altered in the APP/PS1 mouse model of AD, a model known for spatial navigation deficits; 2) the potential involvement of CA3 interneurons in the early hyperexcitability of the CA3 network, a feature shared by both AD patients and the APP/PS1 mouse model. While interneuron firing rates and place cell remapping are mostly
maintained in the CA1 cells during the early phases of plaque deposition, we found several alterations that are present in the CA3 cells. By investigating the interplay between CA3 place cell remapping and the role of interneurons, our research contributes to a deeper understanding of the neurophysiological changes associated with spatial navigation impairments in AD. This knowledge may pave the way for novel therapeutic approaches targeting specific alterations in the hippocampal network.

Measuring and manipulating neuron excitability in a TDP-43 based model of Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease characterized by the progressive degeneration of motoneurons (MNs), leading to paralysis and ultimately death. A hallmark of ALS is the mislocalization and aggregation of the 43-kDa TAR DNA-binding protein (TDP-43), implicated in both sporadic and familial ALS. However, the mechanistic link between TDP-43 dysfunction and changes in MN excitability remains poorly understood. We have established a novel fly model to study ALS by replacing Drosophila TAR DNA-binding protein homolog (TBPH), with wild-type and different variants of mutant human TDP-43 (hTDP-43). A gene replacement strategy based on recombinase-mediated cassette exchange allows us to study the effects of various disease-causing mutations or those known to affect cell biological properties of hTDP-43 in vivo and directly examine and modulate the excitability of neurons. Here we demonstrate that mutant versions of hTDP-43 cause the protein to mislocalize within MNs and other cell types, disrupting its normal cellular location in the nucleus. We are currently using electrophysiological recordings to study changes in the physiological properties of MNs controlling flight in the presence of wild-type or mutated hTDP-43, testing if these mutations disrupt normal MN activity. Given the advantages of our ‘humanized fly model’, this work promises to provide a novel basis for further investigations of the underlying mechanisms of ALS. It has the potential to lead to new research strategies aiming to uncover the factors that contribute to the pathology or disease progression of ALS.

Motor neuron pathology drives spinal circuit defects and phenotype of a mouse model for spinal muscular atrophy with respiratory distress type 1

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Background: The group of spinal muscular atrophies can be subdivided into the classical proximal form, spinal muscular atrophy (SMA), and distal spinal muscular atrophy with respiratory distress type 1 (SMARD1). The main drivers of SMA are motor circuit defects including neuromuscular junction (NMJ) denervation, motor neuron death and loss of sensory proprioceptive premotor synapses. In contrast, little is known about the pathology of spinal motor circuits in SMARD1, which is caused by a deficiency of the DNA/RNA binding protein IGHBMP2. The most established SMARD1 Nmd2J mouse model exhibits vast motor neuron loss and NMJ denervation. However, it is unknown whether spinal motor circuits are affected or which cell types drive the pathology of this incurable disease.

Methods: We applied immunofluorescence in combination with confocal and super resolution analysis to investigate motor circuits of SMARD1 mouse model Nmd2J to quantify motor neuron death, muscle denervation and spinal synaptic loss. To get access to motor neuron function, we performed whole-cell patch-clamp recordings of motor neurons in spinal cords of Nmd2J mice. Furthermore, we injected adeno-associated virus 9 (AAV9) into perinatal mice to selectively restore the disease causing IGHMBP2 protein selectively in motor neurons or proprioceptive neurons to study their pathology of spinal motor circuit and motor phenotype in SMARD1.

Results: First, we compared the pathology of “proximal” motor circuits consisting of the lumbar L1 spinal segment and its axial target muscles with “distal” motor circuits consisting of the L5 segment and distal muscles. We found coincidental α-motor neuron death and muscle denervation within the first two weeks of life which both extended to ~70% at 6 weeks selectively in distal motor circuits, matching the distal phenotype of Nmd2J mice. Similarly, a selective 50% loss of premotor excitatory synapses (C-boutons and proprioceptive synapses) in distal motor circuits was present by 4 weeks when the first motor impairments became apparent. Whole-cell patch-clamp of spinal Nmd2J motor neurons revealed reduced and delayed proprioceptive synaptic transmission. This was accompanied with reduction of Munc13-1 positive presynaptic release sites of proprioceptive synapses. To identify the cause of motor circuit degeneration and dysfunction, we applied a virus that conditionally overexpresses IGHBMP2 (AAV9-IGHBMP2fl/fl) upon Cre recombinase induction. By injecting AAV9-IGHBMP2fl/fl into Nmd2J mice expressing a motor neuron specific Cre, we restored IGHBMP2 selectively in motor neurons. These mice exhibited an almost complete rescue of the entire motor circuit pathology including motor neuron death, proprioceptive synaptic degeneration, synaptic dysfunction and motor phenotype of the Nmd2J mice, demonstrating that motor neuron defects drive SMARD1 pathology.

Conclusion:
Our findings link selective motor circuit pathology, including severe proprioceptive synaptic loss and motor neuron death, to the observed “distal” phenotype of Nmd2J mice and SMARD1 patients. We

develop a novel genetic-viral approach to implement cell type-specific IGHMBP2 expression and demonstrate that motor neurons alone drive SMARD1 pathology. These findings lay the ground for identifying novel disease markers and candidate therapeutic targets to ameliorate this incurable disease.

Acknowledgments:
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Breaking Social Bonds: How LC Degeneration could impact Social Behavior in Parkinson's Progression

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Abstract

Parkinson's disease (PD) is one of the most common progressive neurodegenerative disorders, placing a significant burden on our aging society. While PD was initially characterized as a movement disorder it has become clear that early symptoms are predominantly non-motor. Moreover, PD involves a range of emotional changes that can severely disrupt social functioning in the early stages of the disease. However, the circuit mechanisms and brain areas involved in social impairments remain elusive.

Given that catecholaminergic neurons, particularly noradrenergic neurons in the locus coeruleus (LC), are affected early in PD, we speculated that noradrenergic degeneration could contribute to the social deficits observed in PD. To avoid the trap of hypothesis-driven storytelling, we set out to a data-driven approach that monitors our transgenic mouse model that produces neuromelanin in catecholaminergic neurons (tg-NM), mimicking aspects of PD pathology, at different time points throughout the lifespan of these animals. This model exhibits mild degeneration of the LC in young animals, which progresses to more severe degeneration during adulthood.

We conducted continuous behavioral monitoring of groups of four mice for seven days at multiple stages of disease progression (3, 6, 9, and 12 months). Both male and female mice groups were included, and we tested configurations with mixed genotypes, only wild-type (WT), or only tg-NM animals. Using Long-Mouse Tracking Software, we extracted over 40 different behaviors across several domains from this extensive dataset. We identified alterations in activity patterns, sleep-wake cycles, and several social-related behaviors in tg-NM mice compared to WT controls.

Motivated by these unbiased behavioral observations, we investigated potential histological correlates of the impairments. We quantified noradrenergic innervation to oxytocinergic neurons in the paraventricular nucleus (PVN), dopaminergic neurons in the ventral tegmental area/substantia nigra pars compacta (VTA/SNc), and serotonergic neurons in the dorsal raphe (DR). We observed decreased noradrenergic innervation in these regions, which could impact the function of these key neuromodulatory systems involved in social behavior and emotional regulation.

Our findings suggest that early noradrenergic degeneration contributes to social impairments in PD, potentially through disrupted modulation of other neuromodulatory neurons. Future studies using fiber photometry will investigate whether decreased noradrenergic innervation leads to altered neuronal dynamics within these neuromodulatory circuits. Understanding these mechanisms could inform the development of therapeutic strategies targeting social dysfunction in PD.

Effects of long-term thiethylperazine treatment on Alzheimer's pathology in Tg4-42 mice

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Background: Alzheimer's disease (AD) affects over 1 million people in Germany, a number that will continue to rise rapidly in the coming years due to demographic change. Despite the recent approval of two anti-amyloid monoclonal antibodies, lecanemab (Leqembi®) and donanemab (Kisunla®), in the USA, there is still no universal or easily accessible treatment for. For this reason, research of new therapeutic approaches and strategies is highly important. AD is a progressively advancing disease that is accompanied by an inflammatory reaction, neurodegeneration and brain atrophy. Patients typically suffer from memory loss, cognitive deficits and even complete disorientation. Histopathological correlates such as amyloid plaques, neurofibrillary tangles and an inflammatory reaction accompany the disease. Nevertheless, the exact cause of the disease is not fully understood. Thiethylperazine is an antiemetic drug and was developed in the 1960s. Apart from its therapeutic effects against nausea and vomiting, it also stimulates ABCC1, a transporter considered to be involved in Aβ efflux across the blood-brain barrier. By increasing Aβ clearance, it could therefore be suitable as a potential therapeutic agent against AD. Therefore, this study examined the impact of prolonged thiethylperazine administration on behavioral deficits and pathological changes in a mouse model of AD.

Methods: Tg4-42 animals overexpress Aβ4-42 and show early cognitive deficits and significant neuronal cell loss in the CA1 region of the hippocampus. Wildytpe(WT) and Tg4-42 animals were administered 10mg/kg body weight of thiethylperazine intraperitoneally daily for six months. Subsequently, a series of behavioral tests were conducted to assess motor coordination, learning, anxiety and memory functions. Additionally, immunohistochemical staining of the tissue was performed to examine effects on neurogenesis and interneurons.

Results: There was a significant improvement after thiethylperazine treatment in memory performance in the novel object recognition test of the Tg4-42 animals. In contrast, the spatial learning deficits in the Morris Water Maze could not be restored. The motor deficits observed in Tg4-42 animals during the RotaRod test, which assesses motor coordination, could not be alleviated through thiethylperazine treatment. Anxiolytic effects were seen in three different anxiety tests in transgenic animals but not in wild-type animals. Thiethylperazine caused an increase in neurogenesis in WT but not Tg4-42 mouse model. The treatment had no effect on the number of parvalbumin positive interneurons.

In consequence, although thiethylperazine displays promise in enhancing memory function in Tg4-42 mice, its limited efficacy on spatial learning and motor deficits highlights the necessity for further investigation to elucidate its therapeutic potential in the context of AD.

Effects of Low Dose Δ9-tetrahydrocannabinol (THC) on Alzheimer's Disease Pathology in 5XFAD Mice

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Alzheimer's disease (AD), characterized by memory impairment and cognitive decline, presents as either early-onset familial AD (FAD) or the more prevalent late-onset sporadic AD. The disease is marked by amyloid-beta plaques and neurofibrillary tangles.
This study investigated the effects of low-dose Δ9-tetrahydrocannabinol (THC), the psychoactive component of Cannabis sativa, on AD pathology using the 5XFAD mouse model. This model exhibits accelerated FAD-like pathology, including rapid and widespread Aβ plaque deposition. Treatment started at 1.5 month (preventive), and 5 month (acute) post-natal for 42 ± 2 days. Dosage of THC was 3 mg/kg body weight, and control group was treated by vehicle solution. Previous behavioural study indicated that low-dose THC ameliorated learning and memory deficits in AD mice. Building on these findings, we used immunohistochemistry to examin the impact of THC on critical AD pathologies, including neuroinflammation, Aβ plaque burden, neurogenesis, and synaptic activity. Furthermore, 18F-Florbetaben-PET was performed to analyze cerebral amyloid deposition in vivo. Our results demonstrate that low-dose THC significantly reduced neuroinflammation and Aβ plaque load in 5XFAD mice. However, THC did not significantly impact synaptic activity and neurogenesis. Our findings suggest that THC may offer therapeutic benefits by mitigating neuroinflammation and Aβ accumulation in AD.

Neuronal excitability in entorhinal cortex layer II pyramidal neurons regulates tau propagation in early stage of Alzheimer's disease

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Background: We have recently developed the unique mouse model recapitulating the tau propagation in early stage of Alzheimer's disease (AD). The injection of adeno-associated virus expressing Cre-inducible P301L human mutated tau (AAV-Flex-P301L) in Wfs1-Cre mice, which expresses Cre-recombinase explicitly in ECII pyramidal neurons, induces tau propagation into the CA1 pyramidal neurons. Using this mouse model and a chemogenetic approach, we aimed to determine the effect of neuronal excitability and amyloid pathology on tau transfer to the hippocampal and neocortical regions in mouse brains.

Methods: AAV-Flex-P301L tau was injected into the EC II in APPNL-G-F: TAUKI: Wfs1-Cre, TAUKI:Wfs1-Cre and Wfs1-Cre mice at 7 months old. Tau propagation in the hippocampal and cortex regions were evaluated by immunofluorescence using AT8 phosphorylated and Alz50 misfolded tau antibodies at 3 months post-injection. AAV-Flex-P301L tau was co-injected with Cre-inducible AAV hM3D or hM4D in the ECII in Wfs1-Cre mice at 4 months old and clozapine N-oxide or saline was infused via osmotic pumps following 28 days. Immunofluorescence against HT7 human tau antibody was performed at 1 month post-injection.

Results: We observed AT8+ and Alz50+ neurons in the CA1, subiculum and the visual cortex at 3 month-post injection in APPNL-G-F:TauKI:Wfs1-Cre mice and significantly less in TauKI:Wfs1-Cre mice while p-tau did not advance to the visual cortex in Wfs1 Cre mice. Increased or suppressed neuronal excitability in the medial ECII pyramidal neurons significantly induces or reduces tau propagation to the CA1 compared to the control group respectively, indicating that excitability of ECII regulates the tau transfer efficiency.

Conclusions: Our study demonstrated that amyloid deposition in APPNL-G-F: TAUKI: Wfs1-Cre mice accelerates tau transfer to both CA1 and visual cortex regions and ECII neuronal excitability regulates tau transfer from the ECII to CA1.

Early disease-modifying treatment in a mouse model of Parkinson's disease: Exercise demonstrates its potential

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Aims: Subtle motor and non-motor dysfunctions indicative of beginning Parkinson's disease (PD) are evident before clinical disease diagnosis. Persons of risk developing PD would be willing to determine their risk and change their lifestyle in case of a concrete beneficial approach. Growing evidence indicates the potential of exercise in reducing components of PD-related pathology. We hypothesized that early intervention by exercise has a disease-modifying effect during the prodromal phase in our PD mouse model.

Methods: Two-month-old transgenic mice overexpressing human wild-type alpha-synuclein (Thy1-aSyn) and wildtypes were assigned to three groups receiving different intensity levels of exercise on a treadmill. We assessed motor performance and activity, and took microbiome and brain samples for analysis of microbiota and PD-related pathology.

Results: Transgenic mice showed motor impairment on the challenging beam and vertical pole test, reflecting the expected progression of aSyn pathology at this age. The improved vertical pole performance of trained wildtypes demonstrates the potential of exercise for beneficial effects on gross motor skills (which would rather suggest symptomatic effects), while slightly improved beam performance of transgenics might indicate a disease-modifying potential of exercise. Levels of phosphorylated alpha-synuclein at serine 129, a key feature of PD, were increased in the substantia nigra pars compacta in untrained, but not in trained, Thy1-aSyn mice, indicating a neuroprotective potential of exercise. Intensive training did not induce inflammation, since reactive microglia were not affected. Genotypes did not differ in locomotor open field activity, but untrained transgenics exhibited an anxiety-like phenotype. Intriguingly, intensive training reduced anxiety-like behavior in transgenics to wild-type level and increased bacterial richness and alpha diversity in fecal samples of both genotypes.

Conclusions: These results suggest that exercise alleviates early sensorimotor and even non-motor deficits in Thy1-aSyn mice and demonstrate its potential as an early PD-modifying treatment. Increased fecal microbiota diversity in intensively-exercised mice supports a potential role of the gut-brain axis in the underlying pathological mechanisms of PD.

Neuroprotective Effects of Lycopene: Modulation of Oxidative Stress, Neuroinflammation, and Tryptophan Pathway Metabolites in In Vitro and In Vivo Models

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Neuroinflammation and oxidative stress are critical contributors to neurodegenerative disorders. This study investigates the neuroprotective effects of lycopene through in vitro and in vivo models, focusing on the tryptophan metabolic pathway, particularly quinolinic acid (QA) and kynurenic acid (KYNA). In vitro assays were conducted on SH-SY5Y human neuroblastoma cells to evaluate the protective effects of lycopene against oxidative damage induced by hydrogen peroxide (H2O2) and excitotoxicity triggered by QA. Lycopene significantly mitigated cell death, reduced oxidative stress markers, and restored cellular viability, indicating its potential to counteract neurotoxic insults.

In parallel, an in vivo study utilized a mouse model of neuroinflammation induced by chronic exposure to aluminum chloride (AlCl3). Lycopene treatment effectively attenuated neuroinflammation, as evidenced by a reduction in pro-inflammatory cytokines and oxidative stress markers in brain tissues. Moreover, lycopene modulated the tryptophan-kynurenine pathway by decreasing QA levels and increasing KYNA levels, thereby shifting the pathway toward neuroprotection. Behavioral assessments further confirmed improvements in cognitive and motor functions in lycopene-treated mice.

These findings highlight the dual antioxidant and anti-inflammatory properties of lycopene and its regulatory effects on the tryptophan pathway, suggesting its potential as a therapeutic agent in neurodegenerative conditions characterized by oxidative stress and neuroinflammation.

Molecular imaging of alpha-synuclein as a path towards Parkinson's disease diagnosis

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Parkinson’s Disease is the second most prevalent and fastest-growing neurodegenerative disease caused by the loss of dopaminergic input from the substantia nigra pars compacta to the striatum. After two centuries following its characterization by Parkinson, treatment has remained largely palliative with no prognostic improvement. This is because 'conclusive' clinical symptoms appear very late only after substantial reduction in dopamine has occurred as a result of the degeneration of dopaminergic neurons and their axons. The therapeutic window therefore remains closed to neuroprotective and neurorestorative approaches. In search of realiable biomarkers for early detection and monitoring, alpha-synuclein has emerged as the most promising target. Alpha-synuclein is a major component of one of the pathophysiological hallmarks of Parkinson’s disease called Lewy pathology. Alpha-synuclein is intrinsically disordered under physiological conditions, but can aggregate into different sizes ranging from dimers and tetramers to oligomers, protofibrils, and finally fibrils that are then incorporated into Lewy bodies and Lewy neurites. These aggregates/species have been implicated in various aspects of the disease’s pathology. Oligomers have been identified as the toxic component responsible for cell death while fibrils may be largely responsible for the spread of the pathology across the nervous system. As a result, several previous and ongoing researches have been dedicated to the studying alpha-synuclein, its aggregates and other modifications as potential biomarkers for the disease. Two promising approaches have come out of these. Immunoassays usually based on ELISA have shown that oligomeric and or phosphorylated species are elevated in the CSF and blood components of diseased patients compared to healthy controls. These however, cannot distinguish the various forms of aggregates. We also now have an FDA-approved biomarker based on Seed Amplification Assays (SAAs) using CSF. SAAs are currently validated for only CSF and remains purely qualitative. Hence both the immunoassays and SAAs lack the quantitative segregation of aggregates that can enable disease monitoring and or early diagnosis. Given the nanoscopic sizes of these aggregates, super-resolution imaging is required. One-step nanoscale expansion (ONE) microscopy which enables the visualization of the shapes of individual membranes and proteins on confocal microscopes can provide a relatively simple and inexpensive approach for the characterization of alpha-synuclein aggregates. ONE microscopy has been used to study alpha-synuclein in CSF with similar results as that obtained via immunoassays. However, given the risk associated with the collection of CSF, other readily accessible fluids such as urine and blood would be preferable. I, therefore, decided to employ ONE microscopy but in this case on serum samples. Using alpha-synuclein-specific nanobodies on serum samples and applying ONE microscopy, preliminary results indicate that the proportion of small oligomeric species was higher for Parkinson’s Disease patients compared to the non-Parkinson’s controls. The converse was true for large oligomers and large assemblies which were higher for the non-Parkinson’s controls. Barring a change in these results after expanding to larger sample sizes and different cohorts, the structural identification and quantification of alpha-synuclein species using ONE microscopy has the potential of enabling early diagnosis and monitoring for Parkinson’s disease.

Vascular pathology induced by alpha-synuclein overexpression renders the brain tissue more vulnerable to bacterial endotoxins

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Dysfunction of the blood-brain barrier (BBB) is suggested to play a critical role in the pathological mechanisms and progression of Parkinson's disease (PD). PD-related pathology, such as alpha-synuclein (aSyn) accumulation and inflammatory processes, potentially affect the integrity of the BBB from an early disease stage. Consequently, this may accelerate disease progression by altering the crosstalk of the central and peripheral immune response, especially under bacterial or viral infections. This could result in a self-perpetuating pathophysiology of inflammation and BBB alteration, which contributes to neurodegeneration. Importantly, BBB dysfunction could also affect drug response in PD patients. Therefore, there is urgent need to resolve the underlying molecular mechanisms of BBB dysfunction in PD.

In our initial study, we described microvascular changes during disease progression in a mouse model with overexpression of human aSyn (Thy1-aSyn, line 61, Lau et al., Neurobiol Dis 2023). This model replicates characteristic symptoms of PD, aSyn pathology and dopamine loss. BBB alterations observed in Thy1-aSyn mice included altered transporter protein expression and increased endothelial activation. Striatal capillaries presented with more dysregulated BBB integrity markers compared to cortical capillaries. However, these alterations of BBB integrity did not lead to an overt IgG leakage into brain parenchyma.

Now we present a study that aimed to decipher whether these BBB alterations in Thy1-aSyn mice render this model more vulnerable to additional inflammatory processes such as a single injection of the bacterial toxin lipopolysaccaride (LPS). Two weeks after modelling a transient gut dysbiosis we measured leakage of i.v. injected albumin-Fluorescein-Isothiocyanat-Conjugate (FITC-albumin) in 3 months old Thy1-aSyn and wild type control mice. There was no detectable leakage of FITC-albumin into brain parenchyma but endothelial cells of Thy1-aSyn mice showed positive signal for FITC-albumin. This indicates that the neurovascular unit may react different to peripheral inflammation in Thy1-aSyn animals compared to controls. Furthermore, we aimed to detect differences in peripheral immune cell invasion using Iba1, TMEM119, CD206+ and Lyve1+ to distinguish between microglia and infiltrating macrophages.

Our data provides insights on increased vascular vulnerability of an aSyn overexpression PD mouse model to peripheral gut dysbiosis by enhanced endothelial uptake of FITC-albumin and altered immune cell reaction. This altered peripheral immune cell invasion could drive neuroinflammation and neurodegeneration in PD.

Investigations on proteinopathies along the gut-brain axis in dogs

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Bidirectional connection between the brain and the gut, known as the gut brain axis, is involved in the pathophysiology of proteinopathies and neurodegeneration in Parkinson´s disease (PD). Pathological hallmark of PD is the presence Lewy bodies, which consist of misfolded alpha-synuclein. This protein has also been detected in the intestine. Of note, gastrointestinal symptoms appear early in the disease progression even prior to the classical motor symptoms. The gut microbiome is altered in PD patients and modifications of the microbiome can ameliorate symptoms in animal models of PD. This is in line with studies in epilepsy and other neurological diseases. In spite of this, data is still lacking on whether and how changes in the microbiome lead to detectable morphological alterations in brain tissue. Studies to close this knowledge gap require animal models, however, the short period of disease progression in rodents may hamper the detection of a causative relationship. There are a few studies on age-related neurodegenerative alterations in brains of dogs, but comprehensive studies on pathological hallmarks of PD in dogs are missing.
The aim of our study is to close these knowledge gaps by studying PD-like neurodegenerative alterations in the brain and gut of dogs. We hypothesize that aged dogs develop signatures of proteinopathies in brain and intestine, and that alterations in the gut microbiome correlate with such pathologies.
In the first instance, we aimed to provide a comprehensive picture of proteinopathies in dog brains. Therefore, we took advantage of the large brain bank available at the Department of Pathology and immunostained paraffine-embedded dog brain sections stored up to more than 20 years for proteinopathy, microgliosis and neurodegeneration. Upon extensive establishment of staining protocols for immunfluorescent co-labeling we are currently investigating brain sections and in part intestinal sections from aged dogs that were archived between 2022-2023. Long storage conditions may have altered epitopes preventing staining for certain protein markers. To investigate the potential impact of long storage and paraffine-embedding on staining efficacy, post-fixated brain tissue from 2024 without paraffine-embedding is used to prepare free floating sections for immunostaining for comparison. Where available, these results are going to be correlated with the clinical status on gut microbiome and geriatric symptoms.

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Brain region-specific and systemic transcriptomic dysregulation in a human alpha-synuclein overexpressing rat model

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Synucleinopathies are age-dependent neurodegenerative diseases characterized by alpha-synuclein accumulation with distinct vulnerabilities across brain regions. Understanding early disease stages is essential to uncover initial molecular changes that might enable earlier diagnosis and causal therapy. Here, we profiled longitudinal and brain region-resolved gene expression changes in a rat model of synucleinopathies overexpressing human SNCA. Transcriptomic analyses on gene and transcript level of striatal, frontocortical, and cerebellar tissue in 5- and 12-month-old transgenic (BAC SNCA) and wild type rats revealed that SNCA overexpression leads to age-dependent transcriptomic changes that largely occur region-specific. In frontal cortex, dysregulation of myelination-associated genes agreed with Parkinson patient data as shown before. In addition, BAC SNCA rats displayed more gene expression changes at younger age, with a common and distinctive dysregulation pattern across all three examined brain regions. We also identified a cross-regional set of differential genes with similar perturbation patterns that were affected by SNCA overload. This set was also partially reflected in the gut transcriptome of the same rat model, suggesting a systemic impact of SNCA overload. Taken together, our findings highlight both brain region-specific vulnerabilities and global molecular perturbations associated with alpha-synuclein biology and provide insights into early transcriptomic changes in synucleinopathies.

Electrophysiological and Neurochemical Effects of the Kynurenic Acid Analogue SZR104 in Physiological Conditions and Cerebral Ischaemia: Insights from In vitro Models

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Introduction: The kynurenine pathway of tryptophan metabolism has been a focus of research for its potential therapeutic targets, with multiple research groups, including those at the University of Szeged, actively contributing. Tryptophan metabolism plays a crucial role in maintaining normal brain functions, and an imbalance in its intermediates—particularly the kynurenic acid to quinolinic acid ratio—is implicated in neurodegenerative and mental disorders, such as traumatic brain injury, Alzheimer's disease, Huntington's disease, and schizophrenia. Our research primarily focuses on cerebral ischaemia, investigated through in vitro electrophysiological methods. The ischaemic cascade — a central process of cerebral ischaemia — is heavily influenced by glutamate-induced excitotoxicity. Kynurenic acid, an endogenous NMDA receptor antagonist produced in the kynurenine pathway, has therapeutic potential but limited blood-brain barrier permeability. Kynurenic acid analogues are synthesized by the Department of Pharmaceutical Chemistry, University of Szeged. Among these, SZR104 has twice the blood-brain barrier permeability of kynurenic acid. So, we tested it in an in vitro oxygen-glucose deprivation (OGD) model. Its effects were assessed using paired-pulse (PP) paradigms, long-term potentiation (LTP), and neurotransmitter release (glutamate, acetylcholine) in superfusion experiments.
Methods: hippocampal brain slices were used from Wistar rats (180-220 g). Field potential amplitudes were recorded in the CA1 region of the hippocampus with Schaffer collateral stimulation, and LTP was induced through theta burst stimulation (TBS). Paired pulse (PP) responses were also evaluated. Neurotransmitter release was analyzed in an ex vivo superfusion system, with radiolabeled neurotransmitters collected every 2 minutes.
Results: In the OGD model, SZR104 increased the tolerance of hippocampal pyramidal cells to ischaemia by 2 min and 10 sec compared to kynurenic acid. LTP studies showed that SZR104 improved the maintenance of LTP under physiological conditions compared to the control. Additionally, PP measurements indicated an increase in paired-pulse facilitation (PPF). Further studies are underway to elucidate the underlying mechanisms. In superfusion experiments, while kynurenic acid increased acetylcholine release, SZR104 maintained it at control levels and reduced glutamate release below control levels, unlike kynurenic acid, which significantly increased it.
Discussion: Our results demonstrate that SZR104, a kynurenic acid analogue, enhances ischaemic tolerance in hippocampal CA1 pyramidal cells and sustains LTP maintenance under physiological conditions. Increased PPF suggests involvement of multiple processes, which are currently being explored through additional experiments. Superfusion studies showed differential effects of SZR104 on neurotransmitter release compared to kynurenic acid, suggesting distinct neurochemical actions. These promising in vitro results warrant further in vivo investigation, as presented in another poster by our research group.

Poster Topic

T12: Neuroimmunology, Inflammation and Neuroprotection

T12-2A Sepsis Induces Oligodendrocyte Dysfunction, Changes in Neural Pathways and Brain Barrier Alterations
Nina Hahn, Martin Bens, Christian Geis

T12-3A Sex-Specific Neuronal Autophagy Disruption and Hyperphosphorylation after Neurotropic IAV Infection
Lea Gabele, Shirin Hosseini, Kristin Michaelsen-Preusse, Nele Rieke, Christian Sieben, Martin Korte

T12-1B From autoantibodies to neuropathic pain: a cascade caused by anti-CASPR2 autoantibodies
Margarita Habib, Anna-Lena Wießler, Patrik Fischer, Maximilian Koch, Annemarie Sodmann, Felicitas Schlott, Kathrin Doppler, Carmen Villmann

T12-2B Experimental SAH reveals differences in CBF and CBO in distinct vascular compartments for varying injection velocities and fluids
Katrin Becker, Ute Lindauer, Catharina Conzen-Dilger

T12-3B Circular RNA circKlhl2 modulates TBI response influencing the BDNF pathway
Francesco Roselli, Marica Pagliarini, Zhenghui Li, Florian olde Heuvel

T12-1C Blood-brain barrier integrity and sexual dimorphisms during macrophage invasion of the Drosophila nervous system
Dominik Funke, Bente Winkler, Simone Rey, Christian Klämbt

T12-2C Immunohistochemical investigation of the components of the blood-brain barrier in a mouse model for multiple sclerosis
Hannah Gäb, Greta Hartmann, Hanna Hartwig, Anne-Wienke Nissen, Charlotte Schubert, Manuel Friese, Daniela Hirnet, Christian Lohr

T12-3C Microglial Activation and Complement Dysregulation in Sepsis-Associated Encephalopathy (SAE)
Özge Candemir, Nina Hahn, Ha-Yeun Chung, Jonathan Wickel, Stephan Steinke, Michael Hust, Christine Skerka, Christian Geis

T12-4C Macrophage invasion into the Drosophila brain requires JAK/STAT dependent MMP activation in the blood-brain barrier
Bente Winkler, Dominik Funke, Christian Klämbt

T12-1D C3 and CD14 modulate diffuse but not focal neuroinflammation in TBI associated with polytrauma
Marica Pagliarini, Fan Sun, Zongren Zhao, Markus Huber-Lang, Francesco Roselli

T12-2D Effects of the cannabinoids 2-Arachidonylglycerol and WIN 55,212-2 on primary isolated astrocytic cultures and astrocytic-microglial co-cultures
Franziska Vieregge, Tim Hohmann, Chalid Ghadban, Candy Rothgänger-Strube, Urszula Hohmann, Faramarz Dehghani

T12-3D Modulation of glial inflammatory reactions by GPR55
Annika Hensel, Chalid Ghadban, Candy Rothgänger-Strube, Urszula Hohmann, Tim Hohmann, Faramarz Dehghani

Sepsis Induces Oligodendrocyte Dysfunction, Changes in Neural Pathways and Brain Barrier Alterations

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Sepsis-associated encephalopathy (SAE) is a severe and frequent neurological complication of sepsis causing long-term cognitive dysfunction in more than half of the survivors. While the incidence of sepsis is increasing, the mortality is decreasing, leading to a growing number of patients suffering from chronic SAE. Despite its prevalence, the underlying mechanisms of SAE remain largely unexplored. In this study, we utilized spatial transcriptomics combined with immunofluorescent staining to analyze murine brain tissue samples from a polymicrobial sepsis model. This approach enabled high-resolution mapping of transcriptomic changes while preserving spatial information.

Our findings highlight a significant impact of sepsis on oligodendrocyte populations, with substantial alterations observed in both myelin-forming and mature oligodendrocytes. These changes suggest a pivotal role for oligodendrocytes in SAE pathophysiology, contributing to disrupted myelination and impaired neural connectivity.

We also identified significant disturbances in the cortico-striatal-thalamo-cortical (CSTC) pathway, particularly affecting excitatory neurons in the cortex, thalamus, and medium spiny neurons in the striatum. As this pathway is crucial for associative learning and memory, its disruption may underlie the cognitive deficits associated with SAE.

Furthermore, transcriptomic alterations in the microenvironment of the choroid plexus and vascular leptomeningeal cells suggest their involvement in the pathophysiological mechanisms of SAE. Changes in these regions may contribute to the disruption of the brain's homeostatic functions during sepsis.

Lastly, we observed an inflammatory environment around brain vessels, which could exacerbate neural dysfunction and impair blood-brain barrier integrity, further contributing to the disease process.

By integrating spatial transcriptomics with immunofluorescent staining, we were able to provide a detailed characterization of sepsis-related transcriptomic shifts across brain regions. These findings offer new insights into the cellular and molecular mechanisms of SAE and identify potential targets for future therapeutic strategies.

Sex-Specific Neuronal Autophagy Disruption and Hyperphosphorylation after Neurotropic IAV Infection

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Neurodegenerative diseases are often marked by the accumulation of misfolded or misassembled proteins, which contribute to neurotoxicity. While these diseases are strongly associated with aging, their precise etiology remains unclear. Research has shown that both genetic and environmental factors may contribute to their onset. It has been proposed that persistent viral infections, such as HSV as an environmental factor, could play a role in neurodegenerative conditions like Alzheimer's disease (AD). However, the mechanisms triggering protein aggregation in most sporadic cases remain elusive. One hypothesis suggests that viruses, by hijacking host cellular machinery for replication, cause significant disruptions in cellular proteostasis, leading to the accumulation of misfolded proteins.

A prior study demonstrated that infection with a neuro-adapted influenza A virus (A/WSN/33, IAV), a respiratory virus causing acute infections, can impair autophagosome formation and inhibit autophagic flux in neuronal cultures derived from the substantia nigra. This resulted in the accumulation of α-synuclein and potentially triggered Parkinson's disease.

In this study, we investigate the effects of neurotropic IAV infection (rSC35M, recombinant A/Seal/Mass/1/80 mouse-adapted, H7N7) on the impairment of autophagic flux and the intracellular accumulation of hyperphosphorylated tau in hippocampal neurons, which may contribute to neurodegenerative diseases like Alzheimer's. The relationship between acute respiratory viral infection and protein accumulation in the brain, particularly in a sex-specific manner, is still unclear.

To explore this, we infected sex-divided primary hippocampal cultures for 6 and 24 hours with H7N7 IAV. To better replicate hippocampal physiology, microglia of the respective sex were added to the cultures. Using a tandem reporter construct (mRFP-GFP-LC3), we visualized autophagosome formation, and through immunohistochemistry, we examined the increase in hyperphosphorylated tau. Additionally, we are investigating whether direct infection leads to increased tau hyperphosphorylation.

Preliminary results show impaired autophagy processes after neurotropic IAV infection in both sexes, with strong tau hyperphosphorylation observed in the nuclei and neurites, particularly in directly infected neurons. Understanding how IAV interferes with autophagy and induces tau hyperphosphorylation in a sex-specific context will provide valuable insights into the mechanisms of virus-induced neurodegeneration. These findings could inform future therapeutic strategies to mitigate the neurological consequences of viral infections and prevent the progression of neurodegenerative diseases.

From autoantibodies to neuropathic pain: a cascade caused by anti-CASPR2 autoantibodies

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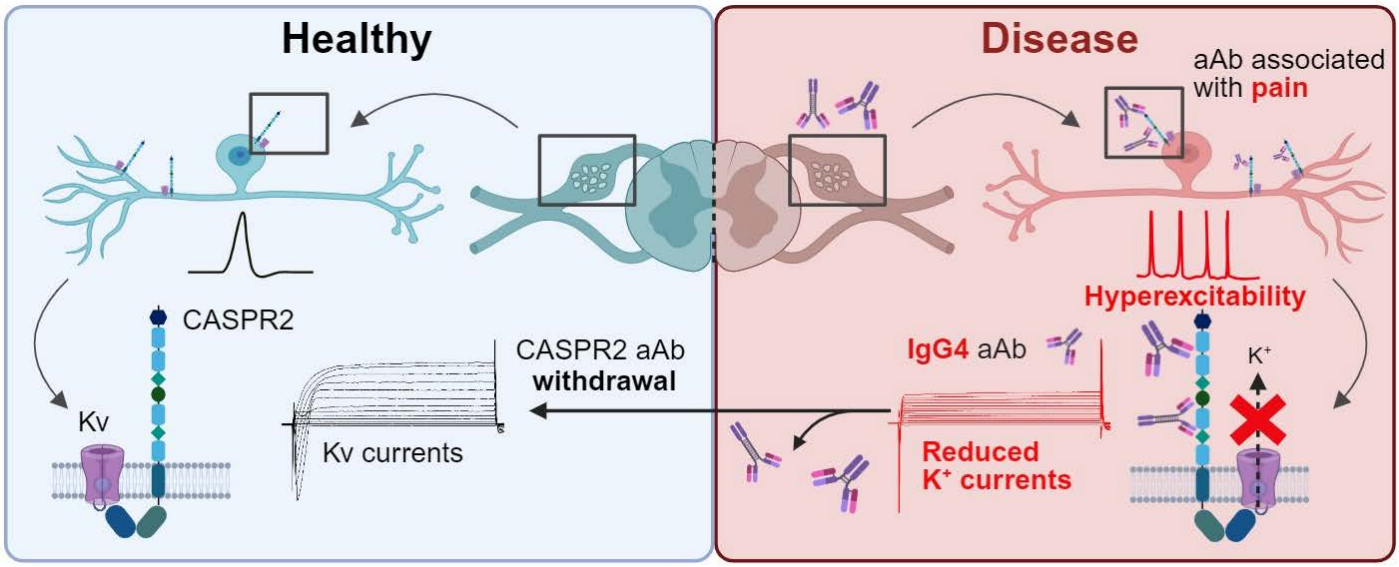
Contactin-associated protein 2 like (CASPR2) autoantibodies (aAb) are not only associated with encephalitis and epilepsy but also with neuropathic pain. The precise underlying pathomechanism how those aAb result in neuropathic pain is incompletely understood.
Not every patient with CASPR2 aAb experiences pain. Further, sera of the patients harbor different IgG subclasses. Almost all have IgG4 aAb, and most of them also carry aAb of at least one other IgG subclass (IgG1, IgG2, or/and IgG3).
Here, we have studied dorsal root ganglia neurons (DRGs) which play a crucial role in pain transmission as gatekeepers by linking the periphery with the central nervous system. On their membrane, DRGs express the voltage gated-potassium channel (VGKC) complex. Voltage-gated potassium channels (Kv) have key functions as they regulate the excitability and restore the resting membrane potential of neurons. As CASPR2 is part of the VGKC complex, the pathomechanism of CASPR2 autoantibodies is hypothesized to originate from an influence on the function of the associated Kv.
To unravel the impact of CASPR2 aAb on the VGKC complex, we analyzed structural and functional changes of the Kv channels as well as effects on the transcriptomics of treated DRG neurons. 4-5 patient sera were pooled to form four major groups: pain IgG4, pain IgGX (IgG4 + IgG1-3), no pain IgG4, and no pain IgGX.
The distance of CASPR2 to Kv1.2 was illuminated through high-resolution lattice SIM^2 microscopy, and structural alterations were discovered subsequent to presence of anti-CASPR2 aAb associated to pain.
Calcium imaging and patch clamp recordings gave insights into the functional consequences of CASPR2 aAb and showed significant alterations of DRG excitability and potassium currents after aAb treatment. Interestingly, the excitability of DRGs in calcium imaging treated with aAb associated to pain was increased, while aAb of the IgG4 only subclass caused the highest reduction of potassium current amplitudes during electrophysiological measurements.
To observe whether the effect of CASPR2 aAbs is direct and can be recovered, calcium imaging and patch clamp recording were performed on DRGs after CASPR2 aAb depletion. The current amplitude of the potassium channels was fully recovered, whereas the excitability of the DRGs remained increased. These findings suggest additional signal cascades being activated contributing to the increased excitability of the DRG neurons, even after aAb binding was released.
To better understand the pathways underlying these structural and functional alterations, DRGs were cultured in microfluidic chambers and transcriptome analysis was performed following incubation with patient sera. By comparing the transcriptome of DRGs treated with aAb groups with and without pain to a control, an upregulation of inflammatory pathways was revealed.
In sum, pain associated aAb alter the structure of the VGKC complex and the transcription profile, and cause hyperexcitability of DRGs. The significance of IgG4 subclass is unraveled by patch-clamp recordings.
This work is supported by the Deutsche Forschungsgemeinschaft KFO5001.


Healthy
Disease
aAb associated with pain
CASPR2
Kv
CASPR2 aAb withdrawal
Kv currents
Hyperexcitability
IgG4 aAb
K+
Reduced K+ currents

Experimental SAH reveals differences in CBF and CBO in distinct vascular compartments for varying injection velocities and fluids

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Background
Subarachnoid hemorrhage/SAH in rare cases is caused by non-aneurysmatic bleeding without increase in intracerebral pressure/ICP and with more benign outcome. Duration and severity of cerebral blood flow/CBF drop and tissue metabolism in SAH correlate with severity of bleeding and predict neuronal damage. Hypothesis of this study was that alterations of CBF and cerebral blood oxygenation/CBO upon injection of blood or saline at varying velocities besides of in microvessels/M are also found in arteries/Aa and veins/Vv.

Methods
In a reanalyzed dataset[1], male wistar rats (body weight 305 ± 30 g) were subjected to cisterna magna injection of either 0.5 ml blood or saline over 1, 10 or 30 min (AB1, 10 or 30, NS1 or 10). For 6 h after injection, CBF and CBO, ICP, blood pressure/BP and body temperature/T were measured. Animals were sacrificed, and histology was done to determine percentage of NeuN positive living neurons in the hippocampus. As statistics, one-way ANOVA, spearman correlation analysis and PLS in R were used.

Results
OxyHb was reduced upon AB, but increased upon NS, with stronger effects of faster injections, while for deoxyHb, the opposite was true. Total hemoglobin/HbT was reduced upon AB and NS, except for an increase in Aa after AB30. For AB1, correlations were close to significance or significant amongst CBF, oxyHb and deoxyHb. CBF and HbT among themselves were closer correlated in M and Vv compared with Aa. ICP had a strong effect on CBF and CBO in M and Vv. The correlation pattern was homogeneous for AB10. Trends towards significance or significant positive correlations were found between ICP, BP and T, and in Vv only, with HbT. Correlations of NeuN with the other parameters differed between Aa, M and Vv. In AB30, the correlation pattern was homogeneous, with values closer to significance compared with AB10. ICP, BP and T showed trends towards significance or significant positive correlations amongst each other. ICP had a strong effect on BP and CBF in Aa. In NS1, values were less close to significance compared with the other groups, including the positive correlations amongst ICP, BP and T. Patterns were heterogeneous and differed markedly between Aa, M and Vv, with a cluster of positive correlations within deoxyHb parameters in Aa, no or significant correlation within oxyHb in M, and a marked cluster of positive correlations amongst deoxyHb, CBF and HbT in Vv. ICP had a strong effect on CBF in Vv and on CBO in Aa and M. Correlations were heterogeneous in NS10. Clusters resembled each other between the three compartments, with values closer to significance in Vv. They were mostly positive within oxyHb, and amongst CBF and HbT, which were also mostly positive for the correlation with oxyHb in Aa and Vv. Correlations with NeuN were mostly far from significance. ICP had a strong effect on BP in Vv.

Conclusion
This study revealed marked variance in CBF and CBO between different injection modalities and mild alterations between vascular compartments. This aligns with the link between metabolic demand of brain tissue, CBF and its microscopic distribution by capillary perfusion. Here, also large vessels were found to be affected by alterations in CBF and CBO under varying bleeding modalities. Differences between

vascular compartments may be explained by differential vessel diameter and amount of perfused microvasculature.

Reference

[1]Conzen et. al., Transl. Stroke Res., 2019

Circular RNA circKlhl2 modulates TBI response influencing the BDNF pathway

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Traumatic brain injury (TBI) causes widespread disruption of synaptic connections and neural networks, which must be re-established for functional recovery to occur. Recently, a new class of non-coding RNA, known as circular RNA (circRNA), has been discovered and may play a role in post-traumatic plasticity and recovery. Given that circRNAs can originate from synaptic genes and appear to be involved in the modulation of synaptic biology, we hypothesize that circRNAs may contribute to synaptic disruption, regeneration, and neuronal vulnerability in TBI.

We assessed synaptic-gene circular RNA expression in hippocampus using RT-qPCR and we validated that circRNAs were significant up-regulated in whole-hippocampus extracts at different timepoints after blunt TBI. Our initial screening revealed the upregulation of circKlhl2 in neurons of the ipsilateral hippocampus of mice 7 days post TBI. To further investigate hippocampal neuronal and synaptic modulation, we performed AAV-mediated overexpression and knockdown in vivo.
Up- or downregulation of circKhlh2 had functional consequences in TBI in particular; overexpression of circKlhl2 is involved in improving hippocampal related tasks in vivo. Moreover, circKlhl2 is involved in the up-regulation of BDNF by sequestering microRNAs related to BDNF mRNA translation. Specifically, circKlhl2 acts as a molecular sponge for miR-30a-5p and miR-30d-5p, thereby regulating BDNF expression.
Finally, to monitor the effect of circKlhl2 OE/KD and its BDNF regulation in neuronal and synaptic plasticity mechanism we investigate how circKlhl2 affects synaptogenesis in vitro, a process that may be relevant to the recovery of synaptic integrity upon trauma.
In conclusion, circKlhl2, through the BDNF pathway, plays a crucial role in the hippocampal recovery processes following TBI.

Blood-brain barrier integrity and sexual dimorphisms during macrophage invasion of the Drosophila nervous system

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The central nervous system (CNS) of Drosophila can be considered as an immune-privileged organ. It is separated from the remaining body by the blood-brain barrier (BBB), which is formed by glial cells and the occluding septate junctions established between surface glial cells. The BBB allows ion and metabolite homeostasis and prevents the invasion of pathogens. Using a novel infection model we could recently demonstrate the infiltration of macrophages into the CNS across an intact BBB during early metamorphosis. Nevertheless, dye uptake experiments revealed a slight but significant increase in BBB permeability during immunity induction. This might hint to the possibility that macrophage transmigration across the BBB leads to transient opening of the otherwise closed septate junctions, and in addition suggests a paracellular route of invading macrophages. To further study how macrophages enter the brain, we initiated an electron microscopic analysis. First results support the notion of a paracellular invasion route. Macrophages come in close contact with occluding junctions of surface glial cells. We will discuss further approaches to dissect how macrophages can regulate opening of occluding septate junctions during their transmigration across the glial barrier. Interestingly, we found sex specific differences in migration across the BBB, with males being more affected than females. First experiments hint to sex specific factors within the glial cells of the BBB as a reason for different migration rates. The group of B. Dauwalder performed single cell sequencing of those cells, revealing sexual dimorphisms in transcription levels of various proteins. To decipher the underlying molecular mechanisms, we are utilizing sequencing data from male and female BBB forming glial cells. Taken together, we show that our model can be used to study especially sex dependent differences of glial cells in vivo.

Immunohistochemical investigation of the components of the blood-brain barrier in a mouse model for multiple sclerosis

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Multiple sclerosis (MS) is an autoimmune, neuroinflammatory disease affecting the central nervous system (CNS). While resulting in a wide range of symptoms, the triggers of this disorder remain unknown. Almost 2.5 million people globally suffer from MS, being affected by neuronal dysfunctions caused by demyelination, axonal loss, gliosis and neuroinflammation. Since an impairment of the olfactory sensory system could be an early warning sign of MS, the olfactory bulb (OB) is of special interest in this study.

Prior research suggests that the blood-brain barrier (BBB) is impaired in both MS and experimental autoimmune encephalomyelitis (EAE), a common animal model to elucidate the pathophysiology of the disease. Therefore, the aim of this immunohistochemical study was to detect possible changes in the integrity of the BBB by staining some of its key components in the OB in mice.

First, the morphology of microglia in EAE was evaluated by using ionized calcium-binding adapter molecule 1 (IBA1) as a marker protein to examine potential changes occurring in neuroinflammatory events. In addition, astrocytes were assessed, as they play an important role in the biochemical control of the BBB, to outline differences in the expression and distribution of their marker proteins glial fibrillary acidic protein (GFAP) and vimentin. Moreover, morphological changes in the astrocytic endfeet in the external plexiform layer (EPL) of the OB were visualized by staining the water channel aquaporin-4 (AQP4). Also, the platelet-derived growth factor receptor β (PDGFRβ) was stained, serving as a selective biomarker for pericytes. Another aim was to visualize collagen IV to mark the basement membrane of the BBB. Lastly, it was of interest if the olfactory ensheathing cells (OECs) that express S100 calcium-binding protein B (S100B) also express IBA1 in EAE, which both can be linked to neuroinflammation.

Our results showed an increase in Iba1 expression and a change in microglia morphology in EAE, indicating neuroinflammation and activation of microglia in the OB. Furthermore, an activation of astrocytes as demonstrated by the upregulation of GFAP and vimentin as a consequence of astrogliosis in EAE was especially observed in the EPL. On the contrary, when comparing AQP4 between EAE and the control group, no significant differences were visible. Also, there was no significant dissimilarity obvious in collagen IV distribution in EAE. In mild EAE it was not possible to confirm a significant smaller number of pericytes or a change in the PDGFRβ expression. Lastly, OECs that wrap axon bundles of olfactory sensory neurons that enter the OB, contribute to the BBB and are considered to promote functional recovery in neuroinflammatory events, did not express Iba1 in controls and EAE.

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Microglial Activation and Complement Dysregulation in Sepsis-Associated Encephalopathy (SAE)

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Sepsis-associated encephalopathy (SAE) is a life-threatening disease affecting more than half of sepsis survivors. The disease progresses from an acute phase with symptoms of delirium, poor concentration, agitation, and hallucinations to a chronic phase with symptoms of anxiety, depression, and cognitive deficits. So far, specific therapeutic options are not available to treat SAE.

Dysregulation of the complement system is implicated in various diseases, including neurodegenerative and autoimmune disorders. In the context of neurodegeneration, the complement system facilitates synaptic pruning through activated microglia. Previous studies done with hippocampal tissue samples from SAE patients´ autopsy and from the murine peritoneal contamination and infection (PCI) sepsis model show increased C1q-tagged synaptic pruning through increased microglial activation. We study how microglia and complement signaling are involved in SAE by using the PCI sepsis model to establish a therapeutic agent.

Our study focused on the classical pathway, which initiates the complement cascade by C1 complex formation assembling C1q, C1r, and C1s subcomponents. Experiments using the intra-hippocampal injection of a specific C1q human blocking antibody demonstrated a reduced synaptic pruning in the CA1 region in the hippocampus. We developed human blocking antibodies targeting the classical complement system. We characterized modified C1q human blocking antibodies by in vitro C3b ELISA assay. C3b ELISA assay shows that the C1q human blocking antibodies lead to reduced classical pathway activation. Furthermore, we performed stereotactic intra-hippocampal injections of modified C1q and C3b human blocking antibodies in the PCI sepsis model to evaluate their specificity and complement inhibition potency in vivo. We used high-resolution 3D airyscan imaging to assess binding of blocking antibodies by immunofluorescent stainings, and novel object recognition (NOR) tests to observe cognitive outcomes following stereotactic intra-hippocampal injections of complement blocking antibody.

In conclusion, complement mediated microglial synaptic pruning is a crucial molecular pathomechanism in SAE. By targeting complement proteins, we can prevent neurocognitive alterations. Moreover, complement pathway-blocking antibodies can be a powerful tool for therapeutic purposes in SAE.

Macrophage invasion into the Drosophila brain requires JAK/STAT dependent MMP activation in the blood-brain barrier

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The central nervous system is well separated from external influences by the blood- brain barrier. Upon surveillance, infection or neuroinflammation, however, peripheral immune cells can enter the brain where they often cause detrimental effects. To invade the brain, immune cells not only have to breach cellular barriers, but they also need to traverse associated extracellular matrix barriers. Neither in vertebrates nor in invertebrates is it fully understood how these processes are molecularly controlled. We recently established Drosophila melanogaster as a model to elucidate peripheral immune cell invasion into the brain. Here we show that neuroinflammation leads to the expression of Unpaired cytokines that activate the JAK/STAT signaling pathway in glial cells of the blood-brain barrier. This in turn triggers the expression of matrix metalloproteinases enabling modulation of the extracellular matrix enclosing the fly brain and a subsequent invasion of immune cells into the brain. Our study demonstrates conserved mechanisms underlying immune cell invasion of the nervous system in invertebrates and vertebrates and could, thus, further contribute to understanding of JAK/STAT signaling during neuroinflammation.

C3 and CD14 modulate diffuse but not focal neuroinflammation in TBI associated with polytrauma

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Traumatic brain injury (TBI) rarely occurs in isolation and often presents alongside other severe injuries, collectively known as polytrauma, which frequently involves hemorrhagic shock. In this study, we used a complex polytrauma model, including TBI, thoracic trauma, bone fractures, and hemorrhagic shock, to investigate the early brain response to polytrauma. Complement factor C3 and the TLR cofactor CD14 are of particular interest due to their key roles in the immune response. C3 is a central protein in the complement system that is essential for amplifying immune signals and driving inflammation, whereas CD14 acts as a co-receptor for Toll-like receptors (TLRs), facilitating the recognition of tissue damage signals and enhancing inflammatory responses. Both are critical for the regulation of neuroinflammation after traumatic injury.

We first conducted a cytokine-focused array three hours post-polytrauma to identify differentially expressed cytokines in sham and polytrauma mice. We then assessed transcriptional responses across different brain regions using qPCR and in situ hybridization, with a focus on both focal and diffuse areas. We also investigated how the absence of C3, CD14, or both influences cytokine responses. Furthermore, in situ hybridization was used to examine TNF-α; production in the neurovascular unit and astrocytic transcriptional responses.

Our study identified a rapid and widespread increase in the levels of the pro-inflammatory cytokine TNF-α; across the brain following polytrauma, which was confirmed via transcriptional analysis and in situ hybridization. TNF-α; was notably elevated in injured, contra-coup, and diffuse brain regions. Using C3 and CD14 knockout models, we observed that their deletion significantly reduced TNF-α; and CCL2 expression in diffuse areas, but had no effect on cytokine levels at the injury site. To explore the mechanisms driving this diffuse neuroinflammation, we focused on the neurovascular complex, hypothesizing its vulnerability to cytokine exposure. In situ hybridization showed that TNF-α; mRNA was concentrated near blood vessels in polytrauma samples from wild-type animals. Further analysis of astrocyte reactivity revealed that GFAP expression, a marker of astrogliosis, was elevated in both diffuse and focal regions. Interestingly, C3 and/or CD14 deletion abolished GFAP upregulation in diffuse regions but had a limited effect on focal areas. Additionally, Emp1 and S100A10, markers of anti-inflammatory astrocytes, were upregulated in diffuse areas of knockout animals, emphasizing the role of CD14 in modulating astrocyte responses.

Our findings indicate that complement factor C3 and TLR cofactor CD14 play significant pro-inflammatory roles in diffuse brain inflammation following polytrauma, while their effects appear limited to focal injury sites. Targeting these pathways using specific inhibitors may offer a promising therapeutic approach for mitigating diffuse brain damage and enhancing recovery outcomes.

Effects of the cannabinoids 2-Arachidonylglycerol and WIN 55,212-2 on primary isolated astrocytic cultures and astrocytic-microglial co-cultures

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Various cannabinoids have been shown to mediate neuroprotective and anti-inflammatory effects via microglia and astrocytes. After neuronal injury or inflammatory events, subsequent activation of microglia leads to their morphological and functional changes. The release of proinflammatory cytokines and the stimulation of microglia and astrocytes exacerbate the neuronal damage.
The aim of this study was to evaluate the effects of co-incubation of the neuroprotective cannabinoids WIN 55,212-2 (WIN, a synthetic cannabinoid) and 2-Arachidonylglycerol (2-AG), the most abundant endogenous cannabinoid in the brain, on glia cell response.
Primary astrocytic and microglial co-cultures and astrocytic cultures from newborn WISTAR rats were treated with WIN and 2-AG alone or in combination. Astrocytic wound healing was assessed in a scratch-wound assay by live cell imaging. Furthermore, the effects of cannabinoids were investigated on morphological changes of glia cells. To study the effects on proliferation, cell cycle analysis was performed by flow cytometry. Lastly, the expression of pro- and anti-inflammatory cytokines on mRNA level and in addition the nitrite oxide secretion were analyzed after LPS stimulation and treatment with cannabinoids.
We observed that the cannabinoid WIN decreased the mean speed of astrocytic wound closure in mixed glial cultures and in isolated astrocytic cultures. 2-AG alone and the combination of WIN and 2-AG showed no alteration of the mean speed. The morphological changes in microglia were evaluated. Cannabinoid WIN alone reduced the LPS-mediated increase in nitrite oxide secretion in astrocytic and microglial co-cultures while 2-AG alone and the co-incubation of WIN and 2-AG had no decreasing effect.
The results of this study will provide an insight to molecular and cellular mechanisms of microglia and astrocytes and their interactions in response to exogenous cannabinoids.

Modulation of glial inflammatory reactions by GPR55

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The mechanisms of neuroinflammation play a crucial role in the pathogenesis of various neurodegenerative diseases. Cannabinoids have been shown to be protective by affecting the activation state of microglia, nevertheless, the underlying molecular mechanism are still poorly defined. Modulating the activity of the orphan cannabinoid receptor Gpr55 was shown to influence inflammation in vivo and to exert neuroprotection in lesioned organotypic hippocampal slice cultures.
In the present study primary astrocytic and astrocytic-microglial mixed cultures were used and their response to stimulation with Gpr55 ligands CID 16020046 and Cannabidiol (CBD) was evaluated. The presence of gpr55 was proven by using real-time quantitative PCR and toxic effect of both ligands examined with MTT assay and propidium iodide staining. Proliferation and nitric oxide secretion were measured in presence or absence of Gpr55 antagonists. The influence on cell proliferation was verified with BrdU staining. Since inducible nitric oxide synthase (iNOS) is responsible for nitric oxide production under inflammatory conditions, the iNOS expression at mRNA and protein levels was investigated at different time points by PCR and Western Blot technique.

The expression of gpr55 at the mRNA level revealed approximately 19-fold higher expression in astrocytes compared to microglia and both cannabinoids showed no toxic effects. BrdU staining demonstrated that pre-incubation with CID 16020046 significantly diminished the number of BrdU-positive cells in comparison to LPS alone. Moreover, an LPS-mediated increase in NO production was significantly reduced by pretreatment (5-60 minutes) with CID16020046 and CBD in mixed astrocytic-microglial cultures but not in primary astrocytes.

These results provide evidence for the involvement of Gpr55 in various aspects of microglial and astrocyte activation in the context of an inflammatory response. Gpr55 thus may represent a promising target for the development of therapeutic concepts in neurodegenerative diseases with a neuroinflammatory background.

Poster Topic

T13: Cognitive, Emotional, Behavioral State Disorders and Addiction

T13-1A Investigating Behavioural Outcomes of Early Life Stress: Insights from a rodent model
Luna Strauch, Pinja Hillman, Claudia Böhm

T13-2A Adolescent-specific acceleration of social sear extinction through social reward system
Sukwon Lee

T13-3A High Consumption of L-Proline Induces Depression-like-Behavior in *Drosophila melanogaster*
Josefine Hoffmann, Burkhard Poeck, Roland Strauss

T13-1B Oxidative and Chronic Mild Stress Induce Depression-Like Behavior in *Drosophila melanogaster*
Helen Marie-Antoinette Holvoet, Burkhard Poeck, Roland Strauss

T13-2B Consequences of adolescent social trauma on social behaviour and neuronal circuitries
Melanie Kabas, Leopold Kinzel, Anna Bludau, Inga D. Neumann

T13-1C Impact of stress and depression on the regulation of actin-binding proteins in the murine hippocampus
Constanze Wenzel, Jonas Cornelius, Kristin Michaelsen-Preusse, Martin Korte

T13-2C The Impact of Social Buffering on Modulating Social Fear: Behavioral and Sex-Specific Insights in Mice
Elif Salur, Iulia Zoicas, Angelika Schmitt-Böhrer

T13-1D Variable light exposure differentially alters midbrain dopamine expression and behaviors in a rodent model of depression
Xiongpeng Weng, Volker Arnd Coenen, Máté Daniel Döbrössy

T13-2D Acute modulation of neuronal networks by medial forebrain bundle DBS in an animal model of depression: Focus on gamma oscillations
Artur Fornol, Lisa Ratz, Yixin Tong, Joana Pereira, Lidia Miguel Telega, Volker Arnd Coenen, Máté Daniel Döbrössy

Investigating Behavioural Outcomes of Early Life Stress: Insights from a rodent model

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Early life adversity (ELA) is one of the main risk factors for the development of psychiatric disorders, yet the neuronal mechanisms linking these experiences to long-term psychiatric vulnerability remain largely unknown. Here we investigate the effects of ELA using the limited bedding and nesting (LBN) model in rodents, aiming to uncover potential biomarkers for stress vulnerability and resilience.

The LBN model induces early life stress by limiting the dam's access to nesting material during the early postnatal period. These conditions have been described to cause erratic maternal behaviour, exposing the pups to a stressful and unpredictable environment early in their life. To assess the behavioural consequences of these early experiences later in life, we employ an array of behavioural tasks, including the Open Field Test (OFT), Elevated Plus Maze (EPM), Novel Object Recognition (NOR), a social interaction task, and a two-alternative forced choice (2AFC) learning task.

Preliminary behavioural analysis revealed no significant differences in anxiety-like behaviours as measured by time spent in the centre during the OFT or in the closed arms of the EPM. During NOR, memory performance as indicated by more time spent with the novel object, was highly variable across animals, in particular in the LBN group.

Upcoming experiments will further examine the behavioural impact of these and other behavioural tasks, particularly the 2AFC learning task and social interaction assays. Moreover, we will use automated tracking of video recordings to perform detailed analyses of interactions between the dam and the pups during the stress phase.
Furthermore, we plan to integrate large-scale neuronal recordings using Neuropixels probes in the prefrontal cortex to examine neural correlates of behaviour during these tasks. Molecular analyses, such as corticosterone measurements, will complement these investigations to provide a more comprehensive understanding of how early adversity influences both behaviour and neural activity.

Adolescent-specific acceleration of social sear extinction through social reward system

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Social anxiety disorder (SAD) is a mental health condition triggered by traumatic social experiences, characterized by intense fear and avoidance of social situations. Individuals with SAD struggle to form normal social relationships due to severe social anxiety, making effective treatment essential for societal well-being. Despite the clinical importance of understanding and treating SAD, the absence of a robust animal model has hindered in-depth research into its mechanisms.
In this study, we developed an animal model to investigate the extinction of social fear and explored the underlying mechanisms. Our results show that adolescent mice exhibit an accelerated extinction of social fear compared to adult mice. However, no differences were observed in the reduction of sociality induced by social fear conditioning between the two age groups, adolescence and adult. Interestingly, we found that the magnitude of social reward was significantly increased following extinction, but only in adolescent mice. This enhanced social reward was notably higher than that observed in the control group, which did not undergo social fear conditioning.
Together, these findings suggest that the accelerated extinction of social fear in adolescence may be driven by an age-specific increase in social reward processing.

High Consumption of L-Proline Induces Depression-like-Behavior in *Drosophila melanogaster*

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Chronic, uncontrollable stress can result in major depressive disorders, which is one of the most prevalent health conditions worldwide. Animal models can help to elucidate the fundamental neurobiological mechanisms underlying this disorder aiming to improve therapeutic strategies. We developed a chronic stress paradigm, which subjects *Drosophila melanogaster* flies to 3 days of repetitive phases of 300 Hz vibrations combined with overcrowding, food and water deprivation, as well as disruption of day time sleep. This treatment induces a depression-like state (DLS), which is characterized by reduced voluntary behavioral activity, including the motivation to climb a just insurmountable gap (risk taking) and to stop for sweets (anhedonia). These behavioral changes are accompanied by decreased serotonin release to the α-lobes of the mushroom body (MB), a major center for associative learning and memory in the adult brain. The DLS can be relieved by feeding the serotonin precursor 5-HTP, the selective serotonin reuptake inhibitor (SSRI) fluoxetine or a 5% sucrose solution (Ries et al. 2017; Hermanns et al. 2022). Feeding sucrose activates serotonin release from the dorsal paired medial (DPM) neurons that innervate the MB (Hermanns et al. 2022). The influence of the microbiome-brain-axis has emerged as a novel topic in etiology of depression, which brings diet into the focus of this line of research. Microbiome and metabolome analysis of patients and mice suggested that increase levels of L-proline as a risk factor in the development of depressive disorders; and high consumption of the proteinogenic amino acid L-proline exacerbates depressive-like-behavior in mice (Mayneris-Perxachs et al. 2022). Here we show that L-proline supplementation also increases sensitivity to vibration stress in *Drosophila*. Remarkably, L-proline reduces climbing attempts significantly even without stress application, when fed in high concentrations. Similar to the DLS induced by vibration stress, the proline-induced DLS lasts for at least 7 days and can be relieved by feeding 5-HTP or fluoxetine. However, feeding 5% sucrose does not ameliorate proline-induced DLS, suggesting that L-proline interferes with the signaling pathway of sugar relief. L-proline reduces GABAergic transmission by inhibiting the GABA synthesizing enzyme glutamate decarboxylase 1 (GAD1; Crabtree et al. 2016). Our data show that reducing GABA production in DPM neurons (co-transmitting 5-HT and GABA) makes flies more sensitive to vibration stress. Furthermore, knockdown of the proline-transporter Slc6a20 in DPM neurons conveys resilience to the proline-induced DLS. These results suggest that L-proline is transported into DPM neurons, where it blocks GAD1 thereby enhancing sensitivity to stress by reducing GABA-ergic transmission. Further research will focus on identifying dietary factors, that are associated with depression, and investigating their specific signaling pathways to improve therapeutic strategies.

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Oxidative and Chronic Mild Stress Induce Depression-Like Behavior in *Drosophila melanogaster*

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Chronic and uncontrollable stress has been linked to various psychiatric disorders, including anxiety and depressive disorders, in both humans and animals. In *Drosophila melanogaster*, prolonged exposure to chronic mild stress induces depression-like behavioral changes, alongside molecular alterations in biogenic amine levels in the brain. To study this, we developed a chronic mild stress model involving three days of repeated short periods of 300 Hz vibrations coupled with overcrowding, disrupted daytime sleep and food deprivation. This treatment significantly reduces the motivation to engage in voluntary behaviors, such as trying to climb an insurmountable gap (risk-taking) or stopping at a sweet-tasting strip (anhedonia), indicating a depression-like state (DLS). These observed behavioral changes are associated with reduced serotonin release to the α-lobes of the mushroom body (MB), a critical center for sensory processing, learning and memory in the fly's central brain. Remarkably, the behavioral deficits can be alleviated by feeding the flies 5% sucrose solution, serotonin precursor 5-HTP, a selective serotonin re-uptake inhibitor (SSRI), or the mood stabilizer lithium chloride (LiCl), over-night among others (Ries et al. 2017; Hermanns et al. 2022). Resilience to the DLS can also be induced by prophylactically supplementing fly food with phytopharmaceuticals before subjecting flies to the stress treatment (Holvoet et al. 2022; Holvoet et al. 2023). Furthermore, we investigate oxidative stress as an alternative stressor, induced by either menadione sodium bisulfite (Jordan et al. 2012) or hydrogen peroxide, and provide further insight into the mechanisms underlying the DLS. We demonstrate that oxidative stress induces phenotypes similar to those of the vibration-induced DLS across various behavioral paradigms. Like the vibration-induced DLS, oxidative stress acts locally within the α/β Kenyon cells of the MB. Additionally, oxidative stress increases neuronal signaling in the MB's γ1/pedc region. Despite these similarities, oxidative stress differs from the vibration-induced DLS at certain levels. For instance, the DLS caused by oxidative stress cannot be rescued by applying 5% sucrose post-stress, suggesting potential damage in the sucrose signaling pathway which includes the appetitive dopaminergic PAM neurons (Hermanns et al. 2022). Future studies will focus on uncovering the distinct mechanisms underlying different stressors and their associated signaling pathways to deepen our understanding of the mechanisms underlying stress-induced behavioral changes.

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Consequences of adolescent social trauma on social behaviour and neuronal circuitries

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Adolescence is a critical developmental phase characterised by high susceptibility to mental disorders, including social anxiety disorder (SAD). Patients suffering from SAD experience intense fear and avoidance of social interactions. These symptoms can be effectively mimicked in adult male and female mice by applying the social fear conditioning (SFC) paradigm. This model of SAD is based on operant conditioning principles, as mice receive a mild electric foot shock upon interaction with a social stimulus (SFC^+), whereas controls can interact freely without punishment (SFC^-). Notably, the evoked social avoidance can be reversed by oxytocin, a neuropeptide with pro-social and anxiolytic attributes, specifically within the lateral septum. In a similar context, oxytocin signalling in the ventromedial hypothalamus (VMH) is crucial for the learning of social avoidance induced by social defeat. However, the precise role of the VMH and the oxytocin system in modulating social fear acquired during adolescence remains to be elucidated.
Here, I studied the acute and persistent effects of adolescent social trauma on social behaviour and the oxytocin system in male and female mice. For this, adolescent mice underwent the SFC paradigm. Either 1d or 28d post acquisition they were exposed to a sex- and age-matched conspecific. Interestingly, although both male and female SFC^+ mice expressed social fear 1d post-acquisition, only male mice still showed social avoidance after 28d and even 56d. Utilizing receptor autoradiography, we revealed reduced oxytocin receptor (OXTR) binding in the VMH of male SFC^+ (compared to SFC^- controls) mice after exposure to a social stimulus 28d post-acquisition. Moreover, we found oxytocinergic fibers near OXTR-positive cells in the ventrolateral VMH using immunofluorescent staining and RNAscope. Some of the oxytocinergic inputs in the VMH originated from the paraventricular nucleus of the hypothalamus (PVN) as identified by retrograde tracing. Thus, our findings suggest that adolescent social trauma induces alterations of the oxytocin system, particularly within the PVN-VMH axis, which might contribute to the persistence of social fear in male mice. However, no alterations in the activation of oxytocinergic PVN cells in response to social exposure were found after adolescent SFC. The activation of VMH cells regarding potential conditioning- or sex-differences in this paradigm is currently under evaluation. To investigate the functional impact of oxytocin within the VMH on social fear we bilaterally infused oxytocin into the VMH of adult male SFC^+ and SFC^- mice 10min prior to social fear extinction. However, we did not reveal any effects on social investigation. Similarly, chemogenetic activation of VMH OXTR-positive cells did not affect social fear extinction 28d after adolescent SFC acquisition.
In conclusion, exposure to adolescent social trauma induces strong social fear in male and female mice, which lasts into adulthood only in male mice and is accompanied by alterations in OXTR binding within the VMH. However, our most recent findings do not support the hypothesis that the oxytocinergic PVN-VMH circuit is involved in the regulation of social fear acquired during adolescence.

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Impact of stress and depression on the regulation of actin-binding proteins in the murine hippocampus

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Chronic stress is known to be a main risk factor for major depressive disorder (MDD). Under physiological conditions, stress triggers the release of hormones like cortisol (humans) or corticosterone (rodents) mediated by the hypothalamus-pituitary-adrenal (HPA) axis, which prepare the body for a "fight or flight" response. While this reaction is beneficial in short-term situations, chronic stress can lead to a persistent overproduction of these hormones. This prolonged exposure can disrupt various functions contributing to the development of MDD.

Chronic stress was shown to lead to shrinkage of the hippocampus, a brain region important for memory formation. The overproduction of stress hormones affects the hippocampus also especially at the cellular level by reducing neurogenesis and inducing spine shrinkage and spine loss. This neurological pathology is associated with cognitive deficits and emotional imbalance, which are common symptoms of major depressive disorder. Given its crucial role in maintaining neuronal structure and function, so far, the actin cytoskeleton appears to be a neglected component in the pathology of MDD.

This research aims to elucidate the molecular mechanisms by which chronic stress affects hippocampal neurons, with a focus on the actin cytoskeleton as key to synaptic changes. Our investigation focuses on analyzing specific actin-binding proteins linked to MDD pathology by exploring how stress-induced changes in signaling pathways and protein expression contribute to neuronal damage and reduced neurogenesis.

Chronically restraint mice showed impaired learning in the Morris Water Maze and dendritic spine loss. Moreover, these mice showed depressive-like behavior as they spent more time in the dark compartment of the dark-light box. Expression level analysis revealed that caldesmon expression was slightly decreased after chronic restraint stress. Current experiments aim to analyze the F-actin/G-actin ratio in the hippocampus and the properties of stress engram neurons.

This understanding could facilitate the development of more effective treatments that target underlying cellular and molecular causes of major depressive disorder.

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The Impact of Social Buffering on Modulating Social Fear: Behavioral and Sex-Specific Insights in Mice

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The conflict between the comforting and threatening aspects of social interactions shapes human behavior. On the one hand, social connections have profound stress-reducing effects, alleviating anxiety, depression, and even physical pain—a phenomenon called social buffering. On the other hand, for individuals with social anxiety disorder, social encounters are often experienced as sources of fear and avoidance. Social anxiety disorder, the most common anxiety disorder, affects 12–16% of the population, with women being disproportionately affected, experiencing the condition at rates 1.5 to twice as high as men. A defining feature of this disorder is the ongoing conflict between the desire for social connection and the fear that such interactions provoke.

Understanding the neurobiological basis of social fear requires well-validated animal models. The social fear conditioning paradigm in rodents offers such a model, replicating key features of social anxiety disorder. In this model, mice learn to associate the approach of an unfamiliar conspecific with mild footshocks, resulting in learned avoidance of social contact. Although social buffering has been shown to reduce anxiety in various contexts, its role in alleviating social fear induced by social interactions remains underexplored.

In this study, we examine the role of social buffering in mitigating social fear while exploring potential sex differences in its effects. Using male and female C57BL/6J mice, we implement tactile, visual, and olfactory forms of social buffering to assess changes in social investigation behavior following social fear conditioning. Our initial findings reveal that males in the control group exhibited reduced social approach behavior and shorter investigation times compared to females. Following social buffering, both sexes displayed increased social investigation during fear extinction, with the most pronounced effects occurring in the early trials. Interestingly, males appeared to derive greater anxiety-relieving benefits from social buffering than females.

These results emphasize the significance of social buffering in modulating socially conditioned fear responses and highlight the importance of investigating sex-specific variations. By advancing our understanding of the interplay between social behavior, fear, and sex differences, this work offers insights that may contribute to the development of more effective and personalized treatments for social anxiety disorder.

Variable light exposure differentially alters midbrain dopamine expression and behaviors in a rodent model of depression

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Background: Depression is a major global health issue. Light exposure has been shown to alleviate depressive symptoms, and the ventral tegmental area (VTA), particularly its dopamine neurons, is strongly linked to depression. However, whether the VTA plays a role in the mechanism by which light alleviates depression remains unclear.

Method: Flinders Sensitive Line (FSL, n=58) and Spague-Dawley (SD, n=65) controls were used in the study. Initial force swimming tests were conducted to phenotype the animals, followed by open field test (OFT), elevated plus maze (EPM), and sucrose consumption test (SCT) to establish a baseline. FSL and SD rats of both sexes were assigned to one of three light conditions: Light+ (19 hours of light, 5 hours of darkness), control (12 hours of light, 12 hours of darkness), and Light- (5 hours of light, 19 hours of darkness), forming 12 total groups (n= 9-16 for each group). Animals were exposed to the light conditions for 2 weeks, during which their locomotor activity, and body weight were continuously recorded. After the 2-week period, OFT, EPM, and SCT were repeated, and the rats were perfused with paraformaldehyde. (PFA), and brain tissues were processed for DAB staining to analyze the VTA and its Tyrosine Hydroxylase (TH) expressing dopamine neurons.

Results: Changing light exposure conditions affected both the locomotor activity and body weight in rats. Light exposure led also to differences in behavioral tests such as the EPM, SCT and OF. Additionally, the light conditions differentially affected midbrain TH expression across the experimental groups: for example, the FSL animals had reduced VTA TH expression under both conditions, but significantly more under the extended dark conditions (Light-).Detailed description of the findings – including differences across the experimental groups and sexes - will be provided in the poster.

Conclusion: Altering light exposure conditions can modify the characteristics of dopamine neurons in the VTA and changes in rat behavior. The FSL animals showed a stronger, more significant response, potentially due to being more sensitive to anxiogenic conditions.

Acute modulation of neuronal networks by medial forebrain bundle DBS in an animal model of depression: Focus on gamma oscillations

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Major depressive disorder (or depression) significantly reduces quality of life, with up to 30% of patients classified as treatment-resistant. Deep brain stimulation (DBS) of the superolateral medial forebrain bundle (slMFB in humans, mfb in rodents), as an experimental therapy, has demonstrated rapid antidepressant effects in both clinical and preclinical studies. However, the underlying mechanisms remain elusive. Gamma oscillations (30-100 Hz), driven by the synchronized activity of GABAergic interneurons, have been connected to states of depression. Recent research found that mfb DBS notably increased low gamma activity in the medial prefrontal cortex (mPFC) of Flinders Sensitive Line (FSL) rats, a rodent model of depression, suggesting that GABAergic interneurons may play an important role in the mechanism of action of DBS.

In this study, we aimed to explore how an acute stimulation protocol, featuring three sessions of 130Hz mfb DBS lasting 30 minutes each, affects neuronal oscillations and gene expression related to GABAergic activity. Using FSL rats as a depression model and Sprague-Dawley (SD) rats as controls, we performed electrophysiological recordings under ketamine-xylazine anesthesia in the mPFC, nucleus accumbens (NAc), and ventral tegmental area (VTA), the key regions of the reward circuitry, following bilateral mfb DBS. Following the recordings, brain tissues were collected for quantitative PCR (qPCR) analysis.

Our results revealed significant increases in high beta (21–30Hz) and more pronounced, low gamma (30–48Hz) oscillations in the VTA of FSL rats compared to SD rats during mfb DBS. A similar trend was observed in the NAc. In the mPFC, we observed high variability among FSL rats, with some animals exhibiting strong increases in low gamma oscillations, while a subgroup showed the opposite response, which could indicate interindividual biological differences within this model modulating treatment response. Additionally, we noted a decrease in low gamma power across all regions in the FSL group over the course of the three DBS sessions, which suggests a potential habituation effect specific to the depression model. Additionally, whether the modulation of gamma oscillations is related to changes in the expression of specific GABAergic markers, such as parvalbumin, is still under investigation by using qPCR.

In conclusion, our findings indicate that acute repetitive mfb DBS modulates gamma oscillations in brain circuits significantly implicated in the pathophysiology of depression, with potential effects on GABAergic interneurons. By elucidating the underlying mechanisms through our electrophysiological and molecular analyses, we propose potential novel stimulation strategies for antidepressant treatments.

Poster Topic

T14: Vision: Invertebrates

T14-1A Multi-scale analysis of swarm initiation and collective behavior in locusts.
Daniele Carlesso, Sercan Sayin, Vishwanath Varma, Iain D. Couzin, Einat Couzin-Fuchs

T14-2A Sensory and Cognitive Rules of Locust Collective Motion
Sercan Sayin, Einat Couzin-Fuchs, Inga Petelski, Mohammad Salahshour, Chi-Yu Lee, Jacob M. Graving, Liang Li, Oliver Deussen, Gregory A. Sword, Iain D. Couzin

T14-3A Differential feature extraction in first order visual interneurons is achieved via distinct cellular and circuit properties
Neel Wagh, Katja Sporar, Junaid Akhtar, Marion Silies

T14-4A Influence of temperature on motion processing in the central brain of bumblebees
Bianca Jaske, Keram Pfeiffer

T14-1B Degenerate connectivity explains functional properties of visual circuitry.
Juan Felipe Vargas Fique, Sebastian Molina-Obando, Marion Silies

T14-2B Characterizing Navigational Strategies in *Drosophila* in Response to Varying Visual Stimuli
Romita Trehan, Hannah Julia Martina Haberkern

T14-3B Neural pathways and computations that achieve stable contrast processing tuned to natural scenes.
Burak Gür, Luisa Ramirez, Jacqueline Cornean, Freya Thurn, Sebastian Molina-Obando, Marion Silies

T14-4B Heterogeneity of synaptic connectivity in the fly visual system – causes and consequences
Jacqueline Cornean, Lena Lörsch, Sebastian Molina-Obando, Marion Silies

T14-1C Linking visual system anatomy to neuronal function in the *Drosophila* motion-detection system
Pradeepkumar Trimbake, Camille Guillermin, Miriam Henning, Marion Silies

T14-2C Emergence of functional diversity in the peripheral visual pathway for the encoding of naturalistic stimuli
Luisa Ramirez, Marion Silies, Julijana Gjorgjieva

T14-3C Neural mechanisms for a stable head direction estimate in dynamic, naturalistic visual environments
Hannah Julia Martina Haberkern, Shivam Chitnis, Marcella Noorman, Philip Hubbard, Tobias Goulet, Ann Hermundstad, Vivek Jayaraman

T14-1D Linking environmental structure and social behavior: a case study on the development of social phenotypes in the desert locust
Madhansai Narisetty, Sercan Sayin, Yvonne Hertenberger, Ahmed El Hady, Einat Couzin-Fuchs

T14-2D Spatio-chromatic visual processing in *Drosophila*
Roshni Pillai, Julia Maria Strauß, Marion Silies, Christopher Schnaitmann

T14-3D The Desert Locust Startle Response: Linking Descending Neurons and Behavioral Dynamics
Hannes Kübler, Yannick Günzel, Einat Couzin-Fuchs

Multi-scale analysis of swarm initiation and collective behavior in locusts.

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The desert locust, a form of grasshopper, are notorious plague insects. Swarms of locusts can extend over several hundred square kilometers with up to 80 million individuals per km2, posing severe threats to global food security. However, locusts do not always swarm. When food is abundant and population density is low, individuals remain cryptic and sedentary ‘solitarious’ grasshoppers. Population upsurges and swarming arise when food scarcity in highly populated areas forces locusts to aggregate around limited resources, causing a transition to the “gregarious” swarm-forming phase. Although behavioral and physiological changes associated with crowding have been widely documented, the biological mechanisms mediating the initiation of swarming and collective migrations are not well understood.

We combine behavioural assays, virtual reality and large-scale laboratory experiments to investigate when and how collective locust marching emerges. We use motion capture technology to continuously monitor the behaviour of solitary locusts during their first exposure to marching bands of gregarious conspecifics for several days. Preliminary findings suggest that, while rapid changes in response to conspecifics can be observed within a few hours, these do not appear sufficient to drive persistent phenotypic changes. Instead, solitary locusts displayed marching-like behaviour only after a few days of crowding. Complementary experiments in virtual reality, where individual locusts are embedded within immersive, responsive, and photorealistic virtual swarms with predefined statistics, provide further insights into how changes in individual interaction maps and kinematics can lead to the formation of coordinated marching events.

Sensory and Cognitive Rules of Locust Collective Motion

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A voracious appetite combined with a high reproductive rate creates a recipe for explosive growth, destined to eventual population collapse once local resources are depleted. Desert locusts escape this Malthusian trap through infamous plagues characterized by long-distance juvenile marching and adult flying swarms. Despite the sustained interest in and socio-ecological impact of locust invasions, the remoteness of swarm locations and limitations of laboratory experiments have, until now, hindered a mechanistic understanding of locust collective behavior.

We addressed this knowledge gap by combining field experiments, panoramic virtual reality, and large-scale behavioral experiments. Sensory deprivation manipulations conducted in Kenya in 2020 revealed the primacy of vision. The analysis of locust behavior immersed in realistic virtual locust bands showed that marching is a product of conspecific pursuit behavior, which was corroborated in arena experiments tracking 2000 locusts in a behavioral arena. Finally, we established that this pursuit behavior is governed by a cognitive process underpinned by a ring-attractor neural model.

We now further delineate the sensory basis of this visual pursuit behavior and consider trade-offs of individual fitness in a collective context. Taken together, our work serves as a keystone for future molecular and physiological studies of locust migrations and, by extension, collective behavior in general.

Differential feature extraction in first order visual interneurons is achieved via distinct cellular and circuit properties

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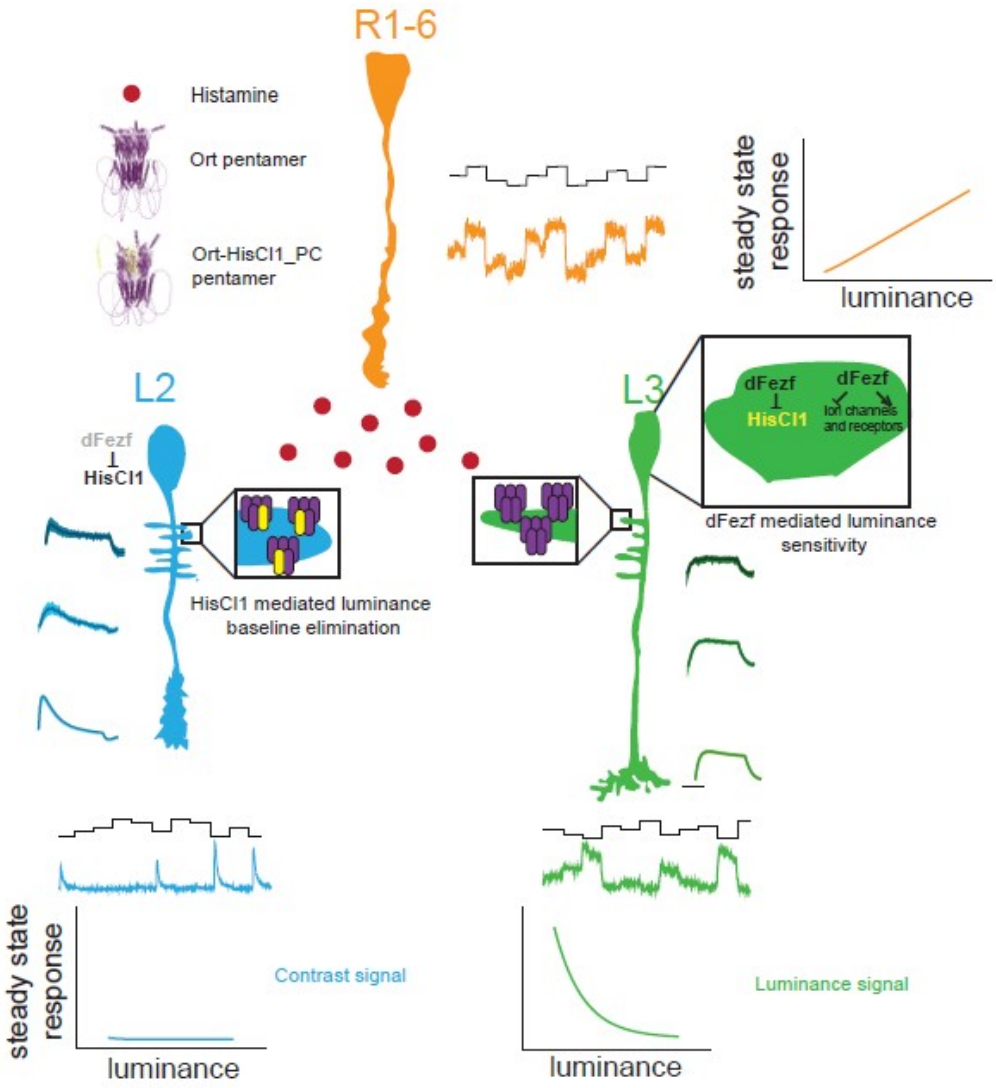
A key function of the visual system is to extract behaviorally relevant features of the visual scene. In the visual system, the stable computation of contrast requires two circuit components: a contrast-sensitive pathway that responds to changes in the visual scene and a luminance-sensitive pathway that corrects contrast computation via a fast luminance gain. In Drosophila, these two pathways diverge downstream of the same histaminergic photoreceptor input in two first-order interneurons: contrast-sensitive L2 and luminance-sensitive L3 neurons. How these two neurons obtain such fundamentally different properties despite receiving the same input is unknown. Here we show that luminance sensitivity in L3 depends on the transcription factor dFezf. In vivo two-photon calcium imaging of dFezf-mutant L3 neurons display physiological properties resembling those of contrast-sensitive L2 neurons. Using cell-type-specific RNAseq we identified differentially expressed ion channels and receptors in L2 vs L3 which are regulated by dFezf. One of the top candidates was the histamine-gated chloride channel HisCl1. To date, lamina neurons were thought to respond to their photoreceptor input via another histamine-gated chloride channel, Ort. Genetic analysis revealed that HisCl1 participates in the elimination of the luminance-sensitive component in L2 neurons, and other genes downstream of dFezf also contribute to establishing L2 properties. Together, our data suggest that distributed coding mechanisms, involving functionally overlapping channels, are at play to ensure robust electrophysiological features. In addition to these distinct cell-autonomous properties of L2 and L3, L2 properties also in part depend on lateral circuitry, as shown by genetic and pharmacological-based approaches. Glutamatergic inhibition mediates the elimination of a luminance-sensitive component in L2 neurons, which is mediated by the glutamate-gated chloride channel GluClα. In summary, our study reveals the cellular and circuit mechanisms underlying the divergent synaptic properties that ultimately achieve behaviorally relevant computations.



Graphical Abstract

Influence of temperature on motion processing in the central brain of bumblebees

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Honeybees and bumblebees can regulate their body temperature independently of ambient temperature (Heinrich, 1974, Science). Nevertheless, there is only a limited number of studies investigating the effects of temperature on sensory processes and neuronal coding. In bumblebees, photoreceptor responses are faster at higher temperatures independent of whether temperature is actively increased during walking or passively due to an external heat source (Rother and Müller et al., 2023, Proc. R. Soc. B). Likewise, in blowflies, photoreceptors (Tatler et al., 2000, J. Comp. Physiol. A) and visual interneurons (Warzecha et al., 1999, J. Exp. Biol.) show faster responses at higher temperatures. Here we investigated the effect of temperature on the tuning of wide-field motion-sensitive neurons in the central brain of bumblebees. Using tetrodes we examined responses to gratings with different velocities (range: 112 °/s to 2705 °/s) under different temperature conditions (range: 23°C to 32°C). While for most neurons an increased head temperature led to a shift of the sensitivity maximum to higher stimulus velocities, for some neurons it remained stable. In some other cases the sensitivity maximum was only affected for one of the movement directions. These different response types might serve different behavioural functions. Neurons that are involved in the control of self-motion might benefit from temperature dependent response properties. Faster forward movement requires a faster processing of visual cues. Nonetheless, other behaviours that rely on optic flow (e.g. measuring distance travelled) require a robust encoding of optic flow information. Hence, neurons that are responding independently of temperature are necessary. Because the underlying biochemical processes are temperature-dependent, mechanisms to compensate for temperature effects and enable a robust encoding are required.

Degenerate connectivity explains functional properties of visual circuitry.

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Visual systems robustly encode visual features under changing contexts, and in the laboratory, visual behaviours are also robust to many genetic disruptions. This argues for redundancy or degeneracy in visual circuits. However, it is not well understood how circuit architecture relates to such robustness. At the level of the connectivity of a network, degeneracy is considered to be a non-repetitive configuration of connections that enables reliable information transmission even when some neurons or connections are missing[1]. In the *Drosophila* visual system, neurons are extensively connected in complex circuits that can potentially support the robustness of visual computations. For example, the core network that computes the direction of movement of bright edges (ON-DS) has been described as a simple circuit motif having the motion sensitive neuron T4 as output[2]. Nevertheless, the connectome shows many more connections than necessary to compute ON-DS[2]. We thus used the power of Drosophila genetics to understand the role of this extra connectivity. We found that ON responses, which are fully lost in mutants of the glutamate-gated chloride channel *GluClα;*[3], are rescued by re-expressing *GluClα;* only in one neuron, Mi1. This result is unexplained by our understanding of the implementation of motion computation (which entails a spatiotemporal signal comparison from at least two different cell types) and argues that the unexplored higher-order connectivity significantly contributes to function. As shown by graph analysis, Mi1 is a highly connected neuron in the visual system. In line with the idea that the complex connectivity of Mi1 played a role in its ability to rescue circuit function, Mi1 in turn rescued other neuron types feeding onto T4. To more generally explore the role of ‘connectedness’ on circuit function we rescued another highly connected neuron (L5) and a low-connectivity neuron (Tm3) and measured the circuit output. Our results are in line with a model in which connectivity predicts the rescue of directional selectivity. To test if partial motifs have degenerate function in comparison to wildtype ON-DS, we recorded the tuning of T4 to different visual variables and extracted its spatiotemporal receptive field. Although partial circuit motives largely recapitulate wildtype function, they fail at low luminance and (preliminarily) show disrupted spatial processing. This evidence argues for a circuit with degenerate connectivity in which the connectivity architecture confers significant robustness to visual computation. This is supported by acute rescue experiments, that argue that the circuit architecture is robust by itself without the involvement of developmental plasticity. We expect further analyses of circuit connectivity and connectome-inspired experiments to deepen our understanding of the role of brain connectivity patterns.

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Characterizing Navigational Strategies in *Drosophila* in Response to Varying Visual Stimuli

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Animals rely on multiple sensory inputs to constantly adapt to changing environments. While humans use advanced technology for wayfinding, insects, such as *Drosophila melanogaster*, heavily rely on visual cues, such as environmental and celestial cues, to navigate. This behavior is essential for basic survival activities like foraging, mating and avoiding predators and may seem simple but is quite complex. Many insects show innate straight-line navigation, but, depending on the sensory conditions, choose between phototaxis, (moving towards or away from light) or menotaxis (walking in a straight line while maintaining an arbitrary heading), for long-distance navigation. EPG (or compass) neurons are essential for menotactic behaviour, allowing the fly to orient itself relative to visual cues and navigate accordingly.

Using a virtual reality (VR)-based behavioral paradigm, we allow *Drosophila* to walk freely on an air-cushioned ball while exposed to varying visual stimuli. By systematically adjusting conditions such as brightness, contrast, and feature size, we aim to determine which visual cues favor specific navigational behaviors. Our analysis focuses on identifying repeating patterns in the flies' walking trajectories and observing when they shift from one navigational strategy to another. For example, flies switch from phototaxis, which dominates in some environments with high-contrast features, to menotaxis when exposed to more gradually changing features like gradients.

Therefore, by characterizing the conditions that lead to phototaxis, menotaxis, or neither of the two we aim to provide insights into the mechanisms governing navigational strategies in flies. A long-term aim of this study is to investigate neural mechanisms underlying these navigation strategies. By silencing or activating specific neurons, we can aim to identify cell types that affect the expression of one navigational strategy over another, which will then enable us to explore the link between sensory input and neural activity. These findings may offer broader implications for understanding navigation in other animals, including mammals, and contribute to the growing body of research on the neural basis of navigation.

Neural pathways and computations that achieve stable contrast processing tuned to natural scenes.

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Natural scenes are highly dynamic, challenging the reliability of visual processing. Yet, humans and many animals perform accurate visual behaviors, whereas computer vision devices struggle with rapidly changing background luminance. How does animal vision achieve this? Here, we reveal the algorithms and mechanisms of rapid luminance gain control in Drosophila, resulting in stable visual processing. We identify specific transmedullary neurons as the site of luminance gain control, which pass this property to direction-selective cells. The circuitry further involves wide-field neurons, matching computational predictions that local spatial pooling drive optimal contrast processing in natural scenes when light conditions change rapidly. Experiments and theory argue that a spatially pooled luminance signal achieves luminance gain control via divisive normalization. This process relies on shunting inhibition using the glutamate-gated chloride channel GluClα. Our work describes how the fly robustly processes visual information in dynamically changing natural scenes, a common challenge of all visual systems.

Heterogeneity of synaptic connectivity in the fly visual system – causes and consequences

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Visual systems are considered homogeneous structures, where highly similar units are organized in columns to retinotopically cover the visual field. The fly eye consists of about 800 single ommatidia and visual columns, with each column encompassing the same columnar neuron types. Each neuron type can be distinguished by its anatomy, by genetic markers, and – in general – by its functional properties, building a clearly structured network. However, by investigating the input connectivity of different visual system interneurons (Tm1, Tm2 and Tm9) using the FAFB EM dataset[1] and FlyWire connectome[2, 3], we found evidence for heterogeneous synaptic connectivity in the visual system: While all neurons analyzed show some degree of variability in their wiring across columns, some neurons of the same type, e.g., Tm9, appear more variable than others (e.g., Tm1 and Tm2). This is in line with previous study[4] showing that these neurons display variable physiological properties in response to the same stimulus[4]. Taken together, we hypothesize that heterogeneous input connectivity leads to variable physiological properties, which argues for a distribution of function within a cell type.

Currently, we are investigating which external and internal factors influence the connectivity of visual system neurons to make them either more or less variable. The developmental temperature of a fly, for example, negatively scales with the input connectivity of these interneurons, which is in line with previous studies on other cell types[5,6]. Furthermore, we observe an increased heterogeneous wiring for multiple neurons in individuals developed at lower temperatures. We explore these and other factors to understand wiring strength and variability. In parallel, we are also exploring the functional consequences of heterogenous properties across the repeating units of the fly eye, and investigate the hypothesis that increased wiring variability even within one cell type can lead to a distribution of function across the fly eye.

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Linking visual system anatomy to neuronal function in the *Drosophila* motion-detection system

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The brain's development sets the foundation for neurons to perform specific tasks in neural computation. These neurons undergo various processes to form intricate neural networks that process unpredictable sensory inputs and produce behaviors aimed at maximizing survival. Motion patterns are the changes in the input scene induced by the self-movement of the animal, termed optic flow, and they are tightly linked with the animal's locomotor behavior. To investigate the role of behavioral constraint on the optimal encoding strategy for visual information in fruit flies we used in-vivo two-photon imaging to record from a population of local-direction selective cells (DS cells) better known as T4/T5 cells. Our data show that the DS cells in *Drosophila* form a population code that is matched to represent optic flow fields generated during translational and rotational self-motion of the fly. Functionally, we identify six subtypes of DS neurons which, on average, encode six axes of motion: in addition to upward and downward motion, these are the two diagonals relative to the front-to-back and back-to-front direction of motion, respectively (Henning et al. 2022).

We are currently exploring how these distinct subtypes and its global motion encoding pattern develop, and how form and function of the fly eye are linked. First, the presence of six functional subtypes well matches the hexagonal arrangement of the ommatidia. We ask if the structural arrangement of the adult compound eye constraints the function of global motion computation regions of the brain. Second, we investigate the development of motion-detecting circuitry exploring the timeline, from the larvae to adult, for the emergence of different DS subtypes. In both approaches, we combine genetic manipulations of either developmental programs or eye structure with functional analysis using in-vivo calcium imaging in the T4/T5 population. Together, we aim to understand how visual morphology instructs and constraints functional properties relevant to visually guided behaviors.

Emergence of functional diversity in the peripheral visual pathway for the encoding of naturalistic stimuli

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In the natural world, animals rely on their capability to accurately extract relevant information from dynamic sensory stimuli, such as odor plumes or moving objects. Understanding how the visual system successfully extracts information from natural dynamic visual inputs to tune behavior requires investigating both the neuronal coding strategies in the early visual system as well as the statistical properties of dynamic natural scenes. For the latter, several studies have shown that features of natural scenes, such as luminance or contrast, have higher order spatial and temporal correlations with a characteristic power-law power spectrum, meaning that relevant information is present at several temporal and spatial scales [1]. Extensive work in visual systems has shown that neuronal coding strategies are organized in a hierarchical processing of information within layers of neurons. Furthermore, recent studies in both vertebrates [2] and invertebrates [3] have provided evidence of a large diversity of functional properties among neurons in the same processing layer. In this work, we propose that the diversification of the visual pathway into different and parallel temporal channels has emerged as a strategy to encode these complex stimulus statistics within behaviourally relevant time scales. To test our hypothesis, we investigate the information coding of peripheral visual networks using natural scene statistics as inputs. Specifically, we derive a normative model to study the functional properties of each neuron in the network and their role in the encoding of natural inputs. We find that naturalistic inputs with different correlation profiles lead to a diversity of temporal scales that optimize the information encoding at early visual stages. Furthermore, we show that networks that share functional properties, such as sustained responses, are less efficient than networks with a combination of transient and sustained responses. Thus, our theory provides a plausible explanation for the early diversification of the visual pathway into different parallel temporal channels.

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Neural mechanisms for a stable head direction estimate in dynamic, naturalistic visual environments

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Tracking head direction is essential for flexible, goal-directed navigation and doing so accurately over extended periods of time requires the use of sensory cues like the sun. Under natural settings, deriving a consistent sense of direction from external cues can be challenging, as cues may temporarily disappear, for example, when clouds hide the sun. Furthermore, when moving through the environment, terrestrial landmarks traverse the field of view, creating conflicts for a head direction estimate. In such dynamic environments, how can a brain extract a consistent head direction estimate?

We address this question in the fly. Using two-photon calcium imaging in head-fixed Drosophila, we monitor the fly's compass-like head direction estimate while the fly explores immersive visual virtual environments. To do this, we developed a novel virtual reality system based on the Unity game engine. We show that flies generalize their frame of reference across different simulated celestial guidance cues: sun-like spots and intensity gradients. Even in environments with approachable landmarks flies can maintain a globally consistent head direction estimate, by selectively tethering neural activity to the available global celestial cues. Thus, the fly compass system is remarkably robust to cue changes and conflicts under conditions that mimic natural scenes.

How is this robustness achieved? Through connectome analysis, we identified potential circuit motifs for selecting sensory cues to generate a robust head direction estimate in complex environments. Further, using calcium imaging we characterized how visual input neurons to the fly's "compass" circuitry dynamically represent the environment and select for reliable compass cues. Finally, we used modelling to test how neural tuning, circuit motifs for cue selection and previously described plasticity in the circuitry contribute to a robust compass estimate.

Linking environmental structure and social behavior: a case study on the development of social phenotypes in the desert locust

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The desert locust, Schistocerca gregaria, has been known for remarkable behavioral plasticity, transitioning between solitary and gregarious phases in response to population density and environmental factors. This study investigates the influence of habitat structure on the development of social phenotype in locusts, focusing on how resource distribution shapes behavior along time. We compared two habitat structures - a centralized and distributed - to assess foraging patterns, activity levels, and social interactions. Using motion-capture technology and advanced video tracking tools, we monitored locust behavior continuously for 21 days to quantify distance walked, speed, and social proximity. Phenotypes at the end of the experiment were further assessed in subsequent behavioral assays comparing response to conspecifics in an unstructured environment. In parallel, molecular investigations were performed to shed light on potential brain wide molecular mechanisms underlying these behavioral changes. For that, locust brains were collected at defined time points throughout the experiment for RNA analysis. Our preliminary results reveal that locusts raised in a centralized habitat exhibited a higher degree of gregarious behavior, while those in distributed habitat structures displayed more solitary behavior. These findings were consistent across both individual and group-level behavioral assays, suggesting that habitat structure significantly influences social phenotype development in desert locusts. Additionally, RNA Sequencing analysis of the locust brains indicated alterations in gene expression associated with these behavioral changes, highlighting the neural and genetic mechanisms that underpin the observed phenotypic differences. Our study underscores the critical role of environmental factors in shaping locust behavior and offers insights into the adaptive strategies of this species in response to habitat variations. It opens up the potential to investigate the neural circuit mechanisms underlying environmentally induced behavioral changes

Spatio-chromatic visual processing in *Drosophila*

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Recent research in *Drosophila* has demonstrated that color opponency, as observed in many vertebrates, is established at the level of photoreceptor terminals. However, the mechanisms by which downstream neural circuits process photoreceptor signals, likely through spatiotemporal computations, remain unclear. To address this, we developed a novel and versatile multi-color visual stimulator for the *Drosophila* visual system. This system, adaptable to the visual systems of various species, enables precise investigation of spatial, temporal, and spectral response characteristics. Using a comprehensive stimulus battery consisting of full-field and spatially structured chromatic stimuli, with discrete stimuli and white noise patterns - we examined the spectral sensitivities and spatiotemporal receptive fields of medulla neurons. Our findings shed light on the neural circuit mechanisms underlying higher-order color processing, such as spatial color contrast.

The Desert Locust Startle Response: Linking Descending Neurons and Behavioral Dynamics

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Swarms of the migratory desert locust *Schistocerca gregaria* can extend over several hundred square kilometers, devouring everything in their path. Despite their immense socio-economic impact, little is known about the mechanisms underlying their collective decision-making processes. Therefore, we combined behavioral experiments of freely walking animals with fine-scaled extracellular recordings of descending neurons and functional whole-brain calcium imaging to shed light on these vital processes.

Navigating complex environments while avoiding threats is central to a locust's survival. To make an informed escape decision, animals rely on individually acquired information, such as the looming silhouette of an approaching predator. Evaluating these external cues is crucial for an effective escape response while conserving valuable energy resources. In our study, we investigate the initiation of individual startle responses in gregarious desert locusts to unravel the underlying decision-making processes. Controlled lab experiments with single, freely moving animals provide insights into the factors influencing an individual's response, revealing a non-linear relationship between stimulus intensity and response probability.

Combining these stimulus-response characteristics with recordings from descending motion-detection neurons gives insights into neuronal evidence accumulation processes. For this purpose, we established a naturalistic trackball setup that allows us to record extracellularly from these neurons while the locusts are tethered walking and jumping in a virtual reality environment.

To further broaden our understanding of the brain regions involved, we developed a protocol for whole-brain functional imaging in virtual environments with single-neuron resolution.

Taken together, we aim to shed new light on the principles underlying the orthopteran escape decision by employing a comprehensive, naturalistic approach, providing insights into the collective decision-making processes of this devastating pest species.

Poster Topic

T15: Vision: Retina and Subcortical Pathways

T15-1A Behavioural studies of vision degeneration in the RD10 mice model
Anna-Lena Linke

T15-2A Visual encoding by retinal ganglion cells in optogenetic models for vision restoration
Varsha Ramakrishna, Tim Gollisch

T15-1B Short-term plasticity of retinal ganglion cell inputs to the dLGN depends on retinogeniculate synapse strength
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T15-2B Modeling spatial contrast sensitivity in responses of primate retinal ganglion cells to natural movies
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T15-1C Cortical feedback alters population activity to improve sensory coding during behavior
Augustine (Xiaoran) Yuan, Wiktor Mlynarski, Laura Busse

T15-2C Protein-lipid binding properties implicate Piccolino in synaptic vesicle tethering at photoreceptor ribbon synapses
Michalina Gadomska, Julia Breuer, Hanna Ehnis, Sina Zobel, Renato Frischknecht, Anna Fejtová, Hanna Regus-Leidig, Johann Helmut Brandstätter, Kaspar Gierke

T15-3C AMPA receptor desensitization decreases input and response gain in the lateral geniculate nucleus
Sonia Ruggieri, Tim Gollisch, Jakob von Engelhardt

T15-1D Knocking out the tectofugal pathway in a bird – the role of AP-2δ in development
Stefan Weigel, Falk Brönnle, Yujunyu Zhang, Hicham Sid, Benjamin Schusser, Harald Luksch

T15-2D Neural basis of visual information integration and decision-making in larval zebrafish
Katja Slangewal, Max Capelle, Florian Kämpf, Armin Bahl

T15-3D Contrast Adaptation in Stimulus Encoding by Retinal Ganglion Cells
Robert Haret, Tim Gollisch

Behavioural studies of vision degeneration in the RD10 mice model

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The retinal degeneration 10 (rd10) mouse is a widely used animal model for retinitis pigmentosa, a group of inherited retinal diseases that result in progressive vision loss. The mice carry a spontaneous mutation in the rod phosphodiesterase (PDE) gene, which results in the degeneration of photoreceptor cells in the retina starting around postnatal day 18. The rd10 model is now a popular choice for studying retinitis pigmentosa due to its slower degeneration compared to other models. However, the behavioural experiments employed to investigate vision degeneration must be adapted. The object of this study was to characterize the degeneration of the photoreceptors and resulting degeneration of vision in the RD10 mice model under different behavioural experimental conditions.
In comparison to normal vision of the Bl6 mice model, our findings revealed that the robust visual deficits of the Rd mice significantly depend on the sex of the mice and the light condition of the behavioural experiment. Together our data suggest, that the choice of the sex of the animal in combination with the experimental condition are critical for this slow-degenerating mice model.

Visual encoding by retinal ganglion cells in optogenetic models for vision restoration

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Retinal degeneration is one of the leading causes of blindness and optogenetics as a potential therapeutic measure has garnered much attention. Naturally light-sensitive molecules like Channelrhodopsin (ChR2) and other engineered ion channels are inserted into the neurons in the inner retina to play the role of light-sensing elements after the loss of photoreceptors. Previous studies have shown responses of retinal ganglion cells (RGCs) in blind animal models with optogenetically modified retinas, mostly based on simple light stimuli. Our study aims to directly compare encoding by RGCs under photoreceptor and optogenetic stimulation in response to spatiotemporally complex and natural stimuli. Furthermore, we would like to estimate an optimal stimulation of such modified retinas to elicit responses like that in a normal retina.

Preliminary experiments using multielectrode array recordings with retinal ganglion cells expressing ChR2 showed light-dependent optogenetically driven responses in the RGCs. Temporal filtering, as assessed via a linear-nonlinear model fitted to ganglion cell responses under flickering stimulation, was much faster under ChR2 activation compared to photoreceptor activation. This is in line with the direct stimulation of RGCs by ChR2 with no photoreceptor or bipolar cell processing. The estimated receptive field sizes of the ganglion cells based on photoreceptor-evoked and ChR2-evoked responses to spatiotemporal white noise had a significant difference in size possibly due to the absence of canonical center-surround receptive field activation under ChR2 stimulation.

We also compared responses of RGCs to natural images under photoreceptor and optogenetic stimulation. We saw that the responses of RGCs under the two conditions were characteristically different which reflected the role of both ChR2-channel dynamics and cellular spiking machinery (cell-type specificity) in generating responses to natural stimuli. Based on these differences, the natural images were modified by methods such as thresholding, scaling and blurring and presented to the RGCs under ChR2 activation to elicit responses like that under photoreceptor activation. We found that certain modified images elicited stronger and also more similar responses to the photoreceptor-evoked responses than unmodified images under ChR2 activation in a cell-type dependent manner.

These findings will help in generating better optogenetic therapies for patients by targeting cell-type specific stimulation and expression of optogenetic constructs in the retina to achieve more natural vision.

Short-term plasticity of retinal ganglion cell inputs to the dLGN depends on retinogeniculate synapse strength

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Retinogeniculate (RG) synapses convey visual information from the retina to thalamocortical relay cells (RCs) in the dorsal lateral geniculate nucleus (dLGN). The dLGN transmits the integrated inputs to the visual cortex. Previous studies revealed a high convergence of retinal axons on a single RC of the dLGN, characterized by a heterogenous connection strength. Only 1-3 of the inputs onto a RC are thought to drive neuron firing (strong inputs). These inputs are characterised by large amplitudes (>1nA) and a pronounced short-term depression of AMPA receptor (AMPAR)-mediated currents. To what extend the remaining retinal ganglion cell projections, which alone might not be able to induce RCs firing (weak inputs), contribute to information processing is not known.

Here, we aim to unravel the projecting retinal inputs and understand their different contribution to RC activation. To do that, we employ patch clamp technique to record AMPAR-mediated currents in RCs of the dLGN combined with minimal and maximal electrical stimulation of the optic tract at different frequency. We observe a negative correlation of short-term plasticity and synapse strength with facilitation of AMPAR currents for the weak inputs and depression for strong inputs at high frequency activation of RG synapses. To investigate the cause of the different short-term plasticity, we examine paired-pulse ratios of NMDAR-mediated currents. In addition, we analyse short-term plasticity of AMPAR-mediated currents in CKAMP44 knockout mice. CKAMP44 is an AMPAR auxiliary protein that slows recovery from desensitization. The data suggest that the differences in short-term plasticity of weak and strong inputs is due to differences in vesicle release probability and AMPAR desensitization, both of which increase with synapse strength.

These results suggest a potential role of weak inputs RG synapses in refining information processing and inputs integration in the dLGN. Considering that weak inputs display facilitation and strong inputs depression, the contribution of weak inputs to signal transmission should increase for strong inputs when retinal ganglion cells fire at high frequency and with many action potentials.

Modeling spatial contrast sensitivity in responses of primate retinal ganglion cells to natural movies

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Retinal ganglion cells (RGCs) encode visual information through complex transformations of light stimuli, influenced by both spatial and temporal dynamics. Traditional models of retinal signal processing, such as the Linear-Nonlinear (LN) model, assume linear integration of light intensity over the cell's receptive field. While effective for simple stimuli, this assumption fails to account for nonlinear computations by RGCs, particularly under naturalistic stimuli. Nonlinear extensions, like subunit models, which divide the receptive field into smaller, nonlinearly combined subfields, are often difficult to fit to experimental data, especially with natural stimuli. Previous work in the salamander retina has shown that spatial contrast sensitivity to flashed images can be partly captured by a model that combines signals from the mean and variance of luminance within the receptive field.

Here, we extend this spatial contrast model to incorporate temporal dynamics and evaluate its performance on ex-vivo electrophysiological recordings from marmoset retinas under artificial and natural movie stimulation. The model quantifies spatial contrast by measuring deviations in pixel intensity from the mean luminance within the receptive field. This is then linearly combined with mean luminance and passed through a non-linear activation function. We show that the model can be fit to experimental data and that it outperforms models with linear spatial integration, especially for cells with larger receptive fields. Finally, we use the model to infer each cell's spatial scale of nonlinear integration and contrast sensitivity. This scale remains consistent under white-noise stimulation but varies with naturalistic movies, likely due to differences in the spatial frequency content of the stimuli.

Our work shows that the spatial contrast model provides a simple approach to capturing nonlinear spatial integration with few parameters. It can be used to assess the cells' functional properties under natural stimulation and provides an easy-to-obtain benchmark for comparison with more detailed nonlinear models.

Cortical feedback alters population activity to improve sensory coding during behavior

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The role of feedback in sensory systems remains a major puzzle in neuroscience. Classical computational theories such as hierarchical predictive coding or hierarchical Bayesian inference postulate abstract normative goals for the role of feedback connections. However, predictions derived from these theories are typically specified at the level of individual neurons. Whether feedback shapes collective properties of neural populations and how such changes may impact encoding of sensory signals remains unknown.

Here we tackle these questions directly by studying the population coding of natural stimuli in the dorsolateral lateral geniculate nucleus (dLGN) of behaving mice. Extracellular electrophysiological recordings were performed in the mouse dLGN, while corticothalamic (CT) feedback from primary visual cortex (V1) was optogenetically suppressed. Our analysis was focused on two aims: i) determining whether CT feedback changes the structure of population activity as opposed to only modulating each neuron independently, and ii) exploring how these changes influence sensory coding.

By comparing distributions of population synchrony to carefully constructed null distributions, we found that population structure changes with feedback. Additionally, feedback employs more unique population states in a way that deviates from the null model prediction. Those unique states are more informative and involve more active neurons compared to the states shared between feedback conditions.

Information theoretic analysis revealed that these population-level changes exert a significant impact on sensory coding. By estimating response entropy we found that when feedback was intact, populations increased the variability of their responses to each movie frame. Furthermore, stimulus-specific information shows that feedback tends to increase coding accuracy for certain temporally localized periods of stimulus. Overall, presence of feedback increases the overall mutual information between stimulus and population activity.

Our findings cannot be immediately reconciled with the existing theories of feedback in sensory coding, which largely focus on individual neurons. Our results suggest therefore a need for expanding the theoretical understanding of the role of feedback modulation. Despite this discrepancy, we demonstrate that cortical feedback does play an important role in shaping efficient and informative population codes in lower levels of the sensory hierarchy.

Protein-lipid binding properties implicate Piccolino in synaptic vesicle tethering at photoreceptor ribbon synapses

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Synaptic ribbons (SRs) are protein structures found in specialized sensory neurons such as rod and cone photoreceptors and cochlear inner hair cells. SRs tether up to several hundred synaptic vesicles (SVs) to sustain fast and continuous exocytosis. How tethering of SVs to SRs is achieved is currently unknown. We previously showed that Piccolino, an integral component of SRs[1,2], binds to RIBEYE, the main component of SRs, via its C-terminus[3]. In this study, we investigate how the N-terminus of Piccolino might interact with SVs.

We discovered an alpha-helical amphiphatic liquid packing sensor (ALPS) motif at the N-terminus of Piccolino as a possible synaptic tethering mechanism. Subsequent protein-lipid binding studies confirmed that purified fragments of Piccolino containing the ALPS motif were able to bind to artificial, SV-like liposomes in a curvature-dependent manner. Blocking the ALPS motif with a specific antibody or removing the ALPS motif from Piccolino resulted in drastically reduced binding to SV-like liposomes, corroborating our hypothesis.

Next, we ultrastructurally analyzed the distribution of SVs at photoreceptor ribbon synapses in Piccolino knock-out (KO) mice. We found a decrease in the density of ribbon-associated SVs in Piccolino KO photoreceptors, compared to wild-type (WT) synapses. In addition, we observed that in Piccolino KO photoreceptors, SVs were located significantly closer to SRs when compared to WT synapses, indicating that SRs are incapable of maintaining the uniform distance between SVs and SRs in the absence of Piccolino. Both findings implicate Piccolino in organizing SVs at photoreceptor SRs. Advanced transmission electron tomography is underway to confirm the absence of tethering molecules in Piccolino KO rod photoreceptors.

Together, our current results suggest that Piccolino achieves SV tethering directly through protein-lipid binding.

AMPA receptor desensitization decreases input and response gain in the lateral geniculate nucleus

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Neural gain control determines how sensory, motor, associative, and cognitive information is represented in the brain. One mechanism underlying neuronal gain is the filter properties of the synapses in which the information is processed. It has been shown that synaptic short-term plasticity (STP) can be influenced by AMPA receptor (AMPAR) desensitization. We therefore hypothesize that desensitization controls gain and tuning properties of neurons. To test this hypothesis, we focused on the visual system and analyzed the role of desensitization (AMPAR) in information processing in dLGN relay cells (RCs). To this end, we generated a computational model of (RCs). Response tuning curves were left-shifted and maximal firing rates increased in a model neuron with AMPARs that recover instantaneously from desensitization. Results from the simulations suggested that AMPAR desensitization decreases not only response but also input gain of dLGN RCs. To test this prediction, we recorded in vivo responses of RCs in a mouse model in which recovery from desensitization of AMPARs is faster than in wildtype mice. Consistent with the data from simulations, we observed that a faster recovery from desensitization leads to a left shift in the contrast tuning curve, increased saturation at lower contrasts, and increased steepness in moving grating tuning curves. In addition, firing rate to the non-preferred stimulus increases in RCs with faster recovery from desensitization. Altogether, we show that desensitization of AMPARs decreases input and response gain, reduces the risk of response saturation, and increases the signal-to-noise ratio.

Knocking out the tectofugal pathway in a bird – the role of AP-2δ in development

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Sensory processing in vertebrates involves pathways that analyze different parameters of sensory input. In the auditory system, for instance, sound is integrated and processed, supporting spatial behavior and higher cognitive functions in the forebrain. The visual system, however, is unique in that it consists of multiple pathways, some of which have distinct and separate roles, such as the accessory optic system. Across all vertebrates, two primary projection systems converge in the forebrain: the tectofugal (or colliculofugal) and thalamofugal pathways. The role of these pathways in visual processing varies across vertebrate classes and species and may depend on the degree of binocular overlap. However, the specific aspects of visual information processed by each pathway remain a topic of debate. In our study, we examine visual processing in chickens, a lateral-eyed vertebrate with limited binocular overlap, using a genetically modified model, the AP-2δ knockout.

AP-2δ is a transcription factor crucial for development. In AP-2δ-deficient mice, the inferior colliculus (IC), a key auditory midbrain center, fails to develop, and retinal ganglion cells (RGCs) are lost, leading to diminished retinal innervation of the superior colliculus [1, 2]. In birds, AP-2δ is expressed in the retina [3], the IC, nearly all auditory hindbrain nuclei, and the optic tectum, but not in the lateral geniculate nuclei [4].

AP-2δ knockout chickens respond to both visual and auditory stimuli yet display deficits in behaviors such as object tracking, exploration, evasive movements, chirping, startle reflexes, and hearing thresholds. Morphological analysis of pre-hatch chickens (E16) revealed alterations in the midbrain, thalamus, and retina, with other brain regions unaffected. In the visual pathways, the RGC layer was reduced, and the optic tectum, a critical midbrain nucleus of the tectofugal pathway, showed disorganized superficial layers, indicating disrupted RGC innervation. Additionally, layer 13, which includes projection neurons of the tectofugal pathway, was significantly reduced. The thalamic target of these cells, the nucleus rotundus, was smaller, although the number of neurons remained unchanged.

In contrast to the tectofugal pathway, the thalamofugal pathway appeared mostly unaffected by the knockout.

In the auditory pathway, the inferior colliculus was entirely absent, and its thalamic target, the nucleus ovoidalis, was smaller but retained a normal number of neurons.

With a disrupted tectofugal system and a largely intact thalamofugal system, the AP-2δ knockout chicken provides a unique model to explore the relative contributions of each visual pathway to visual processing and behavior.

1.Hesse, K., et al. DOI: 10.1371/journal.pone.0023483
2.Li, X., et al. DOI: 10.1186/s13041-016-0244-0
3.Li, X., et al. DOI: 10.1002/dvdy.21744
4.Kettler, L., et al., DOI: 10.3389/fncir.2021.756184

Neural basis of visual information integration and decision-making in larval zebrafish

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Decision-making is a long-studied topic in neuroscience. We have an increasingly good mechanistic understanding of the neural circuits that allow animals to temporarily integrate specific decision variables. However, it remains unclear how these circuits combine, often conflicting, information from multiple sensory channels to form a single decision. Recently, we have described how the larval zebrafish anterior hindbrain integrates visual motion to decide about swimming direction. Other studies, focusing on different sensory stimuli, have identified the same brain area as a central processing structure for sensory-motor control. This raises the hypothesis that the anterior hindbrain forms a general integration hub for decision-making. Here, we employ a combination of behavioral experiments, computer simulations, and two-photon functional imaging to algorithmically and mechanistically describe how larval zebrafish integrate motion and luminance cues. Our behavior experiments and computational simulations argue for a parallel arrangement, in which separate modules temporally integrate information from distinct visual processing streams. Our imaging experiments support these findings, revealing distinct activation patterns with slow temporal dynamics that match the model predictions. These results allow us to build precise neural networks whose connections we test using newly established circuit dissection tools. Together, this means we can describe in mechanistic detail how brains combine and evaluate information extracted from multiple visual features.

Contrast Adaptation in Stimulus Encoding by Retinal Ganglion Cells

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The natural signals encoded at the level of the retina span substantially more orders of magnitude compared to the dynamic range of neurons. Thus, the visual system adapts, i.e. adjust its sensitivity, in order to accurately represent its immediate stimulus. More specifically, retinal neurons adapt to the basic features of the visual input, the mean and variance. Adaptation to changes in the mean light level is highly dependent on the properties of photoreceptor cells while the change in sensitivity as a function of stimulus’ variance (contrast adaptation) is prevalent in retinal ganglion cells (RGCs) as well as in a subset of bipolar and amacrine cells. Upon a change in stimulus contrast, the RGCs modify their temporal filtering properties, adjust their gain and sensitivity and change their average firing response.

Using multi electrode array recordings of mouse RGCs spiking activity I analyse contrast adaptation with the linear – nonlinear (LN) model. The LN assumes linear temporal and spatial integration of the stimulus. Each cell is assumed to have a spatiotemporal filter, which can be calculated by estimating the spike-triggered average (STA) under spatiotemporal white-noise stimulation. These filters are then applied to the stimulus, and the output is passed through an identified non-linearity to yield the neuron’s predicted firing rate. We initially focus on temporal processing. While the LN itself fails to capture adaptation to contrast, by fitting multiple LN models to separate segments of the recording corresponding to the different contrast levels, one can compare the differences owing to contrast adaptation. The change in temporal filtering is represented as the different linear filters of the LN models whereas changes in gain and sensitivity correspond to different slopes and offsets of the non-linearities. Subsequently, we examine extensions of the LN model that can incorporate adaptation to contrast and better explain the RGCs’ responses under different contrast regimes of the stimulus.

Poster Topic

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

T16-1A Reoccurring on-going activation states in local field potentials from human visual cortex
Udo Ernst, Enrique Gabriel Tabilo Romero, David Rotermund, Fabrizio Grani, Eduardo Fernández Jover

T16-1B Neural representation of color in the pigeon visual Wulst
Simon Nimpf, Ann Kotkat, Andreas Genewsky, Laura Busse, David Anthony Keays

T16-2B To follow or not to follow: State-dependent modulation and inversion of the optomotor response in larval zebrafish
Sydney A. Hunt, Ashrit Mangalwedhekar, Armin Bahl

T16-1C SynGAP1 knock down leads to precocious closure of the critical period for ocular dominance plasticity in the mouse visual cortex
Siegrid Löwel, Ariadna Sunyer, Paloma Huguet, Subhodeep Bhattacharya, Oliver Schlüter

T16-2C The role of orexin/hypocretin neuropeptides for vision and visual plasticity in mice
Cornelia Schöne, Jaya Sowkyadha Sathiyamani, Paloma Huguet, Tejas Shaji Nair, Oliver Schlüter, Siegrid Löwel

T16-1D Topographic projections from pulvinar to dorsal and ventral subdivisions of area LIP in the macaque
Sascha A. L. Ziegler, Bashir Ahmed, Andrew J. Parker, Kristine Krug

T16-2D A brain-wide screen reveals a preference for visual objects in the spatial navigation system that refines head direction coding
Dominique Siegenthaler, Henry Denny, Johanna Luise Mayer, Sofia Skromne Carrasco, Adrien Peyrache, Stuart Trenholm, Emilie Macé

Reoccurring on-going activation states in local field potentials from human visual cortex

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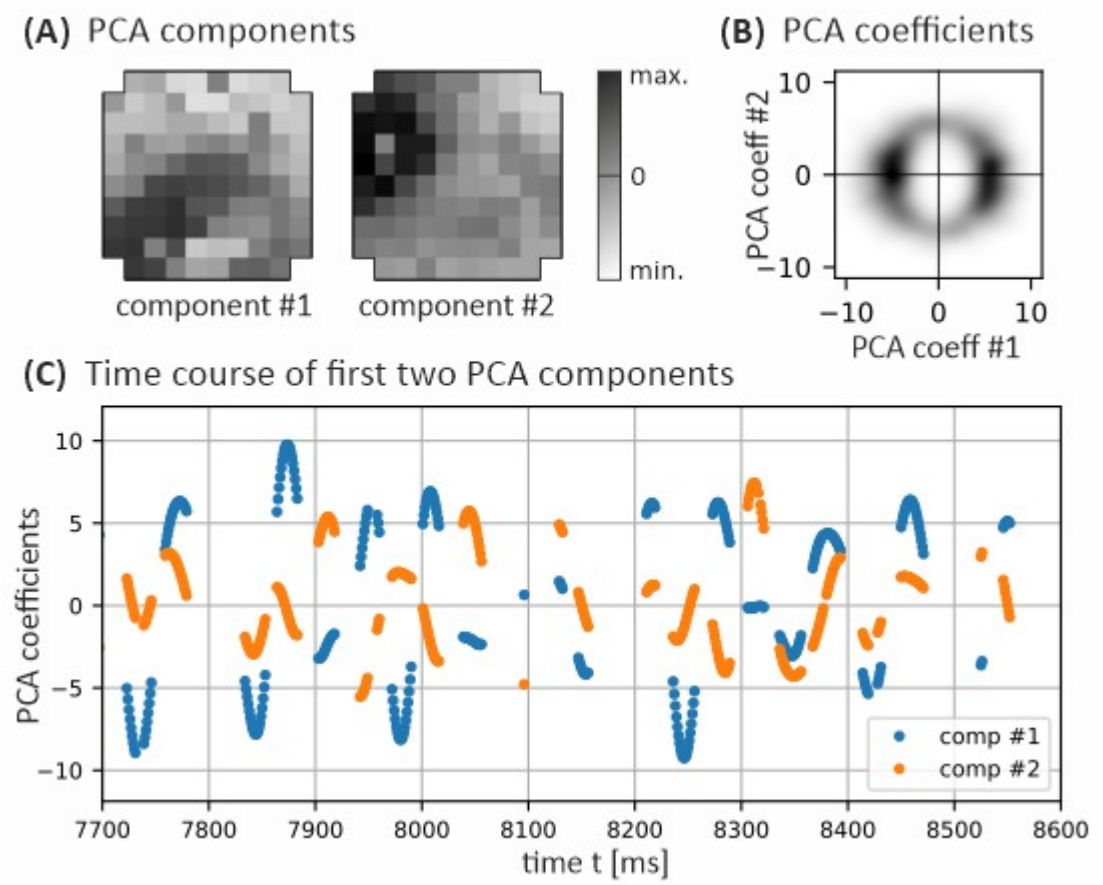
Even in the absence of visual input, the visual cortex exhibits on-going activity. Such activity is not purely random but correlated in space and time. Interestingly, cortical responses to visual stimulation can be better predicted when on-going activity prior to visual stimulus onset is taken into account (Arieli et al., Science 1996). This observation has raised renewed interest in the context of intracortical visual prostheses where electrical stimulation of neural populations in visual cortex aims at restoring simple visual percepts in blind patients. Tailoring artificial stimulation to on-going activity would potentially allow such prostheses to become more efficient and produce more reliable and structured percepts. However, for achieving this goal it is necessary to better understand the structure and dynamics of on-going activity first.

Here we investigated on-going neural activity in the visual cortex of two blind volunteers by developing a novel analysis for characterizing re-occurring neural patterns. In detail, we analyzed 13 (first subject) & 3 (second subject) LFP recordings from Utah arrays implanted in visual cortex (areas V1 and part of V2). The recordings were performed in a passive condition where the subjects were seated, with recording durations from 100 to 600 seconds. Preprocessing was done by using a singular value decomposition to first remove global noise patterns, and then noise specific to the amplifier banks. For analyzing on-going activity, we hypothesized that the cortical dynamics evolves by visiting different characteristic patterns which tend to repeat after some time. To identify such patterns we developed a reoccurrence analysis: For every pattern in an observation sequence, the analysis identifies points in time when a similar pattern reappears. To exclude trivial cases, e.g. when the cortex stays in the vicinity of a specific pattern for an extended time, we require the sequence to first diverge sufficiently far from the original pattern until detecting a reoccurrence.

Our method reveals a wide range in the number of reoccurrences for on-going activity patterns. For in-depth analysis we selected the top 25% of the most frequently occurring patterns and subjected these patterns to a principal component analysis (PCA). It turned out that only two to four PCA components (panel A) strongly contribute to these patterns (i.e. explain individually more than 5% of signal variance). Determining the temporal evolution of the coefficients for the two largest PCA components, we discovered a characteristic ring structure (panel B). In consequence, on-going activity tends to 'circle' through a linear superposition of two dominant modes, with the strength of presence for one mode restricting the presence of the other mode, and vice versa. This interaction was strong and observed consistently over all data sets.

We also generated surrogate patterns with same characteristic length and time scales as in the recorded data, using a constrained random walk in time combined with spatial diffusion. Here the ring structure disappears and the two main components remain uncorrelated. We thus conclude that on-going activity in V1/V2 is not just filtered noise but highly structured, and that it can be explained by a dynamics rotating through a low-dimensional manifold (panel C). Such dynamics might reflect an underlying process which

sequentially activates different orientation domains like observed in animal recordings.



(A) Spatial pattern of PCA components displayed on Utah array. (B) Distribution of PCA coefficients for the components shown in (A). (C) Temporal evolution of dominant patterns over time, in intervals previously identified by reoccurrence analysis.

Neural representation of color in the pigeon visual Wulst

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For many animals, color vision is important for survival, contributing to critical behaviors such as identifying food, detecting predators, and recognizing mates. How colors are processed in the central nervous system of di- and trichromatic vertebrate species (2-3 color cones) has been a topic of considerable research over the last decades. In contrast, the neuronal circuits supporting color vision in tetrachromatic vertebrates (4 color cones), such as birds, remain poorly understood. Through a combination of display engineering, large-scale Neuropixels recordings, and computational methods, we here provide a comprehensive characterization of the neuronal representation of color in the pigeon visual Wulst, the functional homologue of the mammalian primary visual cortex.

We presented full-field colors on a custom 5-channel LED display, covering the avian visual spectrum (300-700 nm) while recording from more than 4000 responsive units in the visual Wulst of awake, head-fixed pigeons. To quantify the diverse response profiles of individual units to the colors, we used a combination of non-linear dimensionality reduction (t-Distributed Stochastic Neighbor Embedding; t-SNE) and a two-step unsupervised clustering approach. This allowed us to identify ~100 unique response types, which could be broadly categorized as color-selective, color-opponent or achromatic. To investigate the neural encoding of color within the visual Wulst, we used CEBRA, a new self-supervised learning algorithm, to find a low-dimensional embedding of responses to the different colors. A classifier trained on the resulting embeddings was able to decode color with high accuracy (~80%, 100 repeats), demonstrating that visual Wulst population activity profiles are distinct between different colors.

Together, our large-scale functional survey highlights the extraordinary diversity and complexity of color responses in the pigeon’s visual Wulst. Ultimately, our findings will allow investigating commonalities and divergences between the evolutionary ancient visual system of birds and our own visual sense.

To follow or not to follow: State-dependent modulation and inversion of the optomotor response in larval zebrafish

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Animals live in a complex and variable world, in which they must flexibly decide how to respond to ever-changing and unpredictable sensory inputs. Such decision-making processes require the brain to integrate information from the surroundings in a state-dependent manner to transform environmental cues into appropriate motor output. The optomotor response in fish and insects is an innately present, stabilizing response to global motion. It has been generally assumed to induce following of motion and to be purely reflexive, making the optomotor response popular for the study of the computations underlying innate behaviors. Previous studies have mainly reported and interpreted behavior from the perspective of trial and animal averages. Whether such averaging approaches are indeed representative of how individuals respond on a trial-to-trial basis remains uncertain. Here, we probe the optomotor response in individual zebrafish larvae at high temporal resolution over the timecourse of multiple hours. We find that fish often exhibit an inverted optomotor response, swimming against the direction of motion, a previously unreported behavior in zebrafish larvae. Using turn angle, swim trajectory, and other swim statistics, we show that this behavior is an active process that cannot be explained by the animal simply ignoring the stimulus and swimming against motion by chance. Analyzing behavior across the population, we identify strong inter-individual differences in the temporal statistics of switching behavior. We find that the inverted optomotor response is also present in head-restrained larvae, enabling us to simultaneously perform functional two-photon microscopy. Focusing on pretectal and anterior hindbrain areas, we identify the neural representations of behavioral state-switching in the context of the optomotor response. Our work will shed light on the precise neuromodulatory mechanisms that give rise to inter-individual and trial-to-trial variability in sensory-motor decision-making. It will also pave the way for future studies to investigate which molecular genetic features give rise to the observed individuality and how behavioral flexibility develops over the lifespan of an animal.

SynGAP1 knock down leads to precocious closure of the critical period for ocular dominance plasticity in the mouse visual cortex

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Neuronal plasticity is a fundamental process in brain development. Understanding the molecular mechanisms involved in it is crucial for finding solutions to neurodevelopmental disorders. This study investigates the role of SynGAP1, a synaptic Ras-GTPase activating protein, in regulating the duration of the critical period (CP) for ocular dominance plasticity (ODP) in the primary visual cortex (V1) of mice. Ocular dominance plasticity induced by monocular deprivation (MD) has been one of the most widely used models to study experience-dependent cortical plasticity. The absence of SynGAP1 was recently shown to speed up the decrease of AMPA silent synapses in layers 2/3 pyramidal neurons of V1 during the critical period, very similar to what has been published about PSD-93 KO mice (Favaro et al, PLoS Biol 16(12):e2006838, 2018). Since precocious silent synapse maturation caused precocious closure of the CP for ODP in V1 of PSD-93 KO mice, we hypothesized a similar result for the SynGAP1 knock-down (KD, shSG) animals.

To image plasticity, we used intrinsic signal optical imaging in mouse V1, a minimally invasive technique. Knock-down of SynGAP1 was achieved by injecting an AAV-shRNA against the protein (shSG) into the visual cortex at postnatal day 2 (control-KD with shLC). Both mice during mid-CP (P24-27) and late-CP (P28-35) were analyzed after a 4 day MD. While V1 of SynGAP1-KD animals in mid-CP showed clear ODP like control-KD V1, ODP was absent in late-CP SynGAP1-KD animals, as hypothesized. Spatial vision (using optomotry) was not different between SynGAP1-KD animals and control mice.

Our findings reveal that SynGAP1 knockdown significantly accelerates critical period closure, suggesting that SynGAP1 is crucial for determining the timing of the critical period for ODP in the visual cortex. Our data thus add to the growing literature emphasizing the important role of AMPA silent synapses for the experience-dependent maturation of V1 circuits.

The role of orexin/hypocretin neuropeptides for vision and visual plasticity in mice

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Lateral hypothalamic orexin/hypocretin (OH) circuits enhance exploratory behaviour and cortical gamma power (Vassalli & Franken 2017, Karnani et al 2020), key physiological variables affecting neuronal responses in the primary visual cortex (V1) (Niell & Stryker 2010). In order to elucidate the functional significance of OH signals for vision we tested orexin KO mice in assays for experience dependent visual plasticity, orientation discrimination and signal transmission within V1.

To test visual plasticity, we used intrinsic signal optical imaging (OI) to assess ocular dominance plasticity (ODP) in V1 of orexin KO mice. Since ODP depends on age and housing conditions, we examined mice both during the critical period (CP) for ODP (P21-P35) and in adults (>P110) with standard cage (SC) housing or running wheel (RW) enrichment during monocular deprivation (MD). During early (P21-P23) and mid CP (P24-27), both SC orexin WT/KO mice showed ODP after 4 days of MD. In contrast, during late CP (P28-P35), ODP in KO was compromised: unlike WT, KO-mice needed 7 days of MD to display ODP, suggesting that CP may close precociously in orexin KOs. Moreover, RW enrichment failed to boost ODP in orexin KO mice. In adult SC WT mice, ODP is absent after 7 days of MD, but can be rescued after RW-enrichment (Kalogeraki et al 2014). In contrast, and as in juvenile KO mice, RW enrichment did not boost ODP in adult KO mice: V1-activity remained dominated by the deprived (contralateral) eye, notably even when KO mice ran similar distances as WT-mice (WT/KO: 3.9±1.2/4.1±1.4 km/d, n=3/3). Therefore, OH circuits promote experience-dependent plasticity and propagate the plasticity boosting effects of RW-enrichment in mouse V1.

We have previously shown that modified maturation of AMPA-silent synapses in both PSD-93 and PSD-95 KO mice alters the timing of the CP for ODP and results in compromised orientation discrimination (Favaro et al 2018). We therefore analysed whether orexin KO modifies AMPA-currents in V1 and causes similar visual deficits. AMPA-receptor(R), NMDAR and GABAR postsynaptic currents between L4-to-2/3 connections were quantified in acute brain slices of late CP orexin KOs. Using minimal stimulation, we observed a 50% reduction in the amplitude of AMPAR-responses, while AMPAR-silent synapse numbers remained unchanged.

Spatial vision of orexin WT/KO mice was analysed by using both optomotry and the visual water task (VWT), a dual choice visual discrimination task. The spatial frequency threshold of the optomotor reflex was similar between WT and KO mice (WT/KO: 0.38±0.002/0.38±0.004 cyc/deg, n=5/5), suggesting that subcortical circuits mediating this reflex remain unaffected in KO mice. Using the VWT, visual acuity was not significantly different between KO and WT (WT/KO: 0.61±0.04/0.53±0.04 cyc/deg, n=7/7). Notably, KOs were worse in orientation discrimination, needing a larger angle difference to discriminate square wave patterns (WT/KO: 20±3°/30±2°, n=7/7, t-test p=0.028).

Thus, basic visual abilities were similar between orexin KO and WT mice, but orientation discrimination was reduced in KOs.

In summary, we conclude that OH neuropeptides are required for V1 experience-dependent plasticity, potentially by strengthening AMPAR mediated excitation in V1.

Ref.:
Favaro et al 2018, PLosBiol 16:e2006838
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Karnani et al 2020 Prog Neurobiol 187:101771
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Topographic projections from pulvinar to dorsal and ventral subdivisions of area LIP in the macaque

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The pulvinar is a large subcortical structure, which has become greatly expanded in primate evolution. While its exact functional role is not fully understood, the pulvinar appears to participate in the integration and modulation of sensory information between cortical areas. Extensive reciprocal connections exist between the pulvinar and virtually all cortical regions, including the lateral intraparietal area (LIP). The dorsal and ventral subdivisions of LIP (LIPd and LIPv, respectively) are structurally and functionally distinct from each other, with LIPd having been linked to visuospatial processing and LIPv to saccade planning—however, it is unknown whether the anatomical connectivity between pulvinar and LIP is differentially related to these subdivisions. To address this question, small volumes (80 nl) of the retrograde tracer, Cholera Toxin B Subunit (CTB low salt, List Biological Labs Inc.), were injected into either LIPd or LIPv of 2 male and 2 female, anesthetized rhesus macaques, using structural MRIs under Brainsight (Rogue Research Inc) control. Between 86-234 hours later, animals were perfused transcardially with 4% paraformaldehyde. Parasagittal sections were cut at 50 µm, and tissue was processed for immunohistochemistry, including Nissl, Gallyas, and anti-CTB staining. Brightfield images of the pulvinar were acquired and examined for retrograde neuronal body labeling. Confirming previous studies, labeled cells were found almost exclusively within dorsal parts of the pulvinar, and this pattern was observed following both LIPd and LIPv injections. However, injection of CTB into LIPd resulted in a greater number of more widely dispersed labeled cells in the pulvinar compared to injection into LIPv, which produced fewer, more clustered labeled cells. In stark contrast to retrograde tracer injections into earlier, dorsal visual area V5/MT, we found almost no labeling in the inferior pulvinar or the LGN, underlining the importance of these connections for purely visual processing. These anatomical connections provide the structural basis for the involvement of the pulvinar in visuo-motor processes. We tentatively suggest that the pulvinar may support the alignment of visual and motor maps, including the integration of information from multiple brain regions for adaptive updating and accurate calibration for sensorimotor links.

A brain-wide screen reveals a preference for visual objects in the spatial navigation system that refines head direction coding

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Animals use visual objects to guide behaviors, from hunting prey, to escaping predators, to navigating through the world. Although much research has focused on the cortical areas responsible for visual object recognition, the influence of visual objects on spatial navigation systems remains largely unexplored. Using functional ultrasound (fUS) imaging in mice, we conducted a comprehensive brain-wide analysis to identify brain areas that were preferentially activated by images of objects compared to scrambled versions of the same stimuli. Interestingly, while visual cortical areas did not show a significant preference, regions associated with spatial navigation were preferentially activated by visual objects. Electrophysiological recordings in the postsubiculum, the primary cortical area of the head direction (HD) system, further confirmed a preference for visual objects, which was present in both HD cells and fast-spiking interneurons. Finally, we found that visual objects dynamically modulate HD cells, selectively increasing firing rates for cells aligned with a visual landmark's direction, while suppressing activity in other directions. These results reveal an interaction between visual object processing and the brain’s spatial navigation system, which refines population-level coding of head direction.

Poster Topic

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

T17-1A Dopaminergic modulation of habituation in the mechano-sensory system in larval zebrafish
Nils Lukas Brehm, Wolfgang Driever, Johann H. Bollmann

T17-2A Probing the impact of the transcription factor Runx1, involved in spiral ganglion neuron subtype specification, on afferent synaptic transmission and neural firing properties
Leon Bösche, Lejla Soše, Nare Karagulyan, Nicola Strenzke, Brikha Shrestha, Tobias Moser

T17-3A Paralemmin-3 – an essential constituent of the submembrane cytoskeleton of auditory hair cells
Christian Vogl, Victoria Christine Halim, Christina Ullrich, Iman Bahader, Makoto F. Kuwabara, Dennis Derstroff, Kathrin Kusch, Nicola Strenzke, Dominik Oliver, Carolin Wichmann, Manfred W. Kilimann

T17-1B Evaluating the Spread of Excitation with Red Light Optogenetic Stimulation of the Auditory Nerve Through Computer Simulations and *In-Vivo* Electrophysiology
Elisabeth Koert, Jonathan Götz, Anna Vavakou, Niels Albrecht, Bettina Wolf, Tobias Moser

T17-2B In-Silico Framework for Benchmarking Optogenetic Hearing Restoration
Lakshay Khurana, Petr Nejedly, Daniel J. Jagger, Lukasz Jablonski, Tobias Moser

T17-3B Evaluating the utility of virtual-channel-based sound-to-neuron stimulation strategy for future optogenetic cochlear implants
Lukasz Jablonski, Antonia Klobe, Lakshay Khurana, Tobias Moser, Gerwald Lichtenberg, Lukasz Jablonski

T17-1C Evaluation of optogenetic therapy for hearing restoration in rodent models of sensorineural hearing loss
Victoria Hunniford, Maria Zerche, Bettina Wolf, Kathrin Kusch, Thomas Mager, Tobias Moser

T17-2C From Sound to Movement: The Neural Backbone of the Acoustic Startle Reflex
Jan Frederik Ahrend, Jana Erlmoser, Christian Vogl

T17-3C A minimal magnetosensory circuit in the pigeon brain
Spencer Balay, Gregory C. Nordmann, Simon Nimpf, Lukas Landler, Erich Pascal Malkemper, David Anthony Keays

T17-1D Assessment of Glutamatergic quantal transmission insufficiency in sensory vestibular functioning.
Ruchi Rajesh Modgekar, Mohona Mukhopadhyay, Aizhen Yang-Hood, Kevin K. Ohlemiller, Maolei Xiao, Mark Warchol, Suh Jin Lee, Rebecca Seal, Susan Maloney, Carla Yuede, Mark Rutherford, Tina Pangrsic

T17-2D Investigating the neural correlates of the magnetic sense in the pigeon
Marco Numi, Simon Nimpf, David Anthony Keays

Dopaminergic modulation of habituation in the mechano-sensory system in larval zebrafish

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Habituation is a basic form of non-associative learning, which results in reduced responsiveness to prolonged or repeated stimuli. The tactile startle reflex (a type of mechanosensory reflex) in zebrafish is a widely-used experimental model for habituation learning. Both the lateral line and trigeminal sensory systems serve distinct roles for encoding mechanosensory stimuli, for example detection of water motion and touch, and are innervated by efferent projections from dopaminergic neurons located in the diencephalon. While neural dynamics during habituation have been studied across several brain areas, a specific role of dopaminergic neurons in this type of behavioral modulation remains elusive.
Here, we focus on the dopaminergic effects on the habituation of the tactile startle response and the underlying neural activity of dopaminergic neurons, specifically in the hypothalamus and posterior tuberculum. Recently, we described how tactile stimuli are processed by the sensory apparatus of the zebrafish head (Brehm et al., 2023). We could identify differences between the lateral line and trigeminal system in terms of velocity tuning curves and found two different functional subtypes of trigeminal afferent neurons.
Now, in behavioral experiments we observe that tactile habituation is sensitive to pharmacological manipulations of dopaminergic signaling. In complementary experiments using functional 2-photon imaging of intracellular calcium and extracellular dopamine signals, we investigate activity correlated with the habituation of behavioral responses observed simultaneously.
We found neurons with distinct activity profiles during the presentation of repeated tapping stimuli that consisted of adapting and non-adapting responses. Taken together, this should help understand whether and how these dopaminergic pathways are connected to habituation.

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Brehm, N., Wenke, N., Glessner, K., & Haehnel-Taguchi, M. (2023). Physiological responses of mechanosensory systems in the head of larval zebrafish (danio rerio). Frontiers in Robotics and AI, 10, 1212626.

Probing the impact of the transcription factor Runx1, involved in spiral ganglion neuron subtype specification, on afferent synaptic transmission and neural firing properties

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Our ability to hear allows us to communicate and connect to the world around us. However, over 430 million people globally face disabling hearing impairment (WHO, 2024). Even though hearing aids and prostheses are widely used, they typically only partially restore hearing. To improve hearing restoration, it is essential to better understand how the auditory system encodes acoustic stimuli.
The auditory sound system responds to an astonishingly wide range of sound pressures, varying over six orders of magnitude. However, it remains unclear how this is achieved on a mechanistic level. While the receptor potential of inner hair cells (IHCs) covers the entire range of audible sound pressures, their postsynaptic counterparts, spiral ganglion neurons (SGNs), only change their firing rate over fractions of it. However, their response functions differ and tile the audible range of sound pressures such that it is fully represented on the SGN population level. The diverse functional properties of SGNs are hypothesized to arise from heterogeneous presynaptic input, molecularly diverse SGN subtypes, and differences in their efferent control.
Single cell transcriptomic analyses revealed three distinct molecular profiles of SGNs, Ia, Ib, and Ic, which led to the notion that they might correspond to the previously defined high, medium, and low spontaneous firing rate (SR) SGNs, respectively. Further, transsynaptic signaling by this three molecularly distinct type I SGNs is believed to play a role in establishing the diversity in IHC synapses.
We investigated how genetic inactivation of Runx1, a transcription factor highly expressed in I b,c SGNs and almost absent in Ia SGNs, influences spontaneous and sound evoked SGN firing in vivo, as well as presynaptic Ca^{2+} channel properties. Recently, it has been shown that the conditional deletion of Runx1 in mice (Runx1cKO) leads to increased abundance of type Ia SGNs and fewer Ib,c SGNs. Therefore, we hypothesized to find a larger population of high SR fibers with lower thresholds. Further, we expected transsynaptic signaling at the afferent synapses to be of type Ia potentially abolishing the gradients of the properties of presynaptic Ca^{2+} influx along the modiolar-pillar axis.
Using spinning disc confocal Ca^{2+} imaging combined with whole-cell patch-clamp, we found that both maximal amplitude and voltage-dependence of the whole-cell Ca^{2+} influx were unchanged in IHCs. However, the pillar-modiolar gradient of the voltage dependence of Ca^{2+} influx at individual IHC active zones (AZs): pillar AZs activating at lower voltages than modiolar ones, was collapsed supporting our hypothesis. In vivo, we observed normal hearing thresholds assessed by recordings of auditory brainstem

responses (ABRs). Preliminary recordings from single SGNs in Runx1cKO mice suggest a higher spontaneous firing rates but mostly unchanged sound evoked firing.
Overall, our findings contribute to a better understanding of how the dynamic range is established. We propose that the transcriptional program of SGNs controlled by Runx1 plays a crucial role in establishing the presynaptic gradient via transsynaptic signaling. Moreover, we find preliminary indication of a greater proportion of high SR SGNs in Runx1 cKO that have a higher abundance of type Ia SGNs. This supports the hypothesis that type Ia correspond to high SR SGNs, which however, needs further testing.

Paralemmin-3 – an essential constituent of the submembrane cytoskeleton of auditory hair cells

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In the mammalian inner ear, cochlear inner hair cells (IHCs) enable accurate and faithful synaptic sound encoding, while outer hair cells (OHCs) perform frequency-specific sound amplification and fine-tuning through their intrinsic voltage-dependent somatic electromotility. This latter process is facilitated by the unique trilaminate structure of the OHC lateral wall, which consists of the transmembrane motor protein Prestin, the subcortical actin- and spectrin-based cytoskeleton, and the cytoplasmic subsurface cisternae. This complex system is essential for both, mechanical rigidity and stability as well as cell expansion and contraction during electromotility. Whereas the structure of the lateral wall is well described, the molecular composition remains largely elusive. Here, we identified paralemmin-3 (Palm3) as a novel protein specifically localized to the lateral walls of auditory HCs that may play a crucial role in connecting the PM to the underlying cytoskeleton. Palm3-KO mice exhibit early-onset and progressive hearing impairment that results from diminished cochlear amplification. Subsequent multiscale morphological analyses revealed structural collapse of OHCs that led to progressive and extensive OHC loss along the cochlear axis. Furthermore, Palm3-KO OHCs exhibit disrupted distribution and attenuated expression of several membrane-associated proteins – including Prestin and alpha2-Spectrin – suggesting a role of Palm3 in plasma membrane scaffolding. In line with this hypothesis, electron tomography of OHC lateral walls revealed significantly fewer and structurally perturbed cisternal structures in Palm3-KO HCs compared to WT littermates. Finally, adeno-associated virus-mediated rescue of Palm3 during early postnatal development partly restored hearing function, enhanced OHC survival, as well as restored OHC cell shape and membrane protein expression. In summary, Palm3 is a protein found in the submembrane cytoskeleton of cochlear hair cells that appears to play an essential role in hair cell biology and hearing.

Evaluating the Spread of Excitation with Red Light Optogenetic Stimulation of the Auditory Nerve Through Computer Simulations and *In-Vivo* Electrophysiology

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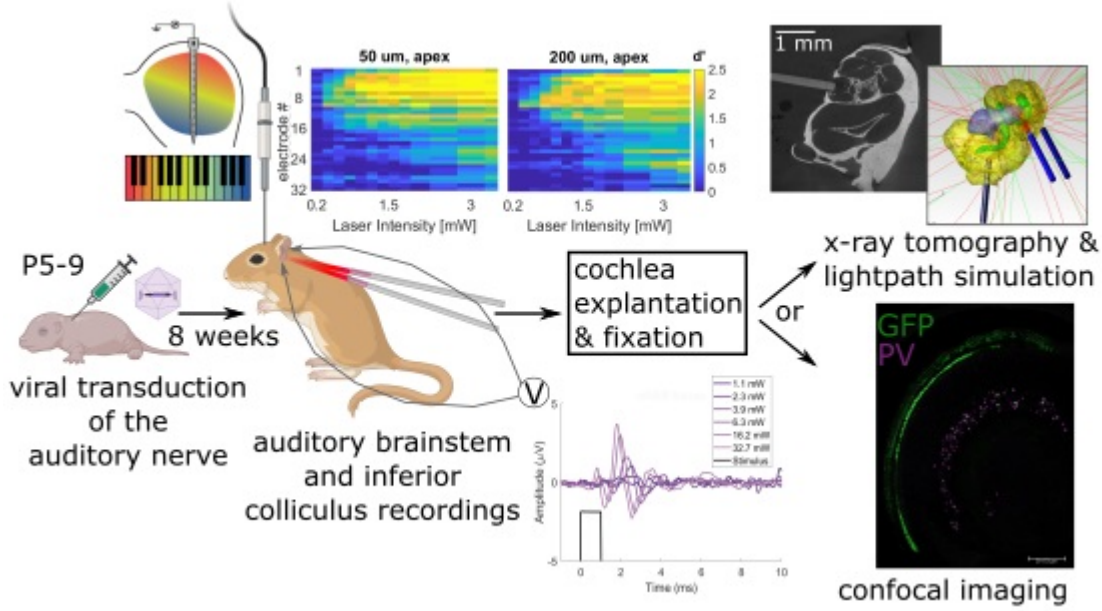
The future optical cochlear implant (oCI) utilizes optogenetic stimulation of the spiral ganglion neurons (SGNs) in the cochlea to improve hearing restoration beyond the state of the art. Preclinical data indicates that this may improve bionic hearing in CI users by more spectrally confined SGN activation compared to electrical stimulation. This study investigates the influence of the light emitter characteristics (one/two laser-coupled glass fibers) on the spatial (spectral) extent of neural activation in red light optogenetic SGN stimulation.

For an initial estimation of the excited cochlear volume, we used 3D modelling based on x-ray data of nine Mongolian gerbil cochleae with sham fiber insertions to simulate light spread and explore relationships between insertion parameters and the irradiance experienced by the SGNs. We then performed *in-vivo* multiunit recordings of inferior colliculus multi-unit activity in anesthetized Mongolian gerbils that postnatally received AAV mediated gene therapy to render their SGNs light sensitive via expression of the red-light activated channelrhodopsin f-Chrimson. During the *in-vivo* recordings, we systematically varied stimulus intensity, fiber diameter, position within the cochlea, insertion angle, and, the inter-fiber distance, and analysed the cochlear spread of excitation (SoE) in the inferior colliculus. Afterwards we determined the density and f-Chrimson-EYFP expression of SGNs using immunohistochemistry and confocal microscopy.

We collected recordings from 13 animals. We observed that light stimulation through an apical cochleostomy activates neurons in low-frequency regions while basal stimulation activates high-frequency regions, in agreement with previous studies. Using f-Chrimson allowed for stimulation with repetition rates up to 350 Hz. Smaller fiber diameters can have a more confined excitation pattern than larger diameters when they point directly at the Rosenthal's canal. This ideal fiber placement is harder to achieve for smaller diameters. Around the radiant flux threshold that is needed to detect a significant change in spike rate (sometimes below 0.5 mW) the SoE was comparable to acoustic stimulation. For high radiant flux the SoE was larger for light stimulation than for the acoustic control with comparable spike rates. Moreover, we sometimes observed two activation bands at high radiant fluxes that was not obvious with blue light stimulation in previous studies.

The aim of this dataset is to help in establishing the design of the future optical cochlear implant and the optogenetic sound coding strategy. We can use the observed results on the activation threshold and spread of excitation for large stimulation intensities to derive requirements on the radiant flux range for light emitters of the future medical device. Moreover, the observed importance of appropriate emitter

positioning needs to be taken into account in finalizing the implant design and possible insertion tools. Ongoing work focusses on the interaction between neighbouring emitters to inform about effects and extent of channel interaction in space and time.



Overview of the experimental workflow

In-Silico Framework for Benchmarking Optogenetic Hearing Restoration

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Background: Optogenetic cochlear implants (oCIs) represent a promising means to better restore hearing in individuals impacted by severe sensorineural hearing loss than possible with electrical cochlear implants (eCIs). The wide spread of current and channel interactions in eCIs limit comprehension of speech in noisy environments and the enjoyment of music. By reducing the spread of neural activation, oCIs promise a greater number of independent stimulation channels.

Methods: A computational framework for the evaluation of oCIs in the human cochlea was developed using four main modules. First, a generic n-of-m sound coding strategy was implemented, which could be easily adjusted to evaluate various parameters. Second, a three-dimensional ray-tracing model of a reconstructed human cochlea was used to investigate light propagation. Third, a biophysical model of spiral ganglion neurons (SGNs) was built to simulate optogenetically evoked firing. Fourth, a similarity measure was developed to compare the input sound spectrograph to the output spikes pattern. Finally, these stages were integrated to generate a comprehensive model capable of processing an audio files dataset and computing a similarity score.

Results: The major findings indicate that the spatial spread of light using µLED- and waveguide-based oCIs is narrower than the electrical current spread. Moreover, the impact of variables such as emitter-to-SGN distance, emitter rotation, and scar tissue formation on the irradiance at SGNs was evaluated. The improved spectral resolution of oCIs compensates for the currently lower temporal fidelity of optogenetically driven firing.

Conclusion: The computational framework provides a valuable resource for researchers to explore the complex interplays of sound processing, light delivery, and optogenetic stimulation. This study supports the notion that optogenetic stimulation of the cochlea could improve the speech understanding of CI users.

Evaluating the utility of virtual-channel-based sound-to-neuron stimulation strategy for future optogenetic cochlear implants

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Electrical cochlear implants (eCIs) are considered the most successful neuroprostheses and represent the state-of-the-art rehabilitation devices for individuals with severe to profound hearing loss. While eCIs users typically achieve fair open-set speech perception in the quiet, their understanding speech in daily situations with background noise and music perception remain limited. The reason is a large spread of electric current from each intracochlear electrode activating a tonotopically broad population of auditory neurons. This limits spectral selectivity of electrical sound encoding to less than 10 perceptually independent stimulation channels. Despite many efforts, this has not improved over a decade. These efforts also include current steering where two channels are simultaneously stimulated at different intensities to create intermediate virtual channels between them reaching total of 120 channels in Advanced Bionics HiRes Fidelity 120 strategy. Clinically, this did not robustly improve the outcome of eCI hearing rehabilitation. On the other hand, optogenetic cochlear implants (oCIs) hold the potential to overcome eCI bottleneck as light can be better confined in the fluid-filled cochlea volume than electric current, promising enhanced spectral resolution. In order to capitalize on the expected increased number of perceptually independent channels for sound coding in future oCI, we adapted the SpecRes strategy (research version of HiRes Fidelity 120) implemented in open-source generic MATLAB toolbox (GMT). Using our recently established in silico framework for benchmarking hearing restoration (FraSCO) we demonstrate the potential and limitations of the modified SpecRes strategy as a starting point for further developing future oCI-tailored coding strategies.

Evaluation of optogenetic therapy for hearing restoration in rodent models of sensorineural hearing loss

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To overcome the wide-spread neural excitation associated with electrical cochlear implants (eCI), stimulation of spiral ganglion neurons (SGNs) using light presents an attractive solution, as light can be better confined in space. Using optogenetics, SGNs are rendered light sensitive by viral delivery of a Channelrhodopsin (ChR) to the cochlea. Previous work provided in vivo evidence to support the clinical potential of the optical CI, but for clinical translation, it is critical to optimize individual components of the gene therapy. To this end, we have employed different rodent models of deafness to investigate the preclinical efficacy of this therapy. Adeno-associated virus carrying ChRs under the control of the human synapsin promotor were introduced to cochleae of adult kanamycin-deafened Mongolian gerbils using a microcatheter inserted into the round window (RW) with a vent at the stapedial footplate; and to transgenic Otoferlin-KO mice at postnatal day 6 using pressure injection through the RW. After 4 to 6 weeks, a laser-coupled fiber (594, 522, or 660 nm) was inserted into the RW and optically evoked auditory brainstem recordings (oABR) were measured. Subsequently, cochleae were extracted for immunohistological analysis. Preliminary results show that kanamycin deafening produced a shift in acoustic ABR threshold of at least 50 dB SPL in adult gerbils. Kanamycin deafening was further validated through the observed loss of hair cells in the immunohistological evaluation. Notably, Otoferlin-KO mice injected with AAV carrying the new powerful ChR variant, ChReef, exhibited sizeable oABR amplitudes and low energy thresholds (170 nJ). Immunohistological evaluation shows robust expression of ChReef in SGNs throughout all turns of the cochlea.

From Sound to Movement: The Neural Backbone of the Acoustic Startle Reflex

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The acoustic startle reflex (ASR) is a fast reactive body movement in response to a loud and unexpected auditory stimulus that has been observed in all mammalian species. In rats, large cochlear root neurons (CRNs) – located between auditory periphery and cochlear nucleus – have been identified as the first neural substrate of the underlying reflex circuit. CRNs receive auditory input from primary afferent spiral ganglion neurons (SGNs) and are subject to modulatory pathways descending from higher brain areas. Their axons mainly target neurons of the contralateral pontine reticular nucleus (PnC) formation, which project onto cranial and spinal motor neurons and are in turn subject to extensive modulatory inputs from various cell types located in higher brain areas. Despite their central role in a reflex circuit directly implicated with individual survival, CRNs are still remarkably poorly characterized in regard to their cellular excitability and activity patterns as well as their molecular composition and morphology in situ. Furthermore, despite behaviorally apparent acoustic startle reflexes in humans, the taxonomic equivalent of CRNs has yet to be identified. In our work, we set out to characterize CRNs in various animal models regarding their morphology, their pattern and identity of somatic innervation as well as their distribution from the modiolar base to the cochlear nucleus. In an attempt to spatially map the entire population of CRNs, we combine immunofluorescent labeling with whole cochlea tissue clearing and 3D reconstructions. Furthermore, we employ various genetic reporter and knockout mouse models alongside neuronal tracer dyes to characterize the afferent input pattern of SGNs and assess their cellular morphology as well as connectivity. Finally, we seek to complement these histological datasets with functional analyses. In this way, we hope to further the understanding of the primary neural base of the ASR, building the base for further investigations in different mammalian species, including humans.

A minimal magnetosensory circuit in the pigeon brain

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The remarkable navigation abilities of the rock pigeon (Columba livia) are facilitated by a sensory system that allows the detection of magnetic fields. While there is compelling behavioral evidence demonstrating the existence of a magnetic sense, the neuronal circuits that process magnetic information in the brain are largely unknown, and the primary sensory cells and transduction molecules have yet to be identified. To address these issues, we combined tissue clearing and light sheet microscopy to screen the pigeon brain for magnetically induced neuronal activity via C-FOS expression. This screen revealed a minimal magnetosensory pathway that involves the central vestibular nuclei in the brainstem and the mesopallium, a multisensory integration center in the forebrain. These regions were robustly activated in magnetic experiments independently performed under white light and in total darkness. Building on these results, we investigated whether the receptive module of the magnetic sense is located in the vestibular system of the pigeon. Physical modelling suggests that the transduction mechanism of magnetoreception could rely on the detection of electric currents induced by magnetic fields in the semicircular canals of the pigeon. Consistent with this hypothesis, we report the presence of a splice isoform of the voltage-gated calcium channel CaV1.3, which is known to play a key role in electroreception, in sensory hair cells of the pigeon inner ear. The development of our screening platform and the identification of stably activated neuronal regions to magnetic stimuli will enable us to preform mechanistic experiments testing CaV1.3's role in magnetoreception.

Assessment of Glutamatergic quantal transmission insufficiency in sensory vestibular functioning.

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The type-3 vesicular glutamate transporter (VGLUT3) in the cochlea facilitates quantal synaptic transmission at the inner hair cell afferent synapses. Similarly, quantal synaptic transmission in vestibular endorgans has also been understood to be glutamatergic, with the expression of VGLUT3 in vestibular hair cells (VHCs). While the genetic deletion of *SLC17A8*, encoding VGLUT3, results in deafness in mice due to the lack of cochlear afferent transmission, the role of VGLUT3 in vestibular function is yet to be elucidated. In this study, we examined the vestibular characteristics of VGLUT3 deficient (*Vglut3-/-*) mice at cellular, system, and behavioral levels. Unlike auditory deficits, *Vglut3-/-* mice showed no significant sensorimotor or balance deficits in tests like RotaRod, inclined screen tests, or open field tests for behavioural analysis. Cellular characterisation revealed strong VGLUT3 immunoreactivity in type-II VHCs and weaker reactivity in type-I VHCs in the utricles and cristae of wild-type (WT) mice, which was absent in *Vglut3-/-* mice. Very sparse VGLUT2 immunoreactivity was noted in the VHCs of WT mice, with no significant upregulation in *Vglut3-/-*mice, while VGLUT1 was not detected in VHCs of either genotype. The absence of noticeable balance issues, continued spontaneous activity without VGLUT3 despite no compensation from VGLUT1/2, and the limited quantal transmission support the idea that non-quantal transmission is the primary mode of neurotransmission between VHCs and vestibular afferent neurons. We propose that non-quantal transmission alone accounts for the seemingly normal vestibular function in *Vglut3-/-* mice.

Investigating the neural correlates of the magnetic sense in the pigeon

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Magnetoreception is a widespread sensory modality that enables animals to detect the Earth's magnetic field. While behavioral evidence strongly points towards the importance of this sensory system for different animals (including many avian species), there are many aspects which are still not understood: 1) Where is the dedicated sensory organ located? 2) How is magnetic information encoded in the brain of these animals? Our lab has previously identified regions in the pigeon brain that show a significant increase in neural activity to the presentation of magnetic stimuli. Our aim is to investigate the neuronal representation of the magnetic sense in two of these areas in the pigeon forebrain: the mesopallium and the hippocampus. To achieve this, we employ high-density, multi-channel Neuropixels probes and developed a pipeline to identify neurons that show a significant response to the stimuli presented.

Poster Topic

T18: Auditory System: Subcortical and Cortical Processing

T18-1A Hearing more than Sound – Shining a light on somatosensory brainstem projections to the auditory midbrain
Falk Brönnle, Aaron Benson Wong

T18-2A Improved temporal processing in the inferior colliculus of mice lacking the extracellular matrix protein brevican
Simone Kurt, Gerhard Bracic, Mira Türknetz, Jutta Engel

T18-3A Auditory Competition or Binaural Decorrelation? A Comparison Between Midbrain Space Maps in the Barn Owl
Roland Ferger, Andrea J. Bae, José L. Peña

T18-4A Origins of the Auditory Brainstem Response (ABR) in Mice: Source Localization with Multichannel Topographic EEG
Xue Wang, Andrej Kral, Rüdiger Land

T18-5A The function of frontal subcortical projections during multisensory task learning
Nilufar Nojavan Lahiji, Irene Lenzi, Björn Kampa, Simon Musall

T18-6A Impact of sub-lethal dosages of the insecticide flupyradifurone on the ascending auditory interneurons in the cricket brain
Marcelo Christian, Manuela Nowotny, Stefan Schöneich

T18-1B Central compensation of neural responses to cochlear synaptopathy can be supported by dendritic spine remodeling through elevated cGMP levels
Joana Ibrahim-Bacha, Dila Calis, Morgan Hess, Csaba Harasztosi, Stefan Fink, Michele Jacob, Peter Ruth, Lukas Rüttiger, Marlies Knipper, Wibke Singer

T18-2B Investigation of the interaction of stress hormone receptors and BDNF for hearing function in the animal model mouse
Leonas Adam, Joana Ibrahim-Bacha, Wibke Singer, Marlies Knipper, Lukas Rüttiger

T18-3B Impact of Otoferlin Mutation on Spontaneous and Sound evoked SGN Activity
Abigail Trebilcock, Han Chen, Fritz Benseler, Nils Brose, Tobias Moser

T18-4B Auditory cortex extracellular matrix density is increased in Mongolian gerbils with tinnitus
Konstantin Tziridis, Holger Schulze

T18-5B Stimulus Onset contributions to Speech Comprehension
Lukas Rüttiger, Jakob Schirmer, Konrad Dapper, Stephan Wolpert, Marjoleen Wouters, Katharina Bader, Wibke Singer, Etienne Gaudrain, Deniz Baskent, Sarah Verhulst, Christoph Braun, Matthias Munk, Ernst Dalhoff, Marlies Knipper

T18-6B Use of OPM-MEG for auditory research
Rodrigo Andrés Donoso-San Martín, Stephan Wolpert, Stefan Fink, Markus Siegel, Paul H. Delano, Christoph Braun, Lukas Rüttiger, Marlies Knipper

T18-7B Characterization of sound evoked responses in neurons of the INLL
Nikolaos Kladisios, Felix Felmy

T18-1C Multifunctional Organization of The Computational Map of Target Distance in Bats
Ali Roustazadeh, Uwe Firzlaff

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Hearing more than Sound – Shining a light on somatosensory brainstem projections to the auditory midbrain

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The inferior colliculus (IC) is a midbrain nucleus playing a major role in the auditory pathway. Its multisensory region, the lateral cortex of the IC (LCIC), was shown to receive inputs from somatosensory regions such as the somatosensory cortex and the dorsal column nuclei (DColN) (Lesicko, 2016). The function of those somatosensory projections is still largely unclear and hypotheses include the suppression of perception of self-generated sound or aiding in orientation towards sound sources (Wu, 2014; Gruters, 2012).

The LCIC can be divided into module and matrix zones using histological staining (anti-GAD, cytochrome oxidase, NADPH-d, AChE), where projections of the somatosensory nuclei terminate primarily into the modules of the LCIC (Lesicko, 2016). However, it is still unknown whether this segregation also applies on a cellular level and whether there is a convergence of the different somatosensory input streams within the IC. Moreover, one of the somatosensory input regions, the DColN was suggested to show a tonotopic and somatotopic map (Lee, 2023). It is still unclear whether these maps are conserved within the efferent projections towards the IC and whether a somatotopic map can be found within the IC. To answer these questions we performed anterograde transsynaptic tracings to localize somatosensory inputs and somatosensory recipient cells in the IC.

Here we used the anterograde transsynaptic properties of adeno-associated virus serotype 1 and a novel surgical approach [adapted from (Joshi, 2022)] to label DColN-to-IC projections and DColN-recipient cells in the IC and other brain areas. We injected AAV1-Cre and AAV1-eGFP into the DColN of Cre-dependent reporter mouse line Ai14 (tdTomato expression) in a B6CBAF1 background. To assess whether there is a conservation of somatotopy, the injection site was systematically varied along the anterior-posterior (AP) extend of the rostral half of DColN. After 3 weeks of incubation time the animals were sacrificed and perfused with 4%-PFA. The brains were dissected and sectioned into 50 µm thick coronal slices. One in 3 slices were DAPI stained, imaged and aligned to the Allen Brain Atlas to study the projection patterns of the DColN.

Our results have revealed a robust contralateral-dominated bilateral projection from the DColN to the IC with extensive projection fibers terminating in the LCIC in a module-like pattern. No obvious correlation between projection pattern and the AP-position of the injection site was seen so far. Future experiments with injections to the caudal portion of DColN will address whether a coarser somatotopic organization exists.

Transsynaptically-labelled cells were observed within the LCIC and the “purely-auditory” central nucleus (CNIC) of the ipsi- and contralateral IC. Furthermore, the DColN injections have shown interesting projection patterns to the forebrain, including known targets of DColN such as the ventral posterolateral nucleus of the thalamus as well as not well-described targets in the cerebral cortex and the hippocampus.

The reliable transsynaptic labeling of the CNIC cells and the suggested projections to cerebral areas indicate more a complex projection pattern than thus far suggested.

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Improved temporal processing in the inferior colliculus of mice lacking the extracellular matrix protein brevican

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The proteoglycan brevican is a major component of the extracellular matrix of perineuronal nets and is highly enriched in the perisynaptic space suggesting a modulatory role in synaptic transmission. Brevican is part of perineuronal nets at various stations of the auditory pathway, e.g. at inner hair cells synapses and at the somata of bushy cells, octopus cells in the cochlear nuclear complex, medial nucleus of the trapezoid body (MNTB) neurons, medial superior olive (MSO) and lateral superior olive (LSO) neurons, neurons of the dorsal nucleus of the lateral lemniscus (DNLL) and some neurons of the inferior colliculus (IC) and the auditory cortex (AC). We have studied the role of brevican for spectral and temporal coding in the IC by using brevican knockout (bcan-/-) mice. To this aim, we performed *in vivo* electrophysiological recordings from neurons of the IC from systemic bcan-/- and bcan+/+ littermates. Responses of IC neurons to pure tones and amplitude-modulated (AM) tones were recorded and characterized as a function of modulation frequency. Hearing thresholds, spontaneous rate, and frequency tuning characteristics of IC neurons of bcan-/- mice were normal. Lack of brevican, however, led to shorter first spike latencies of neuronal responses at modulation frequencies ≥ 70 Hz and to two- to threefold evoked rates in response to AM tone stimulation. Surprisingly, the vector strength and the corresponding correlation coefficients of phase locking ≥ 50 Hz modulation frequency were increased in bcan-/- mice compared to bcan+/+ littermates.
Taken together, our results demonstrate that lack of the extracellular matrix protein brevican improves rather than impairs auditory processing of AM tones. So far, we cannot explain these puzzling results. One problem of this study is the systemic deletion of brevican. Experiments using conditional, region-specific knockout mouse models would help to understand the effects of brevican in the auditory pathway in more detail.

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Auditory Competition or Binaural Decorrelation? A Comparison Between Midbrain Space Maps in the Barn Owl

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The natural environment challenges the brain to prioritize the processing of salient stimuli. This is especially difficult for concurrent auditory stimuli because sound waves interact in a way that the phase of each contained frequency can be altered prior to arrival at the eardrum. Therefore, phase information is altered whenever concurrent sounds consist of overlapping frequency spectra. This interaction depends on the relative phase of two signals and, thus, is different at each ear for spatially separated sound sources, leading to binaural decorrelation. The barn owl, a sound localization specialist, uses interaural time difference (ITD) as primary cue for localizing the azimuth of a sound source. The detection of ITD relies on the binaural correlation and consequently suffers from binaural decorrelation. However, the owl exhibits a circuit called the midbrain stimulus selection network, dedicated to representing locations of the most salient stimulus in circumstances of concurrent stimuli. Previous competition studies using unimodal (visual) and bimodal (visual and auditory) stimuli have shown that relative strength is encoded in the spike response rates. Open questions remained concerning competition between concurrent auditory signals on coding.

To this end, we presented diverse auditory competitors (concurrent flat noise and amplitude modulated noise) and recorded neural responses of awake barn owls in subsequent midbrain space maps, the external nucleus of the inferior colliculus (ICx) and optic tectum (OT, homologue to the mammalian superior colliculus). Other work has shown that binaural decorrelation can explain a decrease in spike response rates in ICx. In this study, we expanded the above experiments to use competing stimuli that were spectrally non-overlapping, ruling out binaural decorrelation, but contained enough frequencies across the owls hearing range to be unambiguously localized.

While both ICx and OT exhibit a topographic map of auditory space, OT also integrates visual input and is part of the global-inhibitory midbrain stimulus selection network. Through comparative investigation of these regions, we show that while increasing strength of a competitor sound decreases spike response rates of spatially distant neurons in both regions, relative strength determines spike train synchrony of nearby units only in OT. Furthermore, changes in synchrony by sound competition in OT are correlated to gamma range oscillations of local field potentials (LFPs), associated with input from the midbrain stimulus selection network. Our results suggest that modulations in spiking synchrony between units by gamma oscillations are an emergent coding scheme representing relative strength of concurrent stimuli, which may have relevant implications for downstream forebrain read out.

We compare results in both midbrain maps according to the effect of spectrally overlapping and non-overlapping stimuli on spike rates and features of the LFP. This further elucidates the essential role of the midbrain stimulus selection network for selecting the most salient stimulus.

Origins of the Auditory Brainstem Response (ABR) in Mice: Source Localization with Multichannel Topographic EEG

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BACKGROUND: The auditory brainstem response (ABR) is widely used to assess auditory brainstem function in mice. However, despite its widespread use, the origins of ABR waves beyond wave I specific to mice remain poorly validated. Remarkably, only two studies in the past 40 years have addressed this issue, which limits the reliability of ABR waves as markers for specific auditory brainstem structures in mice.

METHODS: We used a novel approach to localize ABR wave sources specific to mice by using multichannel topographic EEG recordings. We recorded the topography of binaural click-evoked ABRs using a 30-channel thin-film EEG array from the skull of 15 adult mice combined with 32-channel multielectrode recordings from the auditory cortex. We applied sensor-level analysis and source reconstruction to identify the anatomical origins of ABR waves specific to mice.

RESULTS: The thin-film recorded ABR waves showed a series of distinct spatial topographies. Wave I was strongly lateralized supporting its auditory nerve origin. Waves II/III showed a different lateralized more frontal distribution, supporting a cochlear nucleus and olivary structure origin. This was followed by a distinct wave IV topography at ~4.5 ms with a focused local activity only at electrodes above the inferior colliculus (IC). This IC origin of wave IV was also supported by dipole source reconstruction. Wide-band filtering additionally revealed a late IC wave P0 as a second marker of IC activity at ~9.5 ms, whose latency overlapped with evoked far-field activity in the auditory cortex.

CONCLUSION: The results show that wave IV of the mouse ABR originates from IC activity, providing a validated marker to distinguish pre-IC from post-IC and cortical activity. These findings enhance the understanding of mouse-specific ABR wave origins. Given its widespread use in mouse models, testing higher density EEG to further refine the localization of mouse ABR components is desirable.

The function of frontal subcortical projections during multisensory task learning

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Perceptual decision-making involves transforming integrated sensory inputs into appropriate motor responses. Crucial to this process is the anterior lateral motor cortex (ALM) and its long-range projections to subcortical regions, particularly to the striatum, thalamus, and superior colliculus (SC). Pyramidal neurons within the ALM display heterogeneous activity patterns, ranging from anticipating ramping activity to spiking activity during the response epoch, suggesting that neurons with distinct subcortical projection targets transmit separate information to their respective target regions. However, the specific functional roles of these projection pathways in the learning and execution of sensory-guided behavior remains not fully understood.

To investigate role of the ALM projections to different subcortical targets during auditory-guided decision-making, we used a metabotropic encephalopsin (eOPN3) to selectively inhibit the synaptic release of a specific ALM projection pathways without affecting others. To study the important of ALM projections during learning, mice were first tested in an innately-known motor task and then trained on auditory tasks with increasing difficulty. Our results indicate that projections to the striatum are most important during the early motor skill-learning period but then diminish in importance as expertise is gained. In contrast, projections to the thalamus and SC became more influential in trained mice, suggesting a switch in cortical projection importance from corticostriatal to thalamic- and midbrain-projections to translate sensory information into corresponding behavior.

To further investigate the population dynamics of individual ALM projection neurons, we will label neuronal populations that either project to the striatum or the thalamus with two fluorophores (tdTomato/Alexa647) and conduct chronic 2-photon imaging across cortical layers (L2/3 & L5) while mice learn and perform the same sensory tasks. This approach will provide insights into the temporal evolution of activity changes of projection neurons across cortical layers. Lastly, we will further extend the behavioral task to a multisensory discrimination task to also reveal if decision formation in ALM neuronal populations occurs in a modality-specific manner.

Together, this work aims to uncover how different neural populations in ALM orchestrate the transition from motor skill learning and integration of complex multisensory decisions into behavior across different cortical layers and subcortical projection pathways.

Impact of sub-lethal dosages of the insecticide flupyradifurone on the ascending auditory interneurons in the cricket brain

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Insecticides are an essential part of modern agriculture. However, their extensive use has also been associated with the world-wide decline of the insect fauna over the last decades. Therefore, it is crucial to better understand the impact of modern insecticides on non-target insects with diverse behavioural lifestyles. Here, we analysed the effect of the insecticide flupyradifurone on the auditory pathway of the field cricket *Gryllus bimaculatus*. Flupyradifurone acts as highly selective agonist at achetylcholine receptors in the insect CNS. We recorded the spiking activity of ascending auditory interneurons AN1 and AN2 using a suction electrode at the brain surface. We measured the spiking response to acoustic stimulation (5, 10, 15, 20 and 30 kHz at 70, 75 and 80 dB) while applying different concentrations (10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} mol/l) of insecticide. Flupyradifurone caused a dose-dependent increase of spontaneous spiking activity. In contrast it led to a decrease in stimulus correlated spiking activity of ascending auditory interneurons, peaking in a collapse of activity at the highest measured concentrations. Crickets rely heavily on auditory information for phonotactic mate finding and predator avoidance. Therefore, the disruption of auditory processing may substantially reduce their chances for survival and reproduction.

Central compensation of neural responses to cochlear synaptopathy can be supported by dendritic spine remodeling through elevated cGMP levels

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Hearing loss is increasingly acknowledged as a significant cause of disability and a notable risk factor for cognitive decline and dementia. Many older adults struggle to understand speech in noisy environments, often due to progressive cochlear synaptopathy, which involves the loss of synaptic connections between hair cells and auditory nerve fibers. Previous studies have indicated that cochlear synaptopathy can lead to poorer temporal auditory processing, as manifested by reduced auditory steady state responses (ASSRs). However, in certain instances, deficits in temporal processing can be alleviated by central compensatory mechanisms, such as an enhanced input/output function of auditory brainstem responses (neural gain). This is accompanied by a better hippocampal long-term potentiation (LTP) and long-term depression (LTD) adjustment and upregulation of brain-derived neurotrophic factor (BDNF). In this study, we investigated the cyclic guanosine monophosphate (cGMP) signaling pathway as a potential molecular target involved in central auditory compensation. Mice were administered either a phosphodiesterase 9A inhibitor, which raises intracellular cGMP levels, or a vehicle control. Our results indicate a higher level of cGMP, which favors structural remodeling for central auditory adaptation and points toward the importance of this pathway in compensatory processes.
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Investigation of the interaction of stress hormone receptors and BDNF for hearing function in the animal model mouse

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To date, there are no standardized, scientifically proven treatment approaches for hearing disorders. One reason for this is the variety of different causes for hearing disorders. It is known that factors such as anxiety or stress, but also disturbed signal processing between auditory and non-auditory brain areas, contribute to hearing disorders. The extent to which stress or stress hormones are involved in the interaction between the auditory system and associated brain regions is unclear.
In preliminary studies we could show that a balanced interaction of the stress hormone receptors MR and GR, but also of excitation and inhibition, controlled by neurotrophins such as activity-dependent BDNF, is crucial for intact auditory function. The central deletion of MR or GR has effects on the peripheral auditory system that lead to altered stimulus processing and transmission as well as on central plasticity mechanisms that are important for adaptation to altered input activity. Further studies have shown that the same mechanisms are also associated with changes in activity-dependent BDNF.
Although it is known that glucocorticoids and BDNF share similar intracellular signaling pathways and regulate each other's function at multiple levels, the exact interplay for individual physiological processes such as in our case hearing function is not clear.
In the project presented here, new mouse lines are being investigated that enable both the inducible tissue-specific deletion of one or both stress hormone receptors (MR or GR) and the visualization of activity-dependent BDNF via intrinsically expressed fluorescent proteins (CFP and YFP).
These lines should provide information about the interaction of MR or GR and BDNF and how this can influence auditory function.

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Impact of Otoferlin Mutation on Spontaneous and Sound evoked SGN Activity

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Hearing loss is one of the leading causes of impairment globally and can lead to social withdrawal, higher risks of physical and psychiatric illness, higher rates of unemployment, and poorer educational outcomes. Investigating the genetic alterations that cause hereditary hearing loss is not only essential for improving hearing restoration, e.g. via future gene therapy, but is also an opportunity to understand the molecular physiology of the auditory system. One of the genes commonly affected in recessive sensorineural hearing loss is OTOF, which encodes the protein otoferlin. Numerous pathological variants of OTOF have been identified, causing hearing loss with a broad spectrum of phenotypes including profound to severe bilateral hearing loss known as DFNB96. Work in corresponding mouse models has revealed a crucial and multi-faceted role of otoferlin in inner hair cell (IHC) transmitter release. Otoferlin contains 6-7 C_2 domains of similar structure to the two Ca^{2+} binding C_2 domains in synaptotagmin. It has been shown to be important for several steps of the synaptic vesicle (SV) cycle including SV tethering and priming, Ca^{2+} sensing for SV fusion, and exo-endocytosis coupling. Despite the importance of otoferlin in IHC transmitter release, the precise mechanisms by which otoferlin achieves this function and how the specific C_2 domains are involved remain to be elucidated. The goal of this project was to interrogate the function of the C_2D domain of otoferlin in Ca^{2+} sensing for SV fusion and to examine the impact of presynaptic perturbations on sound encoding at the spiral ganglion neuron (SGN) level. Three mutant mouse strains were generated: $Otof^{D990A}$, $Otof^{D1060A}$, and $Otof^{D990A/D996A}$, by replacing aspartates with alanines in the C2D top loop for aspartates 990, 1060, or both 990 and 996 respectively. Importantly, immunohistochemistry indicated a normal number of afferent synapses per IHC and well maintained otoferlin protein levels in IHCs. In vivo SGN physiology was investigated in the $Otof^{D990A}$ mutant strain using acoustic auditory brainstem responses (aABRs) to measure SGN population responses and juxtacellular recordings from single SGNs. Drastically reduced aABR amplitudes were observed in $Otof^{D990A}$ mice compared to wildtype littermate controls despite similar aABR thresholds, likely indicating reduced synchronicity of SGN responses. This was supported by an elevation in first spike latency (FSL) and variance of FSL in recordings from single SGNs. Both findings indicate a reduction in the temporal precision of SGN sound encoding with the $Otof^{D990A}$ mutation. Moreover, we found spontaneous and sound-evoked firing rates of SGNs to be reduced in $Otof^{D990A}$ mice. Preliminary data suggest that sound-evoked responses were partially recovered with longer interstimulus intervals, indicating a deficiency in rapid SV replenishment to the IHC active zone. These data suggest that the D990A substitution impairs IHC transmitter release potentially at the level of Ca^{2+} triggered fusion but likely also at the level of Ca^{2+} sensitive SV replenishment. Mutations such as this present a unique opportunity to examine the impact of presynaptic perturbations on SGN physiology by not completely ablating otoferlin's function. Next, the other OtofC_2D mutant strains, $Otof^{D1060A}$ and $Otof^{D990A/D996A}$, will be similarly evaluated for effects on SGN sound encoding.

Auditory cortex extracellular matrix density is increased in Mongolian gerbils with tinnitus

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Subjective tinnitus is seen as the pathological result of an interaction of damage to the peripheral auditory system and central neuroplastic adaptations. Here we investigate such tinnitus related adaptations in the primary auditory cortex (AC) 7 and 13 days after noise trauma with possible induction of tinnitus by quantifying the density of the extracellular matrix (ECM) in the AC of Mongolian gerbils (*Meriones unguiculatus*). The ECM density has been shown to be relevant for neuroplastic processes and synaptic stability within the cortex.

We utilized a mild monaural acoustic noise trauma (controlled by auditory brainstem response audiometry) in overall 22 gerbils to induce tinnitus and a sham exposure in 16 control (C) animals. Tinnitus was assessed by a behavioral startle paradigm. Animals were separated for a presence (T) or absence (NT) of a tinnitus percept by the behavioral task.

The ECM density 7 and 13 days after trauma was quantified using immunofluorescence luminance of Wisteria floribunda lectin-fluoresceine-5-isothiocyanate (WFA-FITC) on histological slices of the primary AC, relative to the non-auditory brainstem as a reference area.

At both timepoints, we found that the WFA-FITC luminance of the AC of NT animals was not significantly different from that of C animals. However, we found a significant increase of luminance in T animals' ACs compared to NT or C animals' cortices. This effect was found exclusively on the AC side contralateral to the trauma ear.

These results point to a hemisphere specific process of stabilization of synaptic connections in primary AC, which may be involved in the chronic manifestation of tinnitus.

Stimulus Onset contributions to Speech Comprehension

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Slowing and reduction of auditory responses over age, promoting speech processing and cognitive deficits, are currently controversially discussed as being either related to central brain atrophy or slowing of neural processing from the periphery. We examined young, middle-aged, and older individuals with and without hearing threshold loss using pure-tone (PT) audiometry, short-pulsed distortion-product otoacoustic emissions (pDPOAE), auditory brainstem responses (ABR), auditory steady state responses (ASSR), speech comprehension (OLSA), and syllable discrimination in quiet and noise; amplitudes and latencies of speech EEG responses to syllables, and 4-5 word discrimination tasks using MEG. Speech comprehension deficits were identified dependent or independent of pure tone threshold (PTT) and age. Not only was poor speech comprehension independent of age and PTT linked with differences in cochlear amplifier performance and ABR wave latency shift but also phoneme induced thalamic delay (EEG) and altered attention requiring word discrimination responses in cortical regions (MEG). We furthermore discuss differential changes in amplitude and delays of thalamic or cortical activity in the context of differences in responses to phonemes requiring either temporal fine structure (TFS, < phase locking limit (PLL) or temporal envelope (TENV, >PLL) coding. Data may suggest possible new predictors for altered speech comprehension in quiet and ipsilateral noise that link deficits of the non-adapted, pre-neural input signal to the IHCs stimulus onset to neocortical activity.
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Use of OPM-MEG for auditory research

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Acquired auditory processing disorders including age dependent hearing loss, speech discrimination deficits, tinnitus or hyperacusis, require a personalized diagnosis to assign the individual cause within the auditory hierarchy to either the periphery, subcortical or distinct cortical or cortico-fugal neuronal dysfunctions. The well-functioning feedforward and feedback PV-IN network is an essential precondition for temporal intracortical network function in audition that above all senses relies on high speed of information flow (Zajac IT and Nettelbeck T, 2018). We hypothesize disease-specific deficits in temporal intracortical network function in auditory circuits. Therefore, the diagnostic of those should have a special significance. We used time-sensitive MEG-OPM measurements and aimed to study different auditory stimulus paradigms to detect fast auditory processing in different groups of tinnitus with and without hyperacusis or presbycusis. We expect this method to become an efficient diagnostic strategy to fathom peripheral or central contribution of the distinct auditory impairments in the future to improve individualized targeted interventional therapies. Here we will present preliminary results demonstrating the usability and function of the OPM-MEG for hearing research.

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Characterization of sound evoked responses in neurons of the INLL

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The intermediate nucleus of the lateral lemniscus (INLL) is involved in early cross-frequency integration of auditory information, as has been shown in bats and gerbils. This nucleus is localized in the auditory fibers of the lateral lemniscus between the ventral and the dorsal nucleus. Between the lemniscal nuclei, the dorsal nucleus is best distinguished, while the ventral and intermediate aspects are physiologically rarely separated, although they differ in neurotransmitter content. The biophysical properties of this cell population show vast heterogeneity in sub- and suprathreshold voltage responses. Besides the cross-frequency integration, little is known about the sound evoked response characteristics of the INLL aspect itself. Here, we characterize the sound evoked response properties selectively in INLL neurons by using in vivo single unit recordings in anesthetized gerbils.

Two types of frequency tuning curves exist in the INLL, one with a single best frequency and the other with two best frequencies. Irrespectively of tuning curves, INLL neurons respond either with onset, primary-like or sustained response patterns to pure tone stimulations. Noise stimulations shift the firing behavior in some neurons. The first spike latency and the jitter depend on sound intensity and are generally smaller in onset neurons. Determining the modulation transfer functions from transposed and sinusoidal amplitude modulated stimulations showed that first, INLL neurons have usually a low best modulation transfer frequency that often coincides with a high vector strength. Second, compared to sinusoidal amplitude modulated signals, transposed signals generate higher firing rates with also higher vector strength. Overall, our data indicate less heterogeneity in the sound evoked response patterns as suggested from the vast biophysical heterogeneity. The differences in frequency tuning will be interesting in the light of cross-frequency integration and in the processing of specific environmental sound objects.

Multifunctional Organization of The Computational Map of Target Distance in Bats

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In the auditory cortex (AC) of echolocating bats, the distance to an object (target) is represented in a specialized computational map. Neurons in this map fire action potentials only when echoes from objects, such as obstacles or prey, arrive with a specific delay after the bat emits a call. Unlike structural maps that reflect the topography of peripheral sensors, this target-distance map emerges from neural computations in the auditory system, with each neuron tuned to a specific call-echo delay.

Our previous studies have suggested that, beyond classical delay-tuned neurons, other neurons in the AC play a significant role in resolving multiple objects. However, the relationship between the activities of these different types of neurons remains unclear. In this study, we utilize Neuropixels probes to explore the connectivity and interactions between neurons with different functions in the AC, focusing on layer-specific responses to delay-tuned inputs. We aim to compare delay-tuned responses in the input layers (III–IV) of the AC with those from other layers that mediate interareal (corticocortical/corticofugal) connections and multimodal interactions.

The Neuropixels probes enable simultaneous recordings across all six cortical layers, with their densely placed contacts allowing us to distinguish between responses from nerve fibers (axons, dendrites) and cell bodies. This approach facilitates precise quantification of the contributions of both subcortical structures and cortical neurons to target-distance processing and object resolution in the bat AC.

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Sound Processing in Insects - Temporal Coding and Forward Masking in Spiking Responses

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Processing of auditory information can depend on previous stimulation, which can lead to temporal integration or adaptation. We described in a recent study how each pulse of a complex bushcricket song influences the spiking in response to the subsequent pulse. Bushcrickets (katydids) use acoustic signals for communication. In the species investigated here (*Mecopoda elongata*), the males call and form a chorus that attracts conspecific females. The male song consists of 10-20 broadband pulses (about 2 to 80 kHz) that are stereotypically repeated every two seconds. This temporal information seems to be important for mate finding for the females and chorus formation for the males. The temporal pattern of the song is encoded by the activity of a T-shaped neuron (one, on each side of the ganglion) as one of the auditory output interneurons.

We previously showed that we can monitor with a 32-channel multielectrode array the spiking and local field responses in the prothoracic ganglion in *M. elongata*. At this first processing stage along the ascending auditory pathway, we measured under tonal stimulation a long-lasting increase in the local field potentials (about 100 ms after end of stimulation) that points to intrinsic mechanism in the neuronal activity (Scherberich et al. 2024, jeb245497). Further, we showed that this transient summation in local field potentials over the time course of the conspecific chirp signal together with a reduction in spiking allows for a precise neuronal representation of the temporal structure of the song. By using artificial stimuli that differ in level and inter-pulse timing, we will now test in detail how the spiking depends on the stimulus quality.

Cerebellar activity predicts vocalization in fruit bats

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Echolocating bats exhibit remarkable auditory behaviors, enabled by adaptations within and outside their auditory system. Yet, research in echolocating bats has focused mostly on brain areas that belong to the classic ascending auditory pathway. This study provides direct evidence linking the cerebellum, an evolutionarily ancient and non-classic auditory structure, to vocalization and hearing. We report that in the fruit-eating bat Carollia perspicillata, external sounds can evoke cerebellar responses with latencies below 20 ms. Such fast responses are indicative of early inputs to the bat cerebellum. After establishing fruit-eating bats as a good model to study cerebellar auditory responses, we searched for a neural correlate of vocal production within the cerebellum. We investigated spike trains and field potentials occurring before and after vocalization and found that the type of sound produced (echolocation pulses or communication calls) can be decoded from pre-vocal and post-vocal neural signals, with prediction accuracies that reach above 85%. The latter provides a direct correlate of vocalization in an ancient motor-coordination structure that lies outside of the classic ascending auditory pathway. Taken together, our findings provide evidence of specializations for vocalization and hearing in the cerebellum of an auditory specialist.

Central Processing of Optical Hearing in the Anteroventral Cochlear Nucleus.

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Future cochlear implants may use light instead of electricity to restore hearing in patients with profound hearing loss. Unlike electricity, light can be spatially confined in the cochlea and could therefore achieve a greater frequency selectivity. Excitatory inputs from the spiral ganglion neurons are integrated in the anteroventral cochlear nucleus (AVCN), the first processing unit of the auditory pathway. AVCN neurons are diverse with regard to their morphology, regularity of physiological responses to sound, connectivity and molecular makeup. In this study, we aim to characterise the AVCN neural processing of cochlear optogenetic stimulation and probe the synaptic integration mechanism underlying the observed responses. The auditory pathway of Mongolian gerbil was activated with optogenetic stimulation of spiral ganglion neurons (SGNs) expressing f-Chrimson opsin. We developed a semi-stochastic stimulus to test over 200 combinations of inter-pulse-intervals and light pulses of varying duration. Juxtacellular recordings of SGNs or AVCN neurons were performed sequentially and to monitor the activation of the cochlea, compound action potentials (CAP) were simultaneously measured. We demonstrate the diversity of neuronal responses to optogenetic stimulation at initial stages of the auditory pathway. Our data also shows increase of fidelity of responses in AVCN neurons compared to SGNs. Our findings bring forward our knowledge about input processing in the auditory system and inform the coding strategies for optical hearing restoration.

Developmental refinement of biophysical properties of neurons in the INLL

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The intermediate nucleus of the lateral lemniscus (INLL) consists of a heterogeneous neuronal brainstem population that is involved in early cross-frequency integration of acoustic information. The major organization principle of this nucleus appears to be the membrane time constant (τ_{mem}) instead of the tonotopic maps prevalent within the auditory pathway. Within the neuronal population of the INLL the range of τ_{mem} covers almost three magnitudes building a broad continuum of integration times. Contrary to tonotopic arrangements, the biophysical phenotypes show no apparent spatial organization. Due to the lack of tonotopic cues, it is questioned how these heterogeneous neurons reach their functional maturation. To gain initial insights into the developmental alterations of INLL neurons, we assayed their biophysical properties by in vitro whole cell recordings during postnatal development. The analyzed developmental range covered the time before (postnatal day (P) 6 - 11) and after hearing onset (P 13 – 25), as to identify possible experience dependent influences.

Biophysical properties close to resting potential, like membrane time constant, input resistance and cell capacitance appeared already similar between P6 and nearly matured animals and covered almost the same range of magnitudes. Sub-threshold voltage excursions like sag-potentials as well as the action potential properties, such as height, half-width and speed were largely stable between P6 and nearly matured animals. However, responses to sinusoidal current stimulations showed developmental adjustments after hearing onset. This finding might indicate that the basal biophysical properties of INLL neurons are developed very early and cover already the full range of the cellular heterogeneity. Moreover, functional relevant firing behavior and transient preferences might refine later. The differences in the developmental progression might indicate that experience dependent activity is important in shaping the use-dependent output generation of neurons in the INLL population.

Comparative physiology of action potential generation in neurons of the MNTB

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In the mammalian auditory system, the medial nucleus of the trapezoid body (MNTB) is highly conserved. The input-output structure of these globular cells is dominated by the calyx of Held synapse, which forms a one-to-one connectivity, and a subset of potassium channels that control output generation. Biophysically, MNTB neurons are known for their onset generation of action potentials with short latency and temporal precision. Despite the conserved structure-function relationship of the MNTB, comparative analysis between rodents and bat MNTB principal cells indicated small but significant differences in synaptic size and action potential generation. Here we investigate whether differences in biophysical properties between mammals are the rule rather than the exception. To this end, whole-cell recordings from MNTB neurons in acute brain slices were obtained from adult Mongolian gerbils, Etruscan shrews, and mouse lemurs. The recordings determined the soma size from charging transients and extracted the biophysical properties of MNTB neurons focusing on action potential generation. Soma sizes of MNTB neurons from Mongolian gerbils were largest compared to mouse lemurs and Etruscan shrews. The input resistance of MNTB neurons was about twice as large in Etruscan shrews and mouse lemurs compared to Mongolian gerbils. The membrane time constant was fastest in Mongolian gerbils and slowest in mouse lemurs. Action potential current thresholds were lowest, while the voltage threshold was highest in Etruscan shrews and similar between the other species. The action potential de- and repolarization speed was largest in Mongolian gerbils and smallest in mouse lemurs. The after-hyperpolarization in Mongolian gerbils showed a single deflection, while in mouse lemurs and Etruscan shrews, a second slow after-hyperpolarization was observed. Assaying the temporal precision at rheobase showed that gerbil MNTB neurons are the most precise. Moreover, DTX-k application investigated further differences in the influence of low voltage-activated potassium channels in voltage signaling between the different species. The DTX-sensitive current supports temporal precision of action potential generation in all species, while its role in quenching the number of action potentials is largest in Mongolian gerbils. Taken together, different cellular adaptations exist and are likely based on differentially expressed ion channels. Nevertheless, the overall function of MNTB neurons is conserved, while the amount of temporal precision is species-dependent. The observed differences cannot be explained by soma size scaling and thus represent genuine functional adaptations. Overall, the transfer of gained cellular insights between species might be taken with caution even in highly conserved brain structures.

Neuronal activity patterns in auditory cortex underlying echolocation and communication calls in bats

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Acoustic communication is of critical importance in a large number of vertebrates. The bat *Carollia perspicillata* is a highly social animal and emits sounds using a rich vocal repertoire which can be broadly classified into echolocation and communication calls. Yet to date, neuronal mechanisms underlying these vocalizations are not fully understood. Previous studies indicate that the auditory cortex not only plays a role in sound perception, but also in the preparation and processing of self-produced sounds. To characterize potential neuronal activity during call emission, we performed electrophysiological recordings in the auditory cortex of *C. perspicillata* while they performed spontaneous vocalizations. The data revealed distinct activity patterns related to the type of call the animal was emitting, which emerged already before the onset of the vocalization. Additionally, neuronal firing rates were predictive of multiple call parameters, such as the number of sequentially produced calls or their frequency composition. These results add to the current understanding of neuronal mechanisms underlying vocal control and may have broader implications for other species with high vocalization abilities.

Neuronal representation of vocalisations in the frontal auditory field and the dorsal auditory cortex of the bat Phyllostomus discolor

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Bats are known for their complex vocal repertoire and are considered vocal learners. In these species, the frontal auditory field (FAF) is supposedly placed in a frontal cortico-striatal network for vocal-motor control. The FAF receives input from the auditory cortex (AC) and other auditory nuclei via multiple pathways. So far, not much is known about the transition of vocal information from the AC to the FAF. The AC of bats consists of different sub-fields, amongst which the dorsal fields (dAC) are characterised by precise coding of the temporal envelope of vocalisations. The dAC could therefore be a major source of auditory feed-back information about self-produced or perceived vocalisations to the FAF.
In this study, we used extracellular recordings in anesthetized bats (Phyllostomus discolor) to characterize the basic response properties of FAF (n=92) and dAC (n=142) neurons to pure tone stimuli and investigated how specifically these neurons encoded different types of pre-recorded vocalisations (communication and echolocation calls).
We found that dAC neurons encoded behaviourally relevant call categories and single calls more specifically compared to neurons in the FAF, both in terms of temporal firing patterns and response strength. These findings highlight the role of the dAC in the neural network that governs vocal communication in bats.

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Evaluation of LED-based multichannel optical cochlear implants for refined bionic stimulation of the auditory system

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Worldwide, over 1 million people with severe sensorineural hearing loss use electrical cochlear implants (eCIs) for hearing. Electrical cochlear implants partially restore speech comprehension in most otherwise deaf individuals, yet users still face unsolved challenges primarily routed in insufficient spectral selectivity of electrical stimulation. Promising approaches to overcome these limitations include optogenetic activation of the auditory nerve by optical cochlear implants (oCIs). Preclinical feasibility and improved spectral selectivity of oCIs have been demonstrated in rodents.

Prior work with LED-based oCIs has shown potential for higher spectral selectivity of auditory nerve stimulation and demonstrated the perception of optogenetic stimuli. However, their utility for more complex optogenetic activation patterns remains to be tested.

To address this, we have performed inferior colliculus (IC) recordings of auditory activity in response to optogenetic stimulation of the cochlea by multi-channel LED-based oCIs in Mongolian gerbils.

Recently, oCIs based on commercial LEDs (220 x 270 µm, C460TR2227-S2100, Cree) have successfully been used with preceding AAV-mediated expression of the new powerful channelrhodopsin variant ChReef in spiral ganglion neurons (SGNs). This enables IC responses to low-energy oCI stimulation (238.5 ± 235 nJ) at different sites along the tonotopic axis with near-physiological spread of cochlear excitation (SoE). Building on these findings, we aim to achieve simultaneous but spectrally independent activation of the auditory pathway using multiple channels of the LED-oCI. This involves the design of optical stimulation strategies intended to replicate activation patterns observed with acoustic stimulation.

To provide a framework for optogenetic stimulation, the activation of the inferior colliculus (IC) in the midbrain of adult Mongolian gerbils by refined acoustic stimuli will be investigated. This will, for example, employ narrowband noise stimulation to inform future coding strategies for oCIs.

The results will help to evaluate the efficacy of LED-based optogenetic stimulation of the auditory pathway.

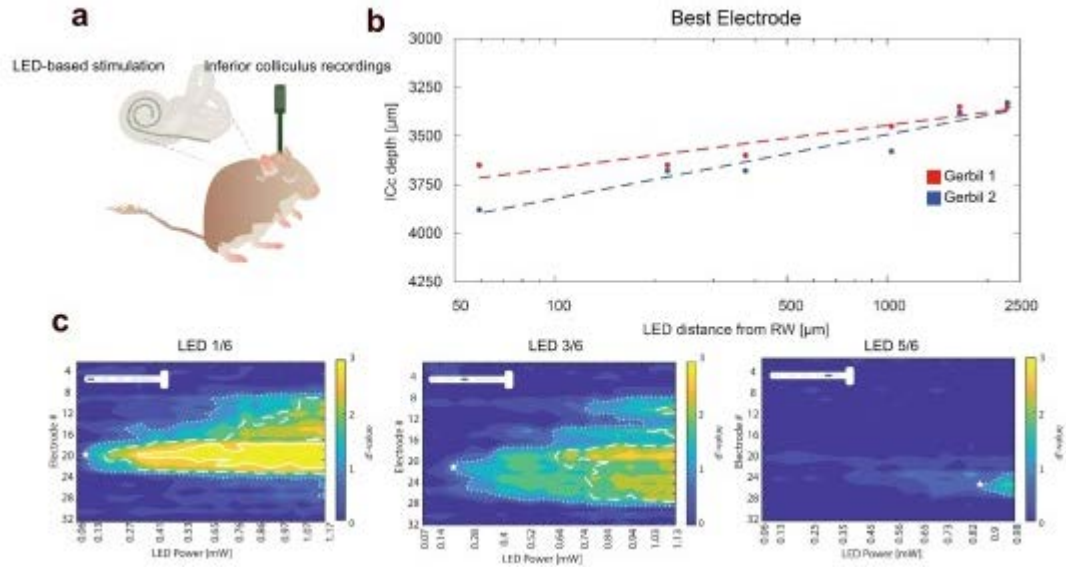

Figure -- LED-based Tonotopic Activation of the Auditory System -- a, Schematic of experimental setup. b, 'Best electrode' where threshold to d' of 1 is reached across LED # stimulated, each LED is indicated as its position along the cochlea from the round window. Average is shown in dashed bold line and individual data points plotted for each LED. Blue and red dots indicate datapoints from stimulation by individual LEDs in different animals. c, Exemplary heatmaps of d-prime across electrodes 1 to 32 (corresponds to most dorsal to most ventral) and across increasing intensities used for each individual LED stimulated. Contour lines for d' of 1,2 and 3 indicated in white dotted, dashed and plain lines respectively. White stars indicate the best electrode , insert on the top left indicates position of LED used for stimulation.

Role of cortical and subcortical regions in learning sound statistics

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Our auditory system is incredibly good at accurately representing stimuli that rapidly change in amplitude and frequency. Neuronal adaptation has been proposed as a central mechanism for enhancing discrimination between the salient foreground stimuli and background noise. This is achieved through the dynamic adjustments in the neuronal response sensitivity based on the statistical structure of the sound.
A critical aspect of this form of statistical learning involves the interplay between feedforward stream and cortical feedback, forming “listening loops” in the auditory brain. To investigate this system we performed simultaneous recordings of the inferior colliculus (IC) and the primary auditory cortex (A1) in both anaesthetised and awake mice.
Using high-density silicon probes we characterized the depth profile of the temporal dynamics of adaptation and learning driven by cortical feedback in the IC region. Additionally, applying the optogenetics approach, we described the role of genetically identified neuronal subtypes involved in the feedforward and feedback interactions during the learning of sound structures.
Furthermore, using sound stimuli of varying complexity and duration, we described the integration-time properties of cortical feedback for learning in the midbrain region.
In conclusion, we investigated the neuronal types and circuit mechanisms that provide 'top-down' information to midbrain structures, responsible for learning sound structure. These findings are critical for better understanding the way the auditory system is able to represent a noisy and constantly changing sound environment, ultimately helping to comprehend the mechanisms underlying the emergence of listening dysfunctions.

Statistical learning in auditory cortex and hippocampus

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The brain is sensitive to the structure of the surrounding environment (statistical learning) as reflected in differential responses to stimuli depending on their relation to other stimuli. For example, both the auditory cortex and hippocampus are sensitive to the probability of appearance of sounds in a sequence, a simple form of statistical learning. Here, we posed the question of whether these two structures are sensitive to different statistical features of the acoustic environment. Using Neuropixels probes to perform the largescale single cell electrophysiological recordings simultaneously in primary auditory cortex (A1) and hippocampus, we systematically presented sound sequences with different levels of complexity to awake or anaesthetized mice. Sound-evoked responses were observed in both A1 and hippocampus. We found cortical and hippocampal responses to surprising sounds and sound omissions, indicating that both A1 and hippocampus are involved in auditory statistical learning. In addition, we analyzed the responses in both structures to statistical structures (e.g. word-like sounds) that require different time windows of integration for their detection. The data, allow us to establish similarities and differences in the handling of sound statistics at two levels of the processing hierarchy.

Advancing optogenetic hearing restoration through cross-modal optimization

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In cases of profound cochlear dysfunction, electrical cochlear implants (eCIs) offer partial restoration of hearing. Nevertheless, the auditory experience with eCIs remains imperfect due to the broad excitation of tonotopically (i.e. spectrally distinct) afferent cochlear neurons by electrical stimulation that limits the number of perceptually different channels available to users. Optogenetics offers a breakthrough by enabling the stimulation of the auditory nerve by an optical cochlear implant (oCI). Light, unlike electrical signals, can be precisely confined in space, resulting in reduced spread of excitation and improved frequency selectivity. Optogenetic stimulation of the auditory nerve differs from electrical stimulation in several key aspects, such as desired higher frequency resolution and currently lower temporal fidelity. Consequently, sound coding strategies used to deliver electrical currents in eCIs cannot be directly applied to oCIs. The current project endeavors to devise tailored strategies for delivering light from multichannel oCIs to the optogenetically modified auditory nerve, following early postnatal AAV-mediated gene transfer. Specifically, our approach involves training convolutional neural networks to predict responses in the auditory midbrain (Inferior Colliculus) to both optical and acoustical stimulation. Subsequently, we leverage these models to perform cross-modal optimization of the optical sound coding strategy, aiming to evoke responses in the Inferior Colliculus that closely resemble those produced by auditory stimuli. Inferior colliculus responses are recorded using a 32 channel Neuronexus probe from anesthetized adult gerbils implanted with a blue creeLED-based oCIs featuring 5-10 channels. Our findings will contribute to refining sound coding strategies for optogenetic auditory prostheses.

Poster Topic

T19: Chemical Senses: Olfaction, Taste, Others

T19-1A SNMP1 is crucial for the detection of both pheromones and plant odorants in the desert locust *Schistocerca gregaria*
Joris Lehmann, Johanna Libnow, Maryam Khosravian, Jürgen Krieger, Jörg Fleischer

T19-2A Early development of the primary olfactory centres and their neurochemistry in *Carcinus maenas* and other malacostracan crustaceans
Johanna A. Seegel, Katja Kümmerlen, Lisa Riehemann, Sophie Raspe, Gabriela Torres, Steffen Harzsch

T19-3A Mechanosensory responses to auditory stimulation recorded at an early processing stage in the stick insect brain
Iob Lambertus Eisele, Volker Dürr, Martin Strube-Bloss

T19-4A Linking neuronal modulation and behavioural responses during olfactory-visual learning in Honeybees
Athil Althaf Aliyam Veetil Zynudheen, Wolfgang Rössler, Martin Strube-Bloss

T19-5A Correlating mouse head-motions with odor plume-encounters in an olfactory-guided navigation task
Mohammad F. Tariq, Scott C. Sterrett, Sidney Moore, Veronica Egger, David J. Perkel, David H. Gire

T19-6A Functional segregation of taste qualities in the zebrafish brainstem vagal lobe is generated and sharpened locally
Sigrun Korsching, Günes Birdal

T19-7A Characterisation of a Hunger State-Dependent Switch in Olfactory Response Behavior
Hari Pradeep Narayanan, Katrin Vogt

T19-1B Columnar processing in the rodent olfactory bulb: 3D Characterization of a putative neuroanatomical correlate of glomerular units
Israt Jahan, Veronica Egger

T19-2B Circuit mechanisms controlling state-dependent food intake in Drosophila
Lara Lederle, Rouven Lukas Ziegler, Janina Brückner, Anna-Lena Eckes, Xinyu Liu, Jan Pielage

T19-3B Ca^{2+} transients in Basal Dendrites of rat Olfactory Bulb Granule Cells
Manon Leygnier, Max Müller, Veronica Egger

T19-4B Synergistic olfactory nerve input and cholinergic neuromodulation activate ERK in rat olfactory bulb vasopressin cells
Nicolas Reichardt, Lisa Kindler, Esteban Pino, Michael Lukas, Hajime Suyama, Veronica Egger

T19-5B AMBROS – Assay for Modular Behavioral Research on Odor and Smell
Fabian Quicken, Simon Hüppelshäuser, Christopher Wiesbrock, Marc Spehr

T19-6B Dissecting neuronal circuits underlying olfactory sensory preconditioning in *Drosophila*
Yogesh Gadgil, André Fiala

T19-7B Inflammatory response in olfactory systems with experimental autoimmune encephalomyelitis
Taekyun Shin

T19-8B Modulation of olfactory bulb LFP activity by HDB cholingergic and GABAergic projections
Yu Jiang, Daniela Brunert, Erik Böhm, Markus Rothermel

T19-1C Functional characterization of target-defined MTCs in olfactory information processing
Siran Sireci, Kim Le, Daniela Brunert, Jan Mayland, Franziska Richter, Pablo Chamero-Benito, Markus Rothermel

T19-2C Evolution of olfactory circuits in the pandan-specialist *D. erecta*
Sinziana Pop, Hui Gong, Zofia Ziolkowska, Lucia L. Prieto-Godino

T19-3C Social distancing: Group behavior and the underlying neural circuits in Drosophila melanogaster larvae
Akhila Mudunuri, Katrin Vogt

T19-4C Expression of olfactory proteins in tarsal neurons of the desert locust *Schistocerca gregaria*
Natalie-Danielle Feige, Maryam Gholamhosseinpour, Jörg Fleischer, Jürgen Krieger

T19-5C Cellular Diversity in the Mouse Accessory Olfactory Bulb: A multidimensional approach to describe single cell types
Andres Hernandez-Clavijo, Uday Rangaswamy, Remo Sanges, Marc Spehr

T19-6C Identification of core genes of clock-controlled pheromone transduction in Manduca sexta
Yajun Chang, Huleg Zolmon, Monika Stengl

T19-7C Processing of behaviorally relevant odors in the posterior tuberculum of zebrafish: bridging olfactory inputs with behavioral outputs
Thomas Offner, Bethan Jenkins, Thomas Frank

T19-8C State-dependent modulation of odor valence and social behaviour via the main olfactory pathway
Jana Marie Sleeboom, Ilona C. Grunwald Kadow, Annika Cichy

T19-1D Stimulus-dependent signal modulation in mouse olfactory signal transduction
Victoria K. Switacz, Daniela R. Drose, Marc Spehr

T19-2D Conserved molecular signatures in hygro- and thermosensory neurons of the two dipteran species *D. melanogaster* and *Ae. aegypti*
Kristina Corthals, Ganesh Giri, Johan Reimegård, Allison Churcher, Anders Enjin

T19-3D Analysis of neuronal morphology in the mouse bed nucleus of the accessory olfactory tract and medial amygdala
Leonie Büsching, Moritz Nesseler, Marc Spehr

T19-4D Representation and Transformation of Temporally Complex Odours in the Mouse Olfactory System
Anantu Sunil, Dyutika Banerjee, Anantha Padmanabhan, Shambhavi Phadnis, Tobias Ackels

T19-5D An olfactory social language in the naked mole-rat?
Mohammed A. Khallaf

T19-6D Impact of Developmental Temperature on *D. melanogaster's* Olfactory Circuit Assembly and Behavior
Leticia Leandro Batista, Pascal Züfle, Ana Sofia de Castro Brandao, Giovanni D`Uva, Christian Daniel, Carlotta Martelli

T19-7D A Functional and Molecular Atlas of the Zebrafish Olfactory Bulb: Connecting Transcriptional Diversity to Behavioral response
Oded Mayseless

T19-8D Anterior olfactory nucleus: an intrinsically mechanosensitive relay for olfaction?
Athanasios Balomenos, Sampurna Chakrabarti, Wenhan Luo, Rosalba Olga Proce, Giovanna Ielacqua, Valérie Bégay, Mireia Pampols Perez, Annette Hammes-Lewin, Hanna Hörnberg, Gary R. Lewin

SNMP1 is crucial for the detection of both pheromones and plant odorants in the desert locust *Schistocerca gregaria*

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The sensory neuron membrane protein 1 (SNMP1) is co-expressed with given odorant receptors in subsets of olfactory sensory neurons (OSNs) in the antennae of insects. Previous studies in holometabolous flies and moths indicated that SNMP1 is critical for proper pheromone-controlled behavior by serving as a co-receptor facilitating ultrasensitive pheromone detection. However, the function of SNMP1 in hemimetabolous insects is largely elusive. Here, we investigated the relevance of SNMP1 for the olfactory system in an important hemimetabolous pest insect, the desert locust *Schistocerca gregaria* (*S. gregaria*). To assess its involvement in olfactory transduction, the CRISPR/Cas technique was employed to generate SNMP1-mutant (SNMP1$^{-/-}$) desert locusts lacking functional expression of SNMP1. Electrophysiological recordings showed that neuronal responses in the antenna towards several *S. gregaria* pheromones were significantly reduced in SNMP1$^{-/-}$ individuals, demonstrating that SNMP1 is essential for the sensitive detection of pheromones in desert locusts.
The co-expression of SNMP1 with a larger number of odorant receptors in OSNs of *S. gregaria* raises the question if the relevance of SNMP1 in the olfactory system of this species is confined to pheromone detection or whether SNMP1 is also vital for the reception of other chemical cues, such as plant odorants that might be important to identify food sources. Electrical recording experiments with antennae of desert locusts revealed diminished neuronal reactivity to some plant odorants in SNMP1$^{-/-}$ animals, suggesting that SNMP1 contributes to the reception of given plant volatiles that could be critical for foraging. Thus, in summary, SNMP1 is involved the detection of both pheromones and plant odorants in *S. gregaria*.

Early development of the primary olfactory centres and their neurochemistry in *Carcinus maenas* and other malacostracan crustaceans

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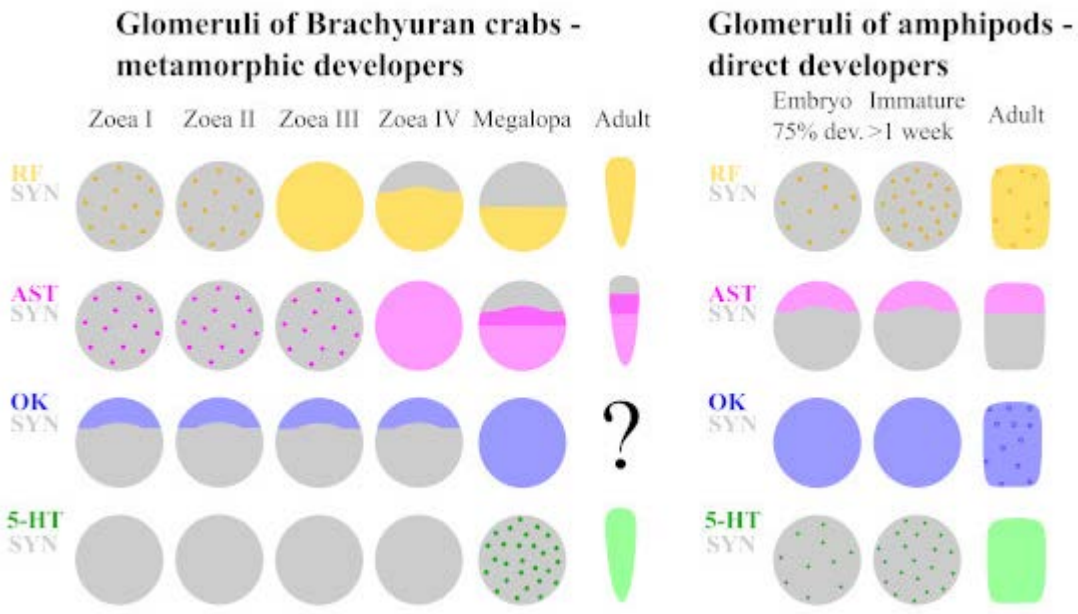
The crustacean central olfactory pathway is a complex system with two brain regions as primary olfactory centres, the so-called olfactory lobes (OL). The fundamental functional differences between the olfactory pathways of different arthropods are a topic of ongoing debate. However, relatively little is known about the development of the OL in malacostracan crustaceans.
The European shore crab *Carcinus maenas* (Brachyura, Decapoda), undergoes metamorphic development with four planktonic zoea, a semi-benthic megalopa and benthic adults. Contrary, *Parhyale hawaiensis* (Amphipoda, Peracarida) develops directly. These divergent lifestyles likely differ in their sensory requirements. Here, we asked the question if these different ontogenies are mirrored in the development of the nervous system, specifically the olfactory pathway.
We used immunohistochemistry to analyse how and when neuroanatomical and neurochemical components of the OL emerge and change in all larval stages of *C. maenas* and in embryos and immatures of *P. hawaiensis* to gain insight into their functions. We stained against the neuropeptides allatostatin (AST), FMRFamide (RF), and orcokinin (OK), and against the biogenic amine serotonin (5-HT). These substances are all neurochemicals known to have a neuromodulatory function in the olfactory lobes of model insect species. In the crustacean OL, they show characteristic patterns both regarding diffuse immunoreactivity in the neuropils and immunoreactive neural somata. We also stained against the pre-synaptic protein synapsin (SYN) to analyse the morphology of the olfactory lobe.
We concluded that the primary olfactory centres of *C. maenas* undergo marked changes from first larva to into adults at several points in time, with the most profound changes occurring between zoea IV and megalopa and thus at the transition from a planktonic to a semi-benthic lifestyle. In contrast to this, in *P. hawaiensis*, even late embryonic stages already show almost all characteristics typical for adults. This indicates that changes in lifestyle and thus in the olfactory environment a crustacean is expected to experience throughout its ontogeny are reflected anatomically and chemically in complex ways in the olfactory pathway. Acknowledgements: We wish to thank Leo Tekotte, Charlotte Astley, and Margot Deschamps from the AWI Helgoland for rearing and fixating *C. maenas* larvae. Supported by the DFG (HA 2540/20).



A glomerulus of the OL in Brachyuran crabs and amphipods: Schematic overview over the development of the morphology and the patterns of immunoreactivity for the five examined neuronal components.

Mechanosensory responses to auditory stimulation recorded at an early processing stage in the stick insect brain

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The common view about the insect antennal lobe (AL) is that it processes olfactory information from antennal receptors. However, occasional studies have reported AL responses to other modalities as well. Mechanosensory stimuli, related to wind or sound picked up by antennal receptors, may arouse neurons already at this early processing level. Here, we test for parallel processing of olfactory and mechanosensory information within the insect AL, similar to what was found in the olfactory system of mammals.

The nocturnal stick insect *Carausius morosus* is equipped with long antennae. The antenna carries a range of sensory structures, providing the animal with chemosensory and mechanosensory information about smell and contacted objects, but also with proprioceptive information about its own posture and movement. Stick insects move their antennae during locomotion, e.g. for tactile sampling of obstacles, but also in apparent alert, stationary phases at night. As antennal movement in such phases is not related to touch, it may be related to sensing of non-contact cues from smell or sound. Here we investigate mechanosensory contributions to early sensory-related activity in the AL. To do so, we inserted an extracellular multi-unit-recording electrode at the posterior rim of the AL and at a depth where we commonly record olfactory neural responses. However, instead of odours, we applied sound stimuli. Since no data exist on sound processing in *C. morosus*, we tested sound stimuli in a frequency range known to be relevant in other insects, e.g. 265 Hz (honeybee: waggle dance), 600 Hz (bushcricket: long-distance signalling) and 900 Hz (antennal hearing is thought to have an upper tone frequency limit below 1 kHz). To account for varying intensity (loudness), each tone was presented in three different amplitudes. We found a variety of response patterns in individual AL-units that clearly depended on tone frequency. Whereas some units showed an ordinary rate code, others revealed a combinatorial pattern of an excitatory response to one, and an inhibitory response to another tone. The latter is reminiscent of glomerular responses to olfactory stimuli within the AL. At the ensemble level, this combinatorial code allows for separation of different acoustical stimuli. This was demonstrated by calculating Euclidean distances among the frequency-dependent population vectors. In general, we found an increasing response strength to go along with increasing stimulus amplitude, suggesting a loudness effect. Searching for the origin of the afferent information, we amputated both antennae just proximal to the pedicel (which houses Johnston's organ) and compared the responses before and after treatment. After ablation, the initially recorded response was gone, suggesting that the stick insect's 'ear' must be located in the antenna. Taken together, our results support the idea of parallel processing of olfactory and mechanosensory information within an early-processing neural circuitry, making the stick insect a promising model to investigate olfactory and mechanosensory integration including proprioceptive feedback for active targeted motor control.

Linking neuronal modulation and behavioural responses during olfactory-visual learning in Honeybees

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To obtain accurate information about the outside world and to make appropriate decisions, animals often combine information from different sensory pathways to comprehensively represent their environment. This process of multi-modal integration, the cross-talk between different sensory modalities, is poorly understood. However, it is a common view that the single elements of a multi-modal stimulus modulate each other by enhancing or suppressing their perception. To study underlying neural circuits, we use honeybees as a model. Honeybees are central place foragers collecting nectar and pollen from flowers about 3 km away from their hive. During their foraging trips they learn to associate the reward value of a flower with its characteristic cues (colour and odour) to be able to revisit this type of flower again and again. Therefore, it is conceivable that the honeybees integrate visual and olfactory cues to form a common percept of that flower. In classical conditioning experiments, we were able to train honeybees to differentiate an olfactory-visual compound stimulus from its single elements and thus build multi-modal reward associations. Thereby, they learn to extend their proboscis (tongue analogue of the honeybee) in expectation of the rewarded stimulus (olfactory-visual compound) and withhold it if the single elements (odour or light) were presented. Lately, we have used that training procedure to compare the behavioural response latencies between a visual, an olfactory, and an olfactory-visual reward association (Strube-Bloss et al., 2023, Front. Physiol.). Indeed, our results support the view that the different modalities influence each other's perception. In our case, the response time to the olfactory modality was decelerated. In contrast, the behavioural response time to the visual modality was accelerated compared to the response time induced by the multi-modal olfactory-visual compound stimulus.
To investigate the underlying neural principals of that multi-sensory integration phenomenon, we concentrate on mushroom body output neurons (MBONs), as they emerge from the multi-modal integration and learning centre, the mushroom body (MB), which receives prominent convergent input from olfactory and visual projection neurons. Using extracellular multi-unit long-term recordings, we explore the MBON responses to olfactory, visual and olfactory-visual stimulation. Furthermore, we adopted the above-mentioned classical multi-modal conditioning experiments to compare the olfactory-visual induced neural activity before and after learning. In addition, we precisely monitor the trained response behaviour by recording the M17 muscle, which innervates the proboscis, as a measure of the reward expectation.

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Correlating mouse head-motions with odor plume-encounters in an olfactory-guided navigation task

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Although much is known about the mammalian brain olfactory structures and the physiological activity within them during passive presentations of odors in head-fixed setups, how olfactory cues shape naturalistic behaviors and their neural underpinnings in freely moving mammals remains poorly understood. One main difficulty of studying naturalistic plume-guided behaviors stemmed from the challenges associated with recreating the complex, stochastic olfactory landscape that animals experience in the wild, and correlating this dynamic olfactory information with the behavior and neural processing. This is because the odor molecules emanating from a source are spread by the turbulent and chaotic motion of the air molecules, resulting in a spatiotemporally varying signal in the form of an olfactory plume. We previously reported a method to record real-time odor information during plume-tracking in mice using head-mounted sensors (Tariq et al., 2021). In addition, Findley et al. (2021) have established head-motions as a key behavioral feature for mice engaged in an odor gradient-dependent choice task.

Here we combine our odor recording method with real-time head motion monitoring, using 3-axis accelerometer recordings and posture tracking, to establish correlations between plume contacts and head-motion changes. We show that mice exhibit robust head-pitch motions in the 5-14Hz range during an odor-guided navigation task, and that these head motions are modulated by plume encounters. Furthermore, mice reduce their angle of travel with respect to the source upon plume contact, suggesting that mice can extract meaningful information about the relative orientation of the source with each encounter. We also establish that mice lower their heads with olfactory information encounters, with a concomitant increase in the frequency of the head-pitch motions. Finally, we establish that the variability of the yaw head angles is reduced after plume encounters with a subtle increase in the rate of yaw turns. Together these findings support a model where mice engage in search behavior prior to plume encounters, then adjust their behavior upon a plume contact to extract meaningful information about the orientation of the odor-source. Similar head motions are also involved in visually-guided tasks, highlighting the importance of head motions for sensory acquisition and motor guidance during free behavior.

Functional segregation of taste qualities in the zebrafish brainstem vagal lobe is generated and sharpened locally

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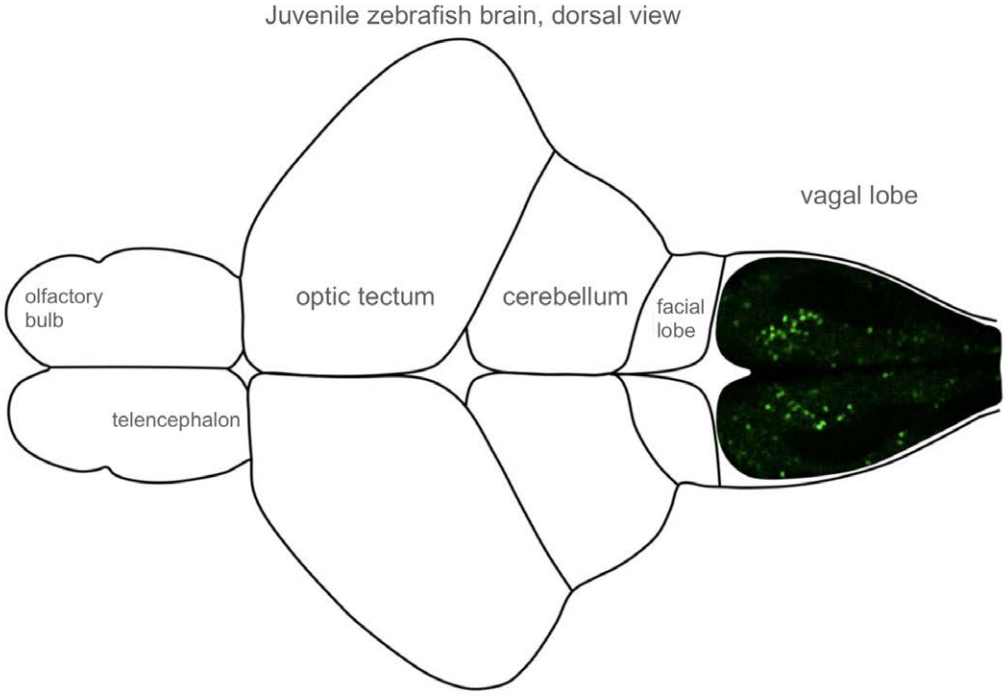
The sense of taste has essential functions in nutrient uptake and toxin avoidance. It has been widely studied at the level of gustatory receptors, taste fibers, and brainstem, the first relay center in(side) the brain. While zebrafish is an important vertebrate model system, there has been no study focusing on taste processing in the vagal lobe of zebrafish. Here we used calcium imaging in the vagal lobe of juvenile zebrafish to provide a comprehensive analysis of different types of neuronal responses to an amino acid mix and a bitter substance, denatonium benzoate. We also analyse the topological distributions of the cells showing responses to these stimuli. Our results show a clear segregation of the locations of neurons that are responsive to the amino acid mix *vs.* those responsive to denatonium benzoate. The cells responsive to the amino acid mix are preferentially located anteroventrally, whereas the cells responsive to denatonium benzoate are located mostly posterodorsally. This is different from the topology of the corresponding receptors (T1Rs and T2Rs, respectively) in the oral cavity, which is very similar for T1Rs and T2Rs. These findings suggest that functional segregation arises *de novo* in the vagal lobe and is not simply propagated from the sensory surface.



Responses to tastants were examined in the vagal lobe of the brainstem.

Characterisation of a Hunger State-Dependent Switch in Olfactory Response Behavior

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Animals need to integrate several multisensory inputs, both synergistic and conflicting, to make appropriate decisions when navigating and foraging.These decisions are often modulated by changes in the animal's internal state, for example, when hungry, sleepy, or aroused. Investigating the functional connectivity between sensory and internal state representations will reveal the spatiotemporal dynamics of decision-making. We investigated the mechanisms by which hunger modulates olfactory preference in Drosophila larvae. We have shown that hunger can switch odour aversion to attraction, where the hunger state information is mediated by a single descending serotonergic neuron, the CSDn, within the larval antennal lobe. Now, we investigate if this is a general phenomenon and if the CSDn also modulates odour preferences with an initially different fed valence. After starvation, attractive odours, such as Ethyl Acetate, become even more attractive and we show that this increased attraction also depends on serotonergic signalling. However, we hypothesise that potentially a different serotonergic subcircuit is required for this attractive odour modulation.

To investigate odour representation in the brain under fed and starved conditions, we utilise a microfluidics device to immobilise larvae for functional 2-photon imaging. This device also allows for an automated and temporally precise presentation of liquid olfactory cues.

Further, we are using our behavioural setup to screen for hunger statedependent modulation of additional attractive and ecologically relevant odours. Unexpected preliminary results suggest that larvae show attraction towards an adult male pheromone already when fed.

Exploring a wider range of olfactory sensory cues will allow us to uncover different modulatory mechanisms and neural circuits underlying flexible decision-making in Drosophila larvae.

Columnar processing in the rodent olfactory bulb: 3D Characterization of a putative neuroanatomical correlate of glomerular units

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The olfactory bulb (OB) plays a key role in processing olfactory information before it is transmitted to higher brain regions. Olfactory sensory neurons in the nose express just one type of olfactory receptor and, in the rodent OB, each activated olfactory sensory neuron type typically converges onto two glomeruli, leading to the activation of their associated principal neurons and interneurons, collectively referred to as glomerular units or columns. These glomerular processing units are emerging as a key element of olfactory coding, similar to the role of columns in other brain regions such as sensory cortices.

We applied tetanic stimulation to the olfactory nerve (50 Hz for 6 min) in 300 µm and 500 µm OB slices from juvenile Wistar rats (PND 14-18). As a marker for the resulting neuronal activity we utilized phosphorylated extracellular signal-regulated kinase (pERK), in combination with the CUBIC tissue clearing method. Thus we could visualize activated neurons in the OB in 3D, revealing cylindrical columnar structures that extended from the glomerular layer to the granule cell layer.
The 3D reconstruction of these columns (N = 20) yielded a mean length along the vertical axis from the mitral cell layer downwards of 476 ± 91 µm (mean ± SD) and a horizontal width of 280 ± 84 µm, respectively. The mean numbers of pERK-positive cells within a column were 167 ± 76 granule cells and 12 ± 5 mitral cells, respectively. There were no significant differences in any of these parameters between the 300 µm and 500 µm slices. Moreover, we applied theta burst stimulation (50 Hz, 5AP @ 5 Hz, 10x), which yielded columns (N = 12) of similar dimensions (length 501 ± 87 µm, width 301 ± 139 µm) and cell numbers (139 ± 62 granule cells, 10 ± 4 mitral cells). Thus, columnar activity is also observed upon a more physiological stimulation paradigm.
For both types of stimulation, usually more than one column was labeled (in 13 out of 19 slices). Notably, in 9 slices we identified columns on the contralateral side of the slice, which we refer to as 'mirror columns'. The synaptic pathways underlying the activation of these mirror columns remain to be elucidated.
Our results align with previous connectivity studies that explored the number and distribution of sister mitral cells belonging to the same glomerulus. Similar columnar organization in the OB has been revealed by a viral transsynaptic tracing technique (Willhite et al., 2006) that might actually be activity-dependent. Thus the columns observed here are likely to reflect the neuroanatomical correlate of glomerular processing units within the OB.

Circuit mechanisms controlling state-dependent food intake in Drosophila

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The ability to appropriately regulate food intake is essential for the survival of all animals. Appropriate feeding decisions are based on a variety of different factors, including the precise integration of sensory stimuli, the internal nutritional state of the animal as well as prior experiences. To date, surprisingly little is known regarding the neuronal circuits that regulate and control state-dependent feeding behavior. The gustatory circuit of the fruit fly Drosophila melanogaster represents an excellent model to explore these circuits due to the unique combination of genetic tools, anatomical knowledge, and a simple response pattern of feeding behaviour.

Here, we describe a novel class of gustatory interneurons (GINvm) which serve as a signalling hub to integrate both sensory and satiety signals to modulate food intake based on the internal state of the animal. We identified a cluster of neurons with an almost identical morphology whose cell bodies are in the ventral-medial suboesophageal zone (SEZ). Morphological analyses demonstrate a unique projection pattern throughout the whole brain with neuronal arborizations branching into the dorsal-medial SEZ and axonal projections reaching the pars intercerebralis (PI) region of the brain. Using genetic targeting and artificial silencing in combination with proboscis extension response (PER) assays and in-vivo Calcium-imaging we show that GINvm-neurons are required for fine-tuning sweet-sensitivity in starved flies at physiological sucrose concentrations through interplay with insulin producing cells (IPCs). We propose that GINvm-neurons represent a gain-control and feed-forward module of information processing within the gustatory system necessary for the integration of taste and hunger signals.

Ca^{2+} transients in Basal Dendrites of rat Olfactory Bulb Granule Cells

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Within the olfactory bulb (OB), granule cells (GCs) mediate recurrent reciprocal and lateral inhibitory synaptic interactions between principal mitral and tufted cells (MTCs) via their apical dendrite that extends toward the external plexiform layer. Moreover, GCs also possess a short brush of basal dendrites within the granule cell layer. Basal dendrites are known to receive inputs from both MTC axonal collaterals and olfactory cortical areas. Recent studies showed that the inputs at those dendrites participate in triggering action potentials. However, the physiological properties of basal dendritic integration were not studied so far.

As a first step, we investigated Ca^{2+} transients evoked by backpropagating action potentials elicited in the soma (sAP), using 2-photon imaging and Ca^{2+} sensitive dye (OGB-1 100µM) in GC whole-cell recordings in juvenile rat OB slices. Transients in the basal dendrites had an average amplitude $(\Delta F/F)_{sAP}$ of 16 ± 8 % (mean±SD, n=142 locations in 23 cells), similar to transient amplitudes in the proximal apical dendrite of the same cells (24 ± 13 %, n=66 locations in 22 cells). Basal spine $(\Delta F/F)_{sAP}$ was similar to that in the adjacent dendrite, just as in the apical dendrite of the same cells, in line with previous observations. However, in contrast to the known increase in apical dendrite Ca^{2+} transients with distance to the soma, there was no significant dependency of $(\Delta F/F)_{sAP}$ on distance in the basal dendrites. Next, we aimed to record synaptic transients $(\Delta F/F)_{syn}$ evoked by proximal electrical stimulation at the basal spines. The failure rate of synaptic transmission at spines that showed at least one response during 10 stimulations (n=14 locations in 8 cells) was high, on the order of 0.80 ± 0.12. Those transients had an average amplitude $(\Delta F/F)_{syn}$ of 36 ± 20 % (n=14 spines in 8 cells), not significantly different from the sAP-mediated transients observed at the same location (30 ± 9 % n=6 locations in 4 cells). Moreover, the transients in the neighboring dendrite were either absent or very small (1.5 ± 3 % n=6 locations in 5 cells), confirming that no global Ca^{2+} conductance was activated during the extracellular stimulation. Spontaneous transients $(\Delta F/F)_{spont}$ were also detected in basal spines. Their amplitude was 51 ± 38% (n=14 spines in 7 cells), not significantly different from $(\Delta F/F)_{syn}$. These observations imply that the known functional differences between basal and apical GC dendrites are also reflected in their respective Ca^{2+} dynamics and synaptic physiology.

Synergistic olfactory nerve input and cholinergic neuromodulation activate ERK in rat olfactory bulb vasopressin cells

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Vasopressin (VP) is a neuropeptide involved in various social behaviors, and its action in the olfactory bulb (OB), the first brain region responsible for olfactory processing, is essential for social discrimination in rats. The OB features an intrinsic vasopressin system, consisting of local vasopressin cells (VPCs), a subtype of superficial tufted cell. We previously found that these VPCs respond to electrical stimulation of the olfactory nerve in acute OB slices with inhibitory postsynaptic potentials, and that the neuromodulator acetylcholine reverts this evoked inhibition to action potential firing in the majority of VPCs. Moreover, we observed that in behaving rats that are exposed to conspecifics, more VPCs are immunopositive to the neural activity marker pERK (pERK-VPC) than in control rats, indicating an increased activity of the bulbar VP system during social interaction. However, it is unclear whether these two observations in VPCs, the increased ERK activation in vivo and the action potential firing in the presence of ACh in vitro, can be actually mapped onto each other.

Therefore, we investigated ERK activation in acute OB slices from transgenic VP-eGFP rats upon either chemical stimulation or tetanic olfactory nerve stimulation. KCl as well as NMDA stimulation increased pERK expression in OB neurons such as mitral cells and granule cells, and both KCl and NMDA caused VPC spiking in whole-cell recordings, but only NMDA slightly increased the percentage of pERK-VPCs. Tetanic olfactory nerve stimulation yielded vertical, column-like ERK activation of neurons across all bulbar layers. In line with our previous observations, the presence of ACh during tetanic stimulation substantially increased percentages of pERK-VPCs within activated glomerular columns as compared to VPCs outside of activated columns, indicating that the columnar pERK-VPCs were activated by coincident synaptic input and cholinergic neuromodulation. This effect was VPC-specific since pERK expression in columnar mitral cells and granule cells was not increased in the presence of ACh. Also, ACh application alone did not increase pERK-VPC activation.

Overall, our results validate pERK expression as a tool to monitor synaptic VPC excitation in the OB, and imply that depolarization of VPCs alone is insufficient to activate ERK. We propose that synaptically-evoked action potential firing – here caused by coincident sensory and cholinergic neuromodulatory input - is a prerequisite for pERK expression in VPCs.

AMBROS – Assay for Modular Behavioral Research on Odor and Smell

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Mammals perceive their environment with a wide range of highly developed senses. They depend on this sensory information to guide appropriate behavioral responses. Olfaction in particular informs individuals about their surroundings, including food and water sources, predator threats, and conspecifics. To facilitate seminatural screening of innate behavioral responses, we introduce AMBROS – the Assay for Modular Behavioral Research on Odor and Smell. AMBROS enables home cage-based, large-scale screening of behavioral changes in mice without experimenter interference. A flexible odor delivery platform allows for presentation of a broad range of chemosensory stimuli, while an automated video recording system records relevant behaviors. Here, we employ urine as a rich source of semiochemicals that conveys essential information on species, sex, social rank, and even health status. We validate AMBROS by demonstrating both attraction to conspecific urine and aversion to predator cues. Together, this setup provides a robust tool for exploring odor-driven behaviors in a controlled, yet naturalistic context. Future work will explore unknown odor cues, such as those related to conspecific health, further expanding the applications of this system.

Dissecting neuronal circuits underlying olfactory sensory preconditioning in *Drosophila*

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Neuronal circuits underlying associative olfactory conditioning, where an odor is paired with a reward or punishment, have been extensively studied in *Drosophila melanogaster*. However, animals can also form associations between coincident relatively neutral odors in the absence of reinforcement. This is can be demonstrated by olfactory sensory preconditioning, a leaning paradigm in which first two relatively neutral odors (A and B) are paired during a preconditioning phase, followed by aversive conditioning where odor A is paired with punishment. In the test phase, flies avoid odor B, suggesting that the valence acquired by odor A extends to odor B, even though it was never directly associated with punishment.

While the neuronal circuits involved in classical olfactory conditioning are well-characterized, those mediating odor-odor associations remain unknown. The insect mushroom body (MB) plays a central role in olfactory learning; receiving olfactory input from the antennal lobes via olfactory projection neurons, and integrating reinforcement signals from dopaminergic neurons to guide behavior through MB output neurons (MBONs). Using thermogenetic and optogenetic tools, we investigate which neuronal classes are essential for forming odor-odor associations.

We found that output from olfactory projection neurons is required during all phases of sensory preconditioning. However, synaptic output from the MB and dopaminergic neurons were not necessary for forming odor-odor associations. Input from dopaminergic neurons was required for the odor-shock pairing phase. These results suggest that odor-odor associations occur upstream of the MB-MBON interface and independent of dopamine signalling contrasting with odor-shock associations. We will discuss potential synaptic sites and neuronal mechanisms underlying odor-odor associations.

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Inflammatory response in olfactory systems with experimental autoimmune encephalomyelitis

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Experimental autoimmune encephalomyelitis (EAE) is a model of CNS autoimmune disease, in which olfactory disorder is also recognized. The inflammatory response in olfactory systems remains to be studied in EAE. The aim of this study is to evaluate inflammatory response in the olfactory systems including olfactory bulbs, olfactory mucosa and vomeronasal organs with EAE by immunohistochemistry. Olfactory tissues were sampled in myelin oligodendrocyte glycoprotein-induced EAE in mice. Formalin fixed tissues were processed for paraffin embedding, and the sections were immunohistochyemically evaluated using various markers including ionized calcium binding adaptor molecule-1 (Iba1) antibody, myeloperoxidase, olfactory marker protein (OMP). In olfactory bulbs with EAE, infiltration of inflammatory cells in subarachnoid space and parenchyma was evident, where glial reaction was also recognized. In olfactory mucosa with EAE, macrophages activation and suppression of olfactory marker protein were confirmed. The inflammatory response was also found in the vomeronasal organs with EAE as does in olfactory mucosa. Collectively, inflammatory response in olfactory systems in the course of EAE is related to dysfunction of all olfactory system as far as EAE is concerned.
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Modulation of olfactory bulb LFP activity by HDB cholingergic and GABAergic projections

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Top-down input from the basal forebrain to the olfactory bulb (OB) can modulate OB output activity: our previous work on single neuron mitral tufted (MT) cell activity during optogenetic activation of GABAergic or cholinergic axons of basal forebrain neurons in anesthetized mice showed that GABAergic and cholinergic fibers had different effects on OB output: cholinergic modulation indiscriminately increases MTC firing while GABAergic modulation is input dependent with low inputs being suppressed while strong inputs are enhanced.

In this study, we analyzed the data with a focus on local field potential activity (LFP). Analyzing LFP power over different frequency bands we found that cholinergic activation did not significantly change LFP power. Activation of GABAergic inputs decreased LFP power across all frequency bands. This decrease was strongest in during low sensory input. Phase amplitude coupling (PAC) between theta and beta bands showed significant effects during both, GABAergic and cholinergic modulation. While activation of cholinergic fibers resulted in a significant increase of coupling, independent of sensory input strength, activation of GABAergic fibers differed between sensory input conditions: when sensory input is low, GABAergic fiber activation resulted in a decrease in coupling, while in conditions with strong sensory input GABAergic modulation resulted in an increase in coupling.

In summary, our findings suggest that PAC in the olfactory bulb mirrors single neuron data from our previous work, confirming that gain modulation of OB output is established by GABAergic rather than cholinergic fibers from HDB. This is, however, not reflected in LFP power changes. Further research is necessary to see how exactly GABAergic modulation acts on OB circuits in order to establish gain modulation.

Functional characterization of target-defined MTCs in olfactory information processing

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Parallel processing of sensory information is a crucial mechanism to decode complex information from the environment, which is well studied in most sensory modalities except chemical senses. The two main types of output neurons of the olfactory bulb (OB), mitral and tufted cells (MTCs) convey odor information to different but partially overlapping regions of the olfactory cortex. However, the question of whether these different regions of the olfactory cortex also receive differential input from the OB remains elusive, yet.

This study aimed to investigate target-specific odor coding of MTCs in the olfactory bulb during odor information processing by the use of adeno-associated virus (AAV)- mediated retrograde tracing from the anterior olfactory nucleus (AON) and the anterior piriform cortex (APC). Using widefield microscopy, the activity of different MTC populations was imaged with virally expressed GCaMP6f. Odor responses of AON- and APC-traced MTC populations showed significant differences in respiration-linked activity but response onset times and spatial response maps were similar between the two populations. Subsequently, we measured single-cell level responses of these populations during odor stimulation using two-photon microscopy. We found that MTCs projecting to AON respond to a wider range of odorants compared to APC projecting cells. On the other hand, MTCs targeting APC were more sensitive to concentration changes. In summary, tracing of MTC populations separated by their target regions showed significant differences in their selectivity and sensitivity, providing support for a role in parallel processing of olfactory information.

Evolution of olfactory circuits in the pandan-specialist *D. erecta*

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When animals adapt to new ecological niches, the olfactory system — used to find food and mates — is placed under enormous evolutionary pressure. In drosophilids host-specialisation evolved repeatedly, accompanied by changes in olfactory circuits. While there is plenty of evidence that olfactory receptors are highly divergent and contribute to novel adaptations, we are yet to comprehend the extent to which nervous systems evolve by changing central neural circuits.

To establish the contribution of neural circuit changes in the evolution of olfaction, we are comparing two fly species adapted to different food sources: *Drosophila melanogaster*, a generalist with a wide preference for rotting fruit, and *Drosophila erecta*, a seasonal specialist on pandan fruit.

We show that pandan purée from fruits collected in the field and single odours identified from the pandan bouquet are more attractive to *D. erecta* than *D. melanogaster* in two behavioural tests: a two-choice odour trap and a high-throughput egg-laying assay.

We identify changes in the underlying neural circuits which explain this behavioural divergence by combining genetic tools in *D. erecta* with in vivo volumetric calcium imaging and neuroanatomy.

Our work highlights how olfactory neural circuits can undergo different evolutionary paths, with changes in both peripheral and central components, as animals adapt their behavioural repertoires.

Social distancing: Group behavior and the underlying neural circuits in Drosophila melanogaster larvae

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Decision-making is complex, as animals not only need to consider information based on their own perception, internal state and experience, but also social cues. Living with conspecifics can be beneficial to gain more information regarding the environment and to access better resources. However, it also increases competition for mates and food. How animals sense their conspecifics and then use this information to modulate their behaviour in a social context is not very well understood. To answer this question, we are investigating group behaviour in larvae of the model organism Drosophila melanogaster. In fly larvae we can make use of state-of-the-art behavioural tracking methods and a rich array of genetic tools to understand behaviour and the underlying neural circuits in detail. Behavioural experiments show that Drosophila larvae avoid their conspecifics in an open arena without any food and disperse more than when they are alone. Being in a group also affects decision-making in different sensory contexts and internal states. Furthermore, social distancing is dependent on social experience during development. Preliminary genetic manipulation experiments suggest that larvae sense each other via multiple sensory systems. To better understand the social dynamics, we make use of an agent-based model which can reproduce behavioural effects. Our results will help to better understand the behavioural algorithms and neural processing mechanisms that underlie social interactions between conspecifics.

Expression of olfactory proteins in tarsal neurons of the desert locust *Schistocerca gregaria*

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Detection of chemical cues from the environment is essential for animals to locate food sources, mating partners, or oviposition sites. For receiving odorous substances, insects are equipped with cuticular and often hair-like structures, termed sensilla, that are associated with olfactory sensory neurons (OSNs) expressing olfactory receptor proteins. Olfactory receptors of the odorant receptor (OR) family form heteromeric complexes with the olfactory receptor co-receptor (ORCO) that is essential for proper function of ORs and is therefore regarded a marker for OR-expressing OSNs. Though the antennae are the main olfactory organs of insects, ORs have also been found expressed in other parts of the body, including legs (tarsi), wings, and the ovipositor. However, the functional relevance of the OR-expressing cells in these body parts is still elusive. Therefore, as a first step to elucidate the functional implication(s) of “extra antennal” OR-positive cells, we aimed at identifying olfactory proteins present in selected tissues of a model organism, the desert locust *Schistocerca gregaria*, an economically important insect pest. Reverse transcription PCR revealed an expression of ORCO in tissues of various body parts, including the tarsi. Conducting fluorescence immunohistochemistry with antibodies against ORCO and a neuronal marker on the tarsi, ORCO was found to be localized in neurons associated with sensilla, indicating a chemosensory function of these tarsal cells. Subsequently, we set out to identify OR types abundant in this tissue by RNA sequencing, leading to a number of ORs expressed in tarsi that could mediate the detection of chemical compounds. To target the potential chemosensory function of OR/ORCO-positive neurons in the tarsi, we intend to employ OR/ORCO knockdown strategies (RNAi) in combination with electrophysiological and behavioral approaches.

Cellular Diversity in the Mouse Accessory Olfactory Bulb: A multidimensional approach to describe single cell types

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The accessory olfactory bulb (AOB) is a specific brain region in the dorso-posterior area of the olfactory bulb that plays a pivotal role in processing of social chemosignals and, thus, regulation of behavior. The AOB receives input from the vomeronasal organ and projects directly to the amygdala/hypothalamus complex. Despite its central role in social signal processing, the cellular composition and functional organization of the AOB is largely unknown. Here, we performed the first AOB-directed single cell transcriptomic analysis from male and female mice. Using snRNAseq technique in an AOB-enriched tissue sample, we describe 24 transcriptionally defined cellular subpopulations in the AOB. Among these, we identify different types of neurons, comprising inhibitory and excitatory neurons, and describe specific marker genes for each subpopulation. Evaluation of marker gene expression patterns by in situ hybridization and immunolocalization confirmed the presence and distribution of different neuron subpopulations described during transcriptomic profiling. Finally, using patch-clamp recordings and high-resolution microscopy, we recorded electrophysiological profiles and morphological characteristics of different cell types in the AOB. Post-hoc analysis of marker gene expression in recorded AOB neurons, allow us to integrate single cell transcriptomics, electrophysiological and morphological profiles to generate a multidimensional description of individual neuronal types in the mouse AOB. This work comprises the first multidimensional study of cellular diversity in the mouse AOB, building a solid foundation for future studies aimed to disentangle the cellular composition and functional organization of the mouse AOB.

Identification of core genes of clock-controlled pheromone transduction in Manduca sexta

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Insect chemosensory transduction is still under debate. Mostly based upon studies in the fruit fly *Drosophila melanogaster* it is assumed that all insect olfactory receptor neurons (ORNs) employ ionotropic chemosensory transduction without G-protein-dependent signal amplification for general odor- as well as for pheromone detection. However, male moths are astoundingly sensitive, apparently detecting single pheromone molecules of their conspecific mates, making it likely that pheromone transduction employes metabotropic cascades allowing for strong signal amplification, comparably to phototransduction. Accordingly, in *Manduca sexta* male hawkmoths, our patch clamp analysis did not find any evidence for olfactory receptor-coreceptor (Orco)-dependent ionotropic pheromone transduction. Instead, with in vitro and in vivo electrophysiological analyses we provide evidence for Go-protein coupled pheromone-transduction cascades involving phospholipase C (PLC) activation in *M. sexta*. Furthermore, our experiments showed that sensitivity and temporal resolution of pheromone transduction expresses zeitgeber time (ZT)-dependent differences correlating with circadian changes in cAMP levels in hawkmoth antennae. To identify the molecular constituents and mechanism of hawkmoth pheromone transduction and its circadian modulation, with qPCR we investigated dynamic transcriptomic changes in adult male hawkmoth antennae at different ZTs comparing sleep- and activity phases of hawkmoths. We found daily changes in the expression of circadian clock genes (eg: *timeless*) proving that hawkmoth ORNs are circadian clock cells with a transcriptional-translational feedback loop (TTFL) clockwork. However, an array of potential candidates involved in pheromone transduction, including 14 G-proteins, 254 ion channels, 43 members of second messenger cascades, and 90 enzymes, exhibited no notable variance across different ZTs, except participants in the control of Ca2+ homeostasis. So far, we found only ZT-dependent expression of *PLCβ 4* in pheromone-stimulated antennae.
In conclusion, we hypothesize that daily or circadian modulation of chemosensory transduction cascades is rather controlled via post-translational feedback loop (PTFL) clocks associated with the plasma membrane than by the TTFL-based circadian clockwork. To further challenge our hypothesis of metabotropic pheromone transduction under PTFL control and to identify signaling cascades present in single ORNs we employ single-nucleus RNA sequencing combined with quantitative PCR (qPCR), and physiological assays. [Supported by DFG grant GRK 2749/1 “multiscale clocks”]

Processing of behaviorally relevant odors in the posterior tuberculum of zebrafish: bridging olfactory inputs with behavioral outputs

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Odors are encoded as spatio-temporal patterns of neuronal activity in the olfactory bulb (OB) network. This primary odor representation is conveyed by extensive axonal projections of mitral tufted cells (MTCs) to a distributed network of brain regions. One of these regions, the posterior tuberculum (PT), receives extensive axonal innervation from a broad set of MTCs in zebrafish. In an ex-vivo preparation of lamprey, it has been identified as an important part of a network involved in odor-motor-transformations. In the genetically tractable zebrafish however, odor responses in the PT have not been characterized so far Using two-photon Ca^{2+} imaging in head-fixed zebrafish larvae, we found that the PT performs a profound transformation of odor responses. As such, responses of PT neurons were more similar to adjacent brain regions, such as the (pre-) thalamic, and hypothalamic areas, than to other target regions of the OB, such as the pallium, subpallium or the habenula. Our observations pave the way to further study the precise role of PT in odor-motor transformations, and the circuit, cellular, and synaptic mechanisms underlying its specific odor response profile.

State-dependent modulation of odor valence and social behaviour via the main olfactory pathway

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A fundamental question in neurobiology is how specific neuronal activity contributes to a behavior or perception. Importantly, the perception of a sensory stimulus depends not only on its physical properties but can be significantly modulated by the internal state as well as experience and context. The olfactory system provides an ideal model to study this modulation of perception and behavior, because it is heavily innervated by all major neuromodulatory centers and chemosensory communication drives many essential behaviors.

Especially, odour valence is a particularly critical feature as it elicits appropriate aversion and attraction – motivated behaviours that are crucial for survival. In addition, odour valence can be significantly modulated by several factors including internal state (e.g., hormonal status). Despite its importance, the mechanisms underlying the encoding and modulation of odour valence remain poorly understood. In particular, how untrained (innate) odour valence is encoded and modulated by non-conditioned changes, e.g. the internal state of the animal, remains largely elusive.

Here, we identified a naturally occurring switch in behavioural valence responses towards a volatile social odour that dependents on the neuroendocrine state. In this model, 1) the response of female mice to the male social cue trimethylamine (TMA) significantly depends on the estrus state; 2) This behavioral switch depends on a defined receptor, TAAR5, which is expressed in the main olfactory system and is specifically activated by TMA; 3) This switch in behavior is specific to TMA. This naturally occurring switch in odor valence provides a unique opportunity to study a non-conditioned, potentially inducible change in perception.

To identify the neural circuits and molecular mechanisms that underly this estrus state-dependent modulation, we use a combination of awake in vivo 2-photon calcium imaging as well as RNA sequencing of specific neuronal subtypes. Moreover, since the valence responses towards TMA are abolished in females lacking TAAR5, we hypothesize that this main olfactory receptor may play a critical role for proper social or reproductive behaviors. To test this, we developed a social interaction paradigm in which the behavior of wild-type and mutant females in different estrus stages towards males is analyzed.

Stimulus-dependent signal modulation in mouse olfactory signal transduction

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Adaptation to prolonged or repetitive stimuli is a critical feature of sensory systems, allowing dynamic adjustment of sensitivity. In olfactory sensory neurons (OSNs), activation of odorant receptors and subsequent G-protein-dependent cAMP signaling are counterbalanced by Ca^{2+}/calmodulin-mediated negative feedback, resulting in sensory adaptation. Many OSNs exhibit high sensitivity with activation thresholds in the (sub)nanomolar concentration range. Thus, OSN sensitivity spans a dynamic range of several orders of magnitude. Whether, beyond adaptation, complementary dose-dependent modulatory mechanisms exist is yet to be identified. Pilot data using Ca^{2+} imaging in dissociated mouse OSNs, using IBMX and forskolin as a "broadband" stimulus, revealed response summation and even potentiation in a dose-dependent manner at short inter-stimulus intervals (ISIs). With increasing stimulus concentrations, the ratio of OSNs with elevated responses decreased, while adaptation became more prevalent. Here, using patch-clamp recordings from OSNs in acute slices, we compare how changing ISIs and stimulus concentrations affect signal modulation, thus altering action potential discharge and, consequently, information transfer to the brain. In line with our pilot data, we primarily found adaptation upon exposure to high stimulus concentrations. Notably, a subset of neurons showed summating responses when stimulated with low stimulus concentrations. Based on these findings, we now aim to explore (*i*) which signaling cascade components are modulated during adaptation versus summation processes, (*ii*) whether dose-dependence is receptor-(in)dependent, and (*iii*) if the maturation stage of OSNs affects the ability of peripheral modulation. Together, we seek to gain insight into how mammalian OSNs shape their odor sensitivity and response strength to decode an extensive range of stimulus concentrations.

Conserved molecular signatures in hygro- and thermosensory neurons of the two dipteran species *D. melanogaster* and *Ae. aegypti*

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Insects rely on sophisticated hygro- and thermosensory mechanisms for survival, however the underlying molecular mechanisms mediating these systems remain poorly understood. Here we conducted a comparative transcriptomic analysis of single nuclei of the hygrosensory and antennal thermosensory neurons in the fruit fly *Drosophila melanogaster* and the yellow-fever mosquito *Aedes aegypti*. This comparative approach allows us to detect conserved molecular signatures of these neuronal populations. The evolutionary conservation of genetic components suggests an essential role in the mediation of sensory processes.

Our study identified 17 shared genes among the top 50 markers in hygro- and thermosensory neuron clusters across both species, including known receptors Ir21a and Ir93a, as well as novel players. Functional validation using a dynamic humidity arena in *D. melanogaster* was used to confirm validity of new genes involved in hygrosensation. Our integrated approach, combining comparative transcriptomics with behavioral analysis, has not only confirmed known components of hygrosensation but also uncovered novel genes, providing new insights into the complex molecular mechanisms of moisture perception in insects. This study sets a valuable precedent for future research in sensory neurobiology and may have implications for vector control strategies.

Analysis of neuronal morphology in the mouse bed nucleus of the accessory olfactory tract and medial amygdala

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In rodents, olfactory information is processed in many areas of the brain. A central hub that controls odor-guided behavior is the amygdala. In pilot studies, we identified axonal projections from both the main and accessory olfactory bulb that converge in the bed nucleus of the accessory olfactory tract (BAOT) and the anteroventral medial amygdala (MeAav). We set out to characterize neuronal morphology within both BAOT and MeAav. To this end, we established an approach to reconstruct single cells in 3D after electrophysiological characterization. Next, we immunohistochemically identified axon initial segments by staining the cytoskeletal protein spectrin β-IV. Accordingly, we differentiate dendritic trees and axonic projections. Furthermore, we identified axons of either somatic or dendritic origin. Moreover, we analyzed additional morphological parameters, such as dendritic tree complexity and primary neuronal orientation, to capture the diversity of cell structures. Finally, all parameters are combined in a hierarchical cluster analysis to identify groups of cells based on their morphological characteristics. Together, detailed morphological profiling describes different cell types in both BAOT and MeAav. These findings offer first hints about the role of different cell types in olfactory integration within the amygdala.

Representation and Transformation of Temporally Complex Odours in the Mouse Olfactory System

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In natural conditions, olfactory cues often occur as spatiotemporally dynamic odour plumes. The temporal profile of odour concentration fluctuations within these plumes contains information about the environment, which can aid animals in performing ethologically relevant tasks such as odour source separation and localisation. This requires the ability to process and extract spatial information from high temporal bandwidth features. Contrary to insects, where olfactory sensory neurons (OSNs) are constantly exposed to the odour environment, the detection and processing of odour fluctuations in mammals has been postulated to be limited to slower time scales, due to lower sampling rates and slower signal transduction kinetics. Despite these limitations, recent studies have shown that the mouse olfactory system can access high-frequency information from temporally complex odours (TCOs). However, an understanding of the relevant temporal features, as well as local and centrifugal circuit motifs involved in processing them is still lacking.

Here, we investigate how TCOs are represented and transformed across the mouse olfactory system, initially focusing on the olfactory bulb (OB). The OB is the first centre of odour processing with extensive interneuron circuitry, a potential computational resource for processing dynamic odour information. We perform dual-colour volumetric 2-photon Ca2+ imaging in transgenic mice expressing GCaMP3 in OSNs and jRGECO1a in projection neurons, called mitral and tufted cells. This approach allows us to simultaneously acquire both input and output signals from the OB. Coupled with a high-speed odour delivery device, we probe the neural correlates of isolated temporal odour features such as pulse interval, onset latency or odour correlation. To fully explore the feature space of TCOs, we recreate previously recorded naturalistic odour plumes. We further perform extracellular unit recordings from projection neurons during targeted opto- or chemogenetic manipulations of different OB interneuron populations (e.g. GABAergic and dopaminergic) using cre-driver lines. Our findings will provide a deep understanding of local circuit mechanisms involved in encoding and modulating TCO information in the mouse OB.

Moreover, we explore how temporal odour features are subsequently translated and integrated into spatial information, and how they are used for behaviour. To this end, we will perform unit recordings from OB downstream areas, such as the piriform cortex and lateral entorhinal cortex. Employing behavioural paradigms simulating naturalistic conditions in freely moving mice in combination with a miniaturised odour sensor and neural recordings will help us understand which temporal features are relevant for olfactory navigation.

An olfactory social language in the naked mole-rat?

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Olfaction plays a crucial role in the survival and social behavior of many species. One exceptionally social mammal is the naked mole-rat, which lives in large colonies led by one dominant female, known as the queen. She is the only breeding female capable of lactating in the colony. We hypothesized that despite its subterranean habitat, the naked mole-rat exhibits a keen olfactory sense which might foster social bonds within the colony and aid in identifying colony members, intruders, and potential threats. However, our understanding of the specific chemical cues governing their social and maternal behaviors remains limited. Here, we examined the chemical signals involved in the social communication of naked mole-rats and the underlying neurobiological substrates. Our chemical analyses of odor profiles from various members have unveiled the presence of a previously unknown queen-specific compound, which has also previously been detected in human breast odors. Electrophysiological recordings indicated that the queen odor is detected by the activation of olfactory sensory neurons in the main olfactory epithelium. Behavioral experiments indicated that females may exhibit attraction to this compound, while males display aversion. Furthermore, our findings reveal that different species of mole-rats exhibit distinct chemical profiles, with the naked mole-rat “queen” odor detected in social species, but absent in solitary species, highlighting the significance of this compound in social communication among African mole-rats. Our findings illuminate the role of olfactory communication in the social dynamics of naked mole-rats, providing valuable insights into the unique social structure and ecological niche of naked mole-rats.

Impact of Developmental Temperature on *D. melanogaster's* Olfactory Circuit Assembly and Behavior

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Poikilothermic animals, such as insects, cannot regulate body temperature, making their development highly sensitive to environmental conditions. In *D. melanogaster*, developmental temperature has been shown to influence pupation time and surprisingly brain connectivity. To understand the origins and consequences of temperature dependent brain wiring, we investigate how developmental temperature affects the olfactory system of the fly at anatomical, physiological, and behavioural levels. Using trans-synaptic labelling tools, we show that in flies developed at 18°C, olfactory receptor neurons (ORNs) have more functional postsynaptic partners than in flies developed at 25°C. This scaling impacts the calcium transient of ORNs, but not of projection neurons (PNs) - ensuring robust odour coding. However, the connectivity of PNs to the circuit's next layer, lateral horn neurons (LHNs), is specific to each tested developmental temperature. This could lead to changes in odour-driven behaviour, which we test in two assays (free walking arena and spherical treadmill). In both, flies developed at 18°C have a stronger approach to odours. To understand the origin of the temperature dependent synaptic scaling, we analysed brain connectivity in flies developed at 12 and 31°C. Across all temperatures tested, the number of synaptic partners scaled exponentially with temperature, similar to the scaling of developmental time-which could derive from similar first principles. We extend a model of development based on Gillooly et al. 2002, hypothesizing that different temperature-dependent metabolic reaction rates limit brain and body development. Our model predicts brain connectivity in flies developed on periodic temperature cycles of different amplitudes and reveals temporal shifts between brain and body development at the anatomical and molecular level, which were tested experimentally. Our work links developmental temperature, circuit assembly, function, and behaviour, offering a novel perspective on how environmental factors shape neural development.

A Functional and Molecular Atlas of the Zebrafish Olfactory Bulb: Connecting Transcriptional Diversity to Behavioral response

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Innate behaviors are crucial for the survival and success of animals, relying on pre-wired neural circuits that transform sensory information into behaviorally relevant outputs. However, a major challenge in understanding these processes lies in bridging transcriptional profiles with functional responses. This challenge is particularly evident in olfaction, where perception is inherently discrete, as odorant molecules can interact with multiple receptors, giving each odor a distinct identity. Unlike the continuous nature of visual stimuli, the discrete combinatorial nature of olfactory stimuli makes topological explanations insufficient for explaining odor-driven behaviors.

To address this, we generated a comprehensive spatial transcriptional and functional atlas of the zebrafish larval olfactory bulb (OB), revealing previously unrecognized cell-type heterogeneity and linking transcriptional clusters to their functional roles. By analyzing odor-driven behaviors, we identified transcriptional clusters shared across odors that elicit similar responses, demonstrating that transcriptionally unique cell types are correlated to odor classification and valence encoding. These findings provide new insights into the molecular and circuit mechanisms underlying sensory-driven behavior, suggesting a functional role for transcriptionally defined cell types.

Anterior olfactory nucleus: an intrinsically mechanosensitive relay for olfaction?

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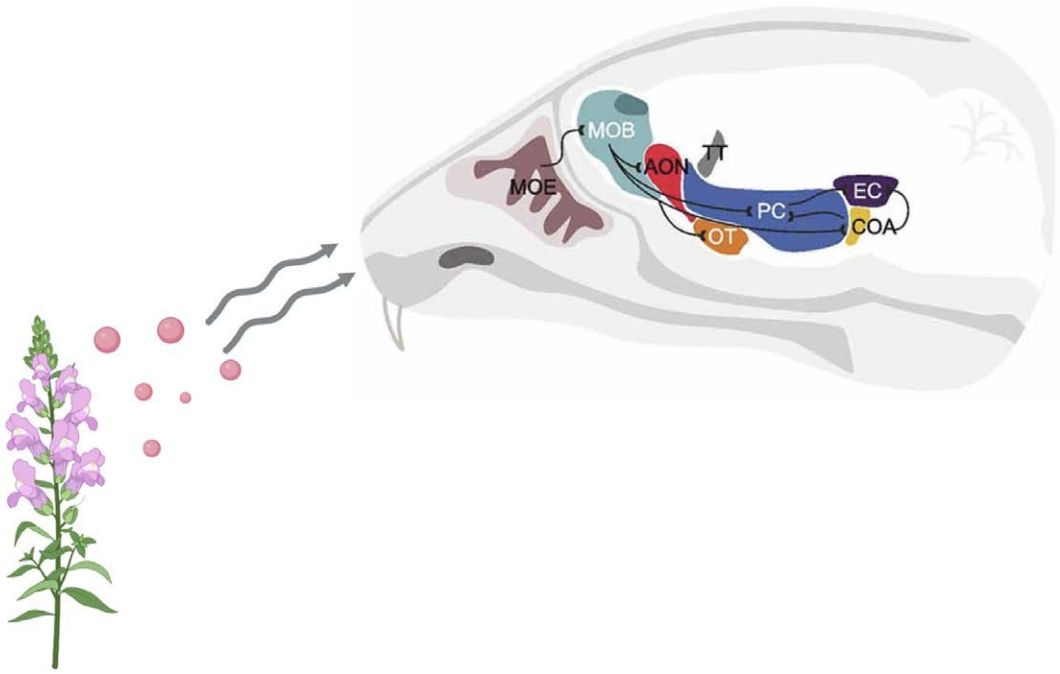
In the mouse olfactory system, mitral and tufted cells in the olfactory bulb receive odor information from the olfactory epithelium and relay this sensory information to the olfactory cortices. Using single molecule Fluorescent in Situ Hybridisation (smFISH), we detected the expression of two mechanosensory genes, Piezo2 and Stoml3, in many cells of the olfactory system, including high levels of expression in the Anterior Olfactory Nucleus (AON). Expression of these two mechanosensory genes in AON was especially prominent in the first two post-natal weeks. This result is consistent with previous observation that Piezo2 fate mapped cells exist in the olfactory system, most prominently in the AON, using mice in which Piezo2-Cre drives tdTomato expression in Piezo2 expressing cells. However, direct physiological evidence for mechanically activated currents in CNS neurons has been limited in the past. We sought to examine the AON population physiologically and to do this we established primary cultures of these neurons. Patch clamp electrophysiological recordings from tdTomato+ AON neurons showed that mechanical stimulation either via pressure or substrate deflection was able to generate robust mechanically activated currents. We thus established a method to isolate and culture primary AON neurons for the first time and we used this model to show that these neurons are mechanosensitive. To investigate the cell-type diversity in this novel in vitro system, we will employ single nucleus RNA sequencing as well as ultra-low input proteomics. Furthermore, we seek to understand the role of AON mechanosensitivity in vivo. We found that a gain-of-function approach by overexpressing Stoml3, a known modulator of Piezo2 channel, in Piezo2+ cells led to impaired odor discrimination in mice without altering the AON structure. Using functional ultrasound imaging we observed activation of AON neurons with airflow-driven mechanical stimulation in vivo. Future work will investigate the airflow-driven activation in the gain-of-function model.



Adapted from Rotermund et al., 2019 using BioRender.com

Poster Topic

T20: Somatosensation: Touch, Temperature, Proprioception, Nociception

T20-1A Navigation with touch
Wenhan Luo, Sampurna Chakrabarti, Lin Wang, Mohammed Ali, Gary R. Lewin

T20-2A Nociception in sharks – an analysis of peripheral sensory nerves
Sampurna Chakrabarti, Athanasios Balomenos, Jasmin Klich, Severine Kunz, Vera Schlüssel, Andrew Gillis, Gary R. Lewin

T20-1B Role of leg-campaniform sensilla in Drosophila melanogaster adaptive walking
Ricardo Custódio, Anna Pierzchlinska, Ezequiel Axel Gorostiza, Till Bockemühl, Gesa F. Dinges, Kai Feng, Ansgar Büschges

T20-2B Thermal encoding by GABAergic interneurons in the posterior insular cortex
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T20-1C Advanced behavioral phenotyping in *Drosophila melanogaster* to establish a model for inhalation toxicology for volatile organic compounds
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T20-2C Load sensors in the fruit fly: detailed analysis of arborisation patterns
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T20-1D Whisker-Mediated Categorization of External Space in Head-Fixed Mice
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T20-2D Establishment of a human induced pluripotent stem cell-based in vitro model for the investigation of sex-specific differences in migraine pathophysiology
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Navigation with touch

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Naked mole-rats (Heterocephalus glaber) are one of the most incredible creatures in the animal kingdom. They are a unique eusocial rodent species with extraordinary longevity and able to communicate with each other with versatile colony-specific dialects and can even survive under extreme hypoxia by switching to fructose as a fuel. Their subterranean burrows are large and complex with multiple branching tunnels, which can spread more than 3km, but mole-rats orientate themselves and navigate without reliance on visual cues. However, the underlying mechanisms used by these animals to navigate have not yet been studied. Our results suggest that naked mole-rats utilize their densely innervated body hairs as tactile navigational tools. Behavioral experiment indicates that the deflection of these hairs as they move down narrow tunnels aids in accurate navigation. Additionally, mechanoreceptors innervating these hairs are tuned to certain deflection angles, probably providing directional information feedback. Moreover, these receptors can also fire continuously for minutes in response to static displacement, potentially providing information on time travelled as the hairs remain bent by tunnel walls during movement. Collectively, our findings illuminate the sophisticated tactile-based navigation system employed by naked mole-rats.

Nociception in sharks – an analysis of peripheral sensory nerves

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Sharks are a group of elasmobranch fish whose ability to detect painful stimuli is debated. Painful chemical, thermal or mechanical stimuli are mostly transmitted through unmyelinated C-fibres (or thinly myelinated A fibres) with small diameter cell bodies in the dorsal root ganglia. Whereas, light touch is transmitted through thickly myelinated A nerve fibres with large diameter sensory neuron cell bodies. In 1993, Snow et al demonstrated that black-tipped shark dorsal root ganglia show a lack of small diameter sensory neurons implicating that sharks are unable to feel painful stimuli. We replicated and extended this finding in Bamboo sharks (Chiloscyllium punctatum) and in hatchlings of Chain catsharks (Scyliorhinus rotifer) using electron microscopy to show a lack of unmyelinated C-fibres in their fin nerves. This is in striking contrast to the peripheral nervous system in almost all mammals studied where C-fibres far outnumber A fibres (by approximately 4 times). Furthermore, we compared electron microscopy analysis of shark fin nerves with mouse saphenous nerves to show that the myelinated fibres in sharks have a significantly larger axonal diameter with poor correlation between myelin thickness and axonal diameter when compared to mice. Comparing the G-ratio (axonal inner diameter to outer diameter) between shark and mouse, we found a population of nerve fibres with low G ratio values, suggesting existence of an unique population of thickly myelinated fibres in sharks compared to mice. Consistent with the peripheral fin nerve data, we found that sensory neurons of Bamboo sharks are unimodal with the peak around 45 µm, which is considered in the large neuron range in multiple mammalian species including mice and sheep. Therefore, we replicated and extended the results from Snow et al. This lack of classical pain-sensing fibres in sharks can be explained if they are indeed unable to feel painful stimuli, or alternatively if some of the large diameter sensory neurons serve as nociceptors with a function in pain sensing. To test this hypothesis, we performed immunohistochemical staining of shark sensory neurons using anti-CGRP antibodies. CGRP is a known peptidergic marker of sensory neurons involved in pain sensing, indeed anti-CGRP antibodies are now an approved migraine medication. Notably, we found that many large diameter shark sensory neurons were immune-positive for CGRP, suggesting that some proteins associated with nociception do exist in sharks. Thus, it is likely that sharks evolved unique strategies to sense painful stimuli through large diameter, myelinated nerves. Future work will generate functional data for the first time to understand pain sensing in sharks using behavioral and electrophysiological tools.

Adult Bamboo Shark

Chain Catshark Hatchling

Role of leg-campaniform sensilla in Drosophila melanogaster adaptive walking

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Movement and the perception that an action occurred are essential for behavior and ultimately shape how animals are able to adapt navigation in complex environments. We focus on how an organism's nervous system acquires and integrates sensory information to efficiently react to its environment. For this, we are investigating, how specific somatosensory information, i.e. signals about load, are sent to local nervous centers of the fruit fly Drosophila melanogaster (D.mel), how this information is processed herein, and what role load proprioception plays in the generation of adaptive locomotor behavior. These sensory organs are campaniform sensilla (CS), mechanoreceptors located throughout the fly's exoskeleton. Here, we focus on the function of the 42 sensors located in each leg (Dinges et al. 2020; Pierzchlińska et al. NWG2024). Preliminary results indicate that activation and inhibition of specific leg CS elicit behavioral effects on walking. Specific kinematic parameters such as leg swing and stance durations were found to be affected. To further dissect this topic, we investigate, how increased load reflects on locomotor behavior in the context of CS optogenetic manipulation. For this we use an established approach (Mendes et al. 2014), i.e. adding weight to the fly´s notum - and, while specific CS are optogenetically inhibited, quantify changes in kinematic parameters during walking. We hypothesize that flies with an increased sensory challenge (carrying up to 2x their body weight), while lacking the mode of proprioception encoded by CS, will show behavioral defects due to a lack of locomotor flexibility. Using Split-Gal4 lines to restrict expression to a small subset of leg CS, we show that inhibiting these CS elicits load-dependent kinematic alterations. For example, a line that labels approximately 25% of leg CS shows that the lack of proprioception produces incremental difficulties in walking with increased load. At the most extreme scenario, inhibiting CS while D.mel carry 2x their body weight produces up to 20% longer stance amplitudes, as well as stance durations that last up to 25% more time. Additionally, using a genetic driver that consistently labels all leg-CS, we observe striking alterations in the leg stepping performance upon optogenetic silencing even without additional load revealing robust kinematic perturbations during straight walking.
These results highlight the contribution of proprioception to walking. The absence of load feedback reveals drastic kinematic changes which are comparable to the increase in body load carried (0x, 1x, and 2x). With our approach, we are able to provide evidence on how CS-encoded proprioception functionally contributes to motor control in walking D.mel.

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Thermal encoding by GABAergic interneurons in the posterior insular cortex

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The processing of sensory information by the neocortex is at the heart of conscious perception and is known to involve a dynamic interaction between synaptically connected GABAergic inhibitory interneurons (INs) and excitatory principal neurons (PNs). One hypothesis is that sensory feature encoding in PNs is supported by different IN types performing specific functional roles. Here we address this hypothesis in the mouse thermal cortex. The thermal system is a fundamental pathway required for accurate object identification, pain perception, and body temperature regulation. It is highly developed in the mouse. However, because the location of a primary thermal cortical region was unclear, how thermal information is processed had been previously poorly understood. Recently, we identified a region of the posterior insular cortex (pIC) that contains somatotopic maps of thermal and tactile information and is required for non-painful thermal perception (Vestergaard et al., 2023; Bokiniec et al., 2023). Two-photon imaging has shown that pIC PNs have a fine-scale and dynamic encoding of skin temperature, but, to date, there is no information on the encoding of temperature by cortical INs. INs could play fundamental roles in thermal processing including the integration of cool and warm information, gain control, modality separation, and spatial integration. As a first step to address these possibilities, here I present our first recordings of somatostatin (SST) and parvalbumin (PV) expressing INs during thermal stimulation. Future work aims to understand the role of cortical INs in thermal processing and perception with the use of a novel two-alternative forced choice (2AFC) thermal perception task.

Advanced behavioral phenotyping in *Drosophila melanogaster* to establish a model for inhalation toxicology for volatile organic compounds

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Chemical pollution is the primary environmental cause of disease and premature death in the world. The annual production of chemicals outpaces the capacity to assess their safety and to monitor human and environmental exposure levels. In particular, air pollution caused 6.7 million deaths in 2019 - 74% of all documented premature deaths due to environmental risk factors. However, suitable models relevant to human and ecological health are rarely available in air pollution toxicology. This project strives to answer whether airborne exposure to volatile chemical compounds alters visual and acoustic behaviors in *D. melanogaster*, establishing its value as a model for adverse health impacts of air pollution.

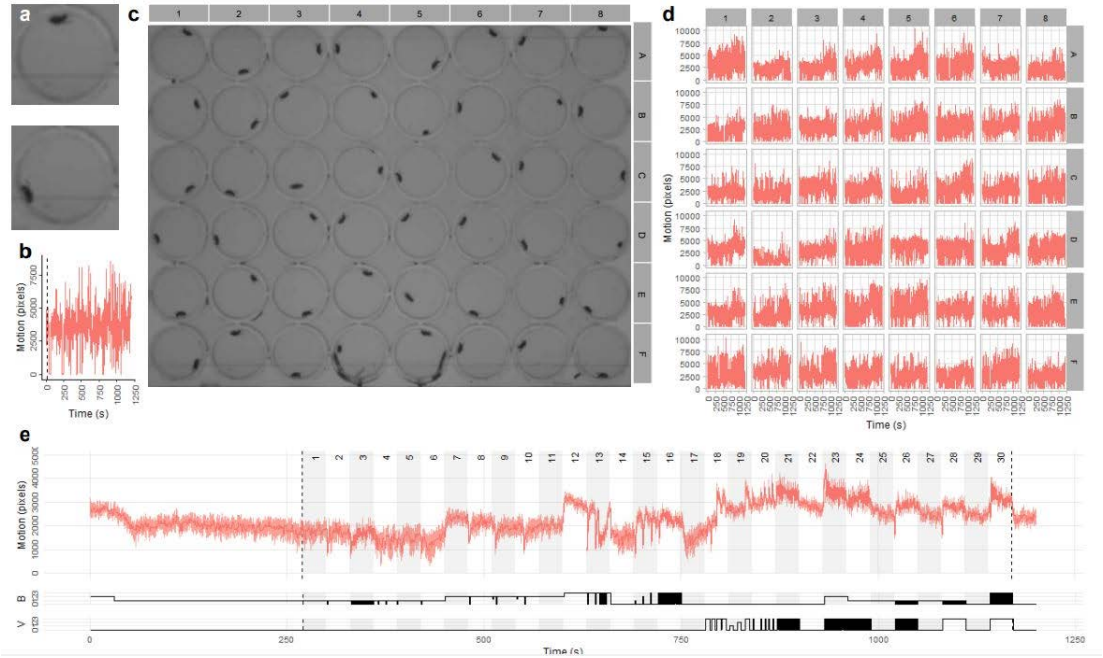

Figure 1. A battery of scalable behavioral assays in adult male flies. (a) Representative images of a wild type fly (WTCS) in a single well of a 48-well plate. Time stamps indicate movement in two subsequent seconds. (b) Representative plot of motor activity (in pixels) over time from a single well during the behavior assay battery. Vertical dashed line at 10 s: time stamps shown in a. (c) Representative image of a 48-well plate during behavioral analysis. (d) Behavioral profiles of 48 wells. (e) Average motion of male flies as a function of time. Data: median 95% CI for 336 time series. Each time series obtained from a well containing a single fly. Wells were tested across seven 48-well plates. Lines above the x-axis: timing, duration and intensity of light (B, backlight) and acoustic (V, vibration) stimuli. Numbers indicate assays in the order of application.

Load sensors in the fruit fly: detailed analysis of arborisation patterns

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Animal's walking is coordinated through adjustments in the relationship between swing and stance phases of each leg, forming intraleg coordination, along with interleg coordination patterns that arise from temporal and spatial interactions between all legs. The locomotor output results from a finely tuned interplay between centrally generated rhythmic activity and sensory feedback from the proprioceptive organs (Bidaye et al. 2018). The fruit fly, *Drosophila melanogaster*, is a convenient model to study the neural control of walking, considering its versatile locomotor behaviour, numerically simple central nervous system and the available neurogenetic tools.

Campaniform sensilla (CS) are load-sensing receptors found throughout the leg, usually in close proximity to the joints. CS are organised into groups and fields, and they vary in their orientation and shape. When the load is exerted on the cuticle, generated forces displace the CS cap. CS neurons convey this information to the central nervous system (Dinges et al. 2021, Tsubouchi et al. 2017). In larger insects, load feedback coordinates the transition between swing and stance phase (Bidaye et al. 2018), which may affect walking speed. Little is known about the role of the CS, including functional differences between the individual types, in locomotor transitions in the fruit fly. Therefore, we optogenetically inhibited CS neurons in freely walking flies. We found that restricting load feedback altered the animals' walking speed. This effect depended on which CS neurons were inhibited.

The behavioural results inspired us to examine the local circuits that integrate load feedback. All CS neurons arborise in the ventral nerve cord (VNC), the insects' equivalent of the spinal cord, in a modality-dependent fashion (Tsubouchi et al. 2017). However, despite the availability of VNC connectome datasets (Phelps et al. 2021, Azevedo et al. 2024, Cheong et al. 2024, Marin et al. 2024, Takemura et al. 2024), data on the arborisation patterns of CS neurons remain limited due to the lack of specific driver lines. In order to connect the gap between the central nervous system and the periphery, we traced the CS neurons in both the VNC and the corresponding legs of each fly, using a stochastic multi-colour labelling method (Multi Color Flip-Out – MCFO, Nern et al. 2015). As a result, we observed individual arborisation patterns of the CS neurons, which were much more diverse than previously assumed. Most of them arborise unilaterally within a neuromere of their entry nerve, with only a small subset of neurons projecting to the neighbouring segments. We describe in detail the arborisation patterns of front leg CS neurons of the femoral field, which correlate with their position in the field. Moreover, we compare these neurons with those found in other CS fields and distinct locations. Finally, by matching the single cell images to EM reconstructed neurons in the connectome datasets, we draw first conclusions on their local connectivity. This knowledge will facilitate both – further connectomics research and functional analyses through optogenetic manipulations of the post-synaptic partners of the CS neurons.

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Whisker-Mediated Categorization of External Space in Head-Fixed Mice

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Rodents navigate their environment and locate nearby objects by scanning their surroundings with their whiskers. Previous studies using head-fixed behavior have begun to decipher the sensorimotor and neuronal mechanisms underpinning tactile object localization. However, these studies focused on object location along a single dimension. In contrast, mice must gather information across two dimensions in horizontal space to generate an egocentric map of their sensory space for navigation. Here, we developed a tactile behavioral paradigm in which both dimensions of object position are relevant. Mice categorized the position of a stimulus in an arbitrarily divided two-dimensional space using their whisker. They readily extrapolated their behavior to newly introduced stimuli closer to the category boundary, suggesting a cognitive representation of tactile space. We further showed that optimal task performance was dependent on the whisker-related primary somatosensory cortex. This behavioral task provides a foundation for unraveling the sensorimotor and neuronal principles underlying tactile spatial representation.

Establishment of a human induced pluripotent stem cell-based in vitro model for the investigation of sex-specific differences in migraine pathophysiology

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Migraine is a very common neurovascular disorder with its underlying mechanisms still not fully elucidated. A variety of genetic variations, including a frameshift mutation in the TWIK-related spinal cord potassium channel (TRESK), have been linked to migraine pathophysiology. While migraine prevalence is similar in both sexes during childhood, incidences increase in women with rising age affecting females three times more often than males. Today, there is prominent evidence that this disparity is mediated by an imparity in sex hormone levels, which was shown to affect the excitability and sensitization of trigeminal nociceptors via the transient receptor potential vanilloid 1 (TRPV1). For the investigation of the interplay of sex hormones, TRPV1 and TRESK signaling in migraine pathophysiology, we used a reliable protocol for reprogramming human dermal fibroblast (HDF) into induced pluripotent stem cells (iPSCs). Subsequent differentiation into nociceptive neurons serves as a basis for our novel human cellular *in vitro* model expressing nociceptor-specific ion channels and the G protein-coupled estrogen receptor 1 (GPER1) likewise. The abundance of nociceptor-associated ion channels and GPER1 was detected by fluorescence microscopy. Moreover, functionality and the sensitivity towards GPER1 agonists were investigated by electrophysiological recordings in a HEK293-based cellular model and iPSC-derived nociceptive neurons. Concomitantly, a sex-matched migraine patient and healthy control cohort was established. Within this study fibroblast cultures were generated and used for whole genome sequencing analysis in order to identify novel migraine-related genetic variants with sex-specific differences. Furthermore, fibroblasts are currently used for the reprogramming into patient-specific iPSCs. Using this iPSC-based *in vitro* model and the established cohort of sex-parietal migraine patients and healthy controls, we intend to investigate the molecular mechanisms underlying the pathophysiology of migraine, focusing in particular on the role of sex hormones and migraine-associated ion channels.

Poster Topic

T21: Motor Systems

T21-1A Unravelling the neural control of forward and backward stepping of an insect leg
Angelina Ruthe, Philipp Rosenbaum, Silvia Daun, Ansgar Büschges

T21-2A Role of pretectal dopaminergic neurons during spontaneous locomotion in zebrafish
Shagnik Chakraborty, Wolfgang Driever, Johann H. Bollmann

T21-3A Pharmacological analysis unveils similarities in load processing between two joints in two stick insect species
Matthias Gruhn, Mascha Driesch, Anna Haberkorn, Christopher Körsgen, Ansgar Büschges

T21-4A Cholinergic modulation of striatal synaptic transmission after short- and long-term deep brain stimulation of the entopeduncular nucleus in an animal model of paroxysmal dystonia
Marco Heerdegen, Fabiana Santana-Kragelund, Denise Franz, Stefanie Perl, Anika Lüttig, Henning Bathel, Angelika Richter, Rüdiger Köhling

T21-5A Prediction of Dystonic Attacks in a Hamster Model of Dystonia via Single-Channel EEG
Valentin Neubert, Rahul Bordoloi, Monique Zwar, Olaf Wolkenhauer, Rüdiger Köhling

T21-1B Carrion crows learn to use stick-tools with high efficiency and skill
Felix W. Moll, Julius Würzler, Andreas Nieder

T21-2B Brain-wide latent population activity integrates action and goal expectation
Yangfan Peng, Carl Lindersson, Sasha Tinelli, Jeffrey Stedehouder, Rahul S. Shah, Armin Lak, Charlotte J. Stagg, Andrew Sharott

T21-3B Descending control of walking direction in Drosophila
Jan M. Ache, Sander Liessem, Fathima Mukthar Iqbal, Aleyna Meric, Ezequiel Axel Gorostiza, Federico Cascino-Milani, Till Bockemühl, Ansgar Büschges, Stefan Dahlhoff

T21-4B Analyzing individual locomotion behavior in Drosophila larvae
Marit Praetz, Luis Garcia-Rodriguez, Christian Klämbt

T21-5B Sleep Disruption Improves Performance in Simple Olfactory and Visual Decision-Making Tasks

Paula Pflitsch, Nadine Oury, Kumaresh Krishnan, William Joo, Declan G. Lyons, Maxim Quirijn Capelle, Kristian Herrera, Armin Bahl, Jason Rihel, Florian Engert, Hanna Zwaka

T21-1C Muscle control of multi-modal courtship signals in *Drosophila*
Melanie Stenger, Elsa Steinfath, Kimia Alizadeh, Jan Clemens

T21-2C Vocal Strategies for Territorial Defense and Mate Attraction in Nightingales
Niels Hein, Giacomo Costalunga, Daniela Vallentin

T21-3C Symmetry break and leg specific roles during curve walking in *Drosophila*
Ezequiel Axel Gorostiza, Divya Sthanu Kumar, Ricardo Custódio, Nino Mancini, Till Bockemühl, Kei Ito, Salil Bidaye, Ansgar Büschges

T21-4C Auditory feedback influences syllable repetition in birdsong
Jacqueline Laura Göbl, Dmitry Kobak, Lena Veit

T21-5C Social context affects adaptive song sequence learning in songbirds
Lioba Fortkord, Lena Veit

T21-6C Parallel sensorimotor pathways control landing in *Drosophila*
Sander Liessem, Samuel Asinof, Aljoscha Nern, Marissa Sumathipala, Han S. J. Cheong, Tess Oram, Mert Erginkaya, Chris J. Dallmann, Gwyneth M. Card, Jan M. Ache

T21-1D Real-Time Segmentation and Classification of Birdsong Syllables for Learning Experiments
Nils Riekers, Lena Veit

T21-2D Kinematics of walking initiation in *Drosophila melanogaster*
Fabian Jakobs, Moritz Haustein, Till Bockemühl, Ansgar Büschges

T21-3D Kinematic synergies of leg stepping in walking fruit flies, *Drosophila melanogaster*
Moritz Haustein, Ansgar Büschges, Till Bockemühl

T21-4D Speed-related changes in kinematic variability in walking *Drosophila* in the context of stability and interleg coordination
Till Bockemühl, Vincent Godesberg, Ansgar Büschges

T21-5D Structure-function analysis of cell types mediating corollary discharge signaling in larval zebrafish
Katharina Lischka, Johann H. Bollmann

T21-6D Analysis of the local search behavior in Drosophila melanogaster larvae
Jessica Kromp, Tilman Triphan, Andreas S. Thum

Unravelling the neural control of forward and backward stepping of an insect leg

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Walking is a well-studied behaviour that is generated by the interaction of descending input from the brain with local premotor circuits in the spinal or ventral nerve cord as well as sensory feedback from the legs of an animal. Changing walking direction from forward to backward asks for modifications in these premotor circuits generating leg movements. Thus far, the mechanisms underlying the generation of backward stepping of the legs are only merely understood. Stick insects constitute a suitable model to study such mechanisms as size and structure of their leg muscle control system allows the application of a variety of electrophysiological methods in semi-intact preparations. In stick insect middle legs, forward and backward stepping is known to be based primarily on the activity of the Thorax-Coxa joint motor neurons and muscles protractor and retractor coxae that move the leg back and forth, while activity of the more distal leg joints remains roughly the same (Rosenbaum et al. 2010). In forward stepping, protractor coxae generates swing and retractor coxae stance. The reverse is the case for backward stepping. Here we aim to unravel, how local premotor networks, in particular the population of nonspiking interneurons (NSIs) of the different leg joints with load feedback from leg load sensors, i.e. campaniform sensilla (CS), contribute to generating a forward and backward stepping motor output. We performed intra- and extracellular recordings of MNs and NSIs combined with stimulation of CS during forward and backward stepping of the stick insect middle leg in a semi-intact preparation. As expected, NSIs supplying MNs of the distal leg joints exhibit qualitatively similar activity patterns in both stepping directions. NSIs supplying MNs of the ThC joint, however, show different types of activity patterns. Most of them change their activity pattern between both stepping directions, serving a synaptic drive to ThC MNs appropriate for each walking direction. Individual NSIs appear to be silenced by a hyperpolarization in membrane potential and some NSIs showed a similar activity pattern in forward and backward stepping. Stimulating load sensors on the leg, i.e. CS, during stepping in both stepping directions revealed that CS input contributes instrumentally to the membrane potential modulation of premotor NSIs. Our findings emphasize the decisive role of CS feedback for establishing motor output for forward and backward stepping.

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Role of pretectal dopaminergic neurons during spontaneous locomotion in zebrafish

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In the absence of salient sensory cues, animals execute sequences of motor actions in order to explore and forage. Neuromodulatory systems, such as the dopaminergic (DA) system, modulate neural circuits controlling motor activity during spontaneous locomotion. In the diencephalon of zebrafish, there is a cluster of pretectal DA neurons, which project to the optic tectum; the role of these neurons in controlling or modulating locomotion, however, is poorly understood. Here, we address the structure and function of these DA cells to better understand their role in spontaneous swimming.
We first investigate the projection pattern of pretectal DA neurons into the optic tectum by implementing an intersectional fluorescence labelling approach using transgenic lines that express different fluorescent proteins in the two different neuronal populations. We observe axons of the pretectal DA neurons mainly in the deep layer of the tectal neuropil, close to where axons of tectal projection neurons run to leave the tectum caudally on their way to premotor regions in the mid- and hindbrain. To further investigate a possible functional role in motor control, we measure temporal relationships between activity in these neurons and swimming behaviour. Specifically, we use 2-photon imaging of Ca2+ and DA signals in combination with genetically encoded sensors of neural activity (GCaMP and GRAB sensors for calcium and DA sensing, respectively) expressed in pretectal DA neurons and their putative post-synaptic target sites in the optic tectum. Simultaneously, spontaneous locomotion is recorded using peripheral motor nerve recordings. We observe swim-associated neuronal activity and DA release during spontaneous motor events, which suggests a possible role of these neurons in modulating visuo-motor transformation in the tectum. Thus, our findings provide insights into a possible neuromodulatory mechanism in a midbrain structure that is known to control essential sensory-guided behaviours.

Pharmacological analysis unveils similarities in load processing between two joints in two stick insect species

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Mechanosensory feedback such as load feedback through campaniform sensilla (CS, e.g. Ritzmann & Zill 2017) is essential for insects to adapt posture and locomotion and shape the motor neuron (MN) output to behavioral needs (Bidaye et al. 2018). However, the influence and functional connectivity of load signals on the different MN pools of an insect leg is still largely unknown.
Previous studies have shown through anatomical and electrophysiological studies in protractor and retractor coxae MN (ProCx and RetCx) of the stick insect species *Carausius morosus* that local load can be distributed to diverse MN pools but is channeled through local inhibition (Haberkorn et al. 2019, Schuckel et al. 2019). Here, we aimed at investigating if this distribution of CS influences is specific only for the thorax-coxa joint of *C.morosus*, but instead also present in another leg joint, and whether this form of regulation of influences is also present in the related stick insect species *Medauroidea extradentata*.

For this purpose, we extracellularly recorded the response of ipsi- and contralateral ProCx and RetCx MNs and that of the MNs of the next distal coxa-trochanter joint (levator and depressor trochanteris, LevTr and DepTr), upon stimulation of trochanteral CS G1-4 and femoral CS G5. This was done in both species and in regular saline as well as in saline containing the GABA-A blocker picrotoxin (PTX), which has previously been shown to unmask excitatory connections between CS and MN pools (Schuckel et al. 2019). When recording LevTr and DepTr MNs, we also always recorded ipsilateral ProCx and RetCx activity as control for effective stimulation. The leg stump of the resting animal was stimulated in anterior and posterior direction.
Activation of the trCS and feCS activated ProCx and RetCx MNs of *M.extradentata* in a similar way as described before for *C.morosus*, both in saline and in PTX (Haberkorn et al. 2019; Schuckel et al. 2019). Anterior deflection exclusively activated ProCx MNs and posterior deflection RetCx MNs, and PTX application caused activation of both MN pools in both stimulus directions as well as contralateral activation of both MN pools. For the CxTr joint, we found that load feedback from the trCS and feCS in *C.morosus* activates DepTr MNs upon anterior and posterior deflection of the leg stump. In contrast, neither stimulation lead to activation of LevTr MNs. Application of PTX lead to the additional recruitment of fast DepTr MNs in both stimulation directions, and to activation of fast LevTr MNs upon posterior but not anterior deflection. Load stimuli to trCS and feCS were largely processed in a similar way in *M.extradentata*, with the difference that, also in control conditions, anteriorly and posteriorly directed stimuli lead to responses of LevTr MNs. In both species, PTX application also led to additional activation of contralateral DepTr and LevTr MNs in both stimulus directions, which is never the case without PTX.
Our findings suggest that there are minor differences in the processing of the same load information from TrCS and FeCS in the CxTr joint between the two stick insect species. However, in both species, processing of CS influences onto thoracic and coxal MNs is dependent on GABAergic inhibition and thus similarly regulated.
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*contributed equally to the work

Cholinergic modulation of striatal synaptic transmission after short- and long-term deep brain stimulation of the entopeduncular nucleus in an animal model of paroxysmal dystonia

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Acetylcholine released by striatal cholinergic interneurons plays a pivotal role in modulating striatal activity, ultimately influencing the function of the basal ganglia. Alterations in cholinergic signalling may lead to movement disorders, and consequently, anticholinergic medication is a well-established treatment option for hyperkinetic movement disorders, such as dystonia. In cases of medication-resistant, generalised dystonia, deep brain stimulation (DBS) has emerged as a key therapeutic intervention. However, the precise mechanisms underpinning DBS remain unclear. Given the delayed therapeutic effects of DBS in dystonia patients, typically observed after several weeks or months, it is hypothesised that long-term adaptive restructuring of the basal ganglia network occurs. Furthermore, it has been speculated that striatal cholinergic signalling may contribute to these long-term adaptive changes.

To explore the temporal dynamics of striatal modulations induced by DBS, we compared the membrane properties of striatal medium spiny neurons and the spontaneous glutamate release from corticostriatal synapses in dtsz hamsters subjected to short-term and long-term DBS. These hamsters represent a phenotypic model of dystonia, closely mimicking the clinical features of the human movement disorder. Bilateral stimulation electrodes were implanted in the entopeduncular nucleus (EPN; the human equivalent of the GPi). For long-term DBS, the electrodes were connected to STELLA, a software-driven implantable modular stimulation device, for a continuous period of over 11 days. Short-term DBS was conducted for 3 hours using an external stimulator. DBS (130 Hz, rectangular pulses of 50 µA and 60 µs) and sham-DBS were performed in vivo in freely moving dystonic animals. Neuronal and synaptic activity were assessed using whole-cell patch clamp recordings from striatal medium spiny neurons in acute parahorizontal slices. The potential effects of short- and long-term DBS on cholinergic synaptic transmission were evaluated by applying acetylcholine (100 µM) dissolved in an artificial cerebrospinal fluid recording solution.

Our findings reveal distinct effects of short- and long-term DBS on neuronal excitability. Short-term DBS increased action potential frequency, whereas long-term DBS reduced it. Moreover, miniature excitatory postsynaptic currents (mEPSCs) resulting from spontaneous glutamate release were attenuated exclusively after short-term DBS, a reduction that was no longer observed after long-term DBS. Notably, both short- and long-term DBS interacted with cholinergic signalling, as mEPSC frequencies were elevated following the application of acetylcholine in slices from stimulated hamsters. These results suggest that DBS induces complex homeostatic modulations, affecting cellular excitability, spontaneous synaptic transmission, and acetylcholine signalling.

Prediction of Dystonic Attacks in a Hamster Model of Dystonia via Single-Channel EEG

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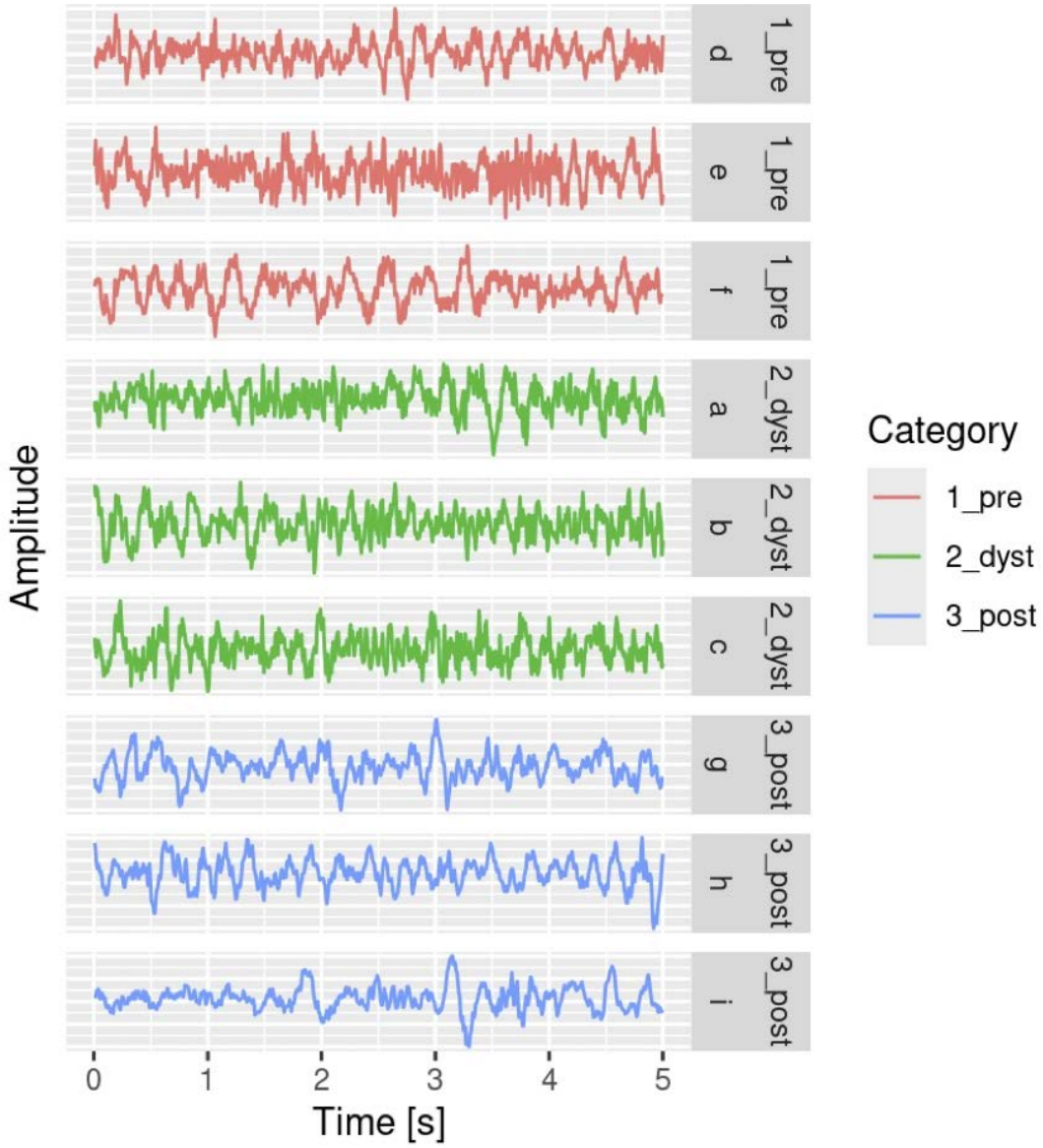
Objective:
Dystonia is a neurological disorder characterised by sudden episodes of impaired movement, arising from disruptions in cortical, basal ganglia, cerebellar, and thalamic networks. Unlike Parkinson's disease, dystonia lacks a consistently effective treatment. Deep brain stimulation (DBS) of the globus pallidus in dystonic animal model, the "dt^{sz}" hamster, has shown promise in reducing the frequency of dystonic attacks. The ability to predict imminent attacks using electrophysiological biomarkers could enable real-time activation of stimulation devices.

Methods:
In a pilot experiment using wireless transmitters, EEG recording electrodes were implanted subdurally in a dt^{sz} hamster (-0.6 mm AP, +2.2 mm ML of bregma). Continuous EEG and video recordings were captured over 7 days, and dystonic episodes were identified through manual video screening based on behavioural changes. The EEG signals were segmented into 10-second intervals for the pre-attack (20 minutes prior), peri-attack, and post-attack (10 minutes after) phases. A variety of linear and non-linear time-series features were extracted, and a random forest classifier was trained to distinguish between these phases.

Results:
A total of 10 dystonic attacks were visually detected, with durations ranging from 15 seconds to 19 minutes (mean: 3.8 ± 4.6 minutes). The classifier, trained to detect the dystonic attack phase, achieved a Matthews correlation coefficient (MCC) of 0.11. Aggregating classification results in 5-minute intervals based on a 51% probability threshold did not improve performance.

Conclusion and Outlook:
Despite the extraction of numerous interpretable features, our classification efforts have thus far been unsuccessful. We hypothesise that the cortical region recorded may be too broad to yield a meaningful single-channel EEG signal. We plan to implant depth electrodes near the globus pallidus internus in a larger cohort of animals, which we expect will provide a higher signal-to-noise ratio, fewer signal sources, and insights into inter-individual similarities and differences. Our ultimate goal is to identify predictive yet computationally simple features for integration into a stimulation device, enabling robust real-time prediction of dystonic attacks in human patients.



Single-channel subdural EEG recorded from the cortex of a dtsz hamster.

Carrion crows learn to use stick-tools with high efficiency and skill

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Tool use is fundamental to our lives. Throughout the animal kingdom, tool use is rare, and its underlying neural mechanisms have not been characterized. Flexible tool use requires the skill to reliably handle and use a tool accurately, while also being able to adapt as needed, but how the brain achieves this balance between stability and flexibility remains unknown. To gain traction on this issue, we trained carrion crows (Corvus corone) to use a beak-held stick-tool to retrieve food rewards from beneath a Plexiglas plate in a fully automated apparatus. By using state-of-the-art computer vision methods (DeepLabCut), we precisely quantified their tool use related movement trajectories. We found that the similarity between movement trajectories increases across several weeks within a given task condition (i.e., food reward position). Individual crows developed idiosyncratic trajectories, suggesting varying levels of behavioral lateralization. Despite the emergence of stereotypical movement sequences, the crows remained remarkably flexible, immediately adapting to occasional performance errors – such as when the stick-tip accidentally lost contact to the pellet – by executing compensatory movements. Additionally, we observed that the crows frequently adjusted the orientation of the stick tool within their beaks to ensure precise alignment after retrieving it from the apparatus. Taken together, we demonstrate both the reproducibility and the sensory feedback-driven adaptability of the crows' tool use. This new behavioral paradigm will enable us to identify the neuronal underpinnings of tool use control in the crow brain, uncovering network coding principles underlying skilled action sequences.

Brain-wide latent population activity integrates action and goal expectation

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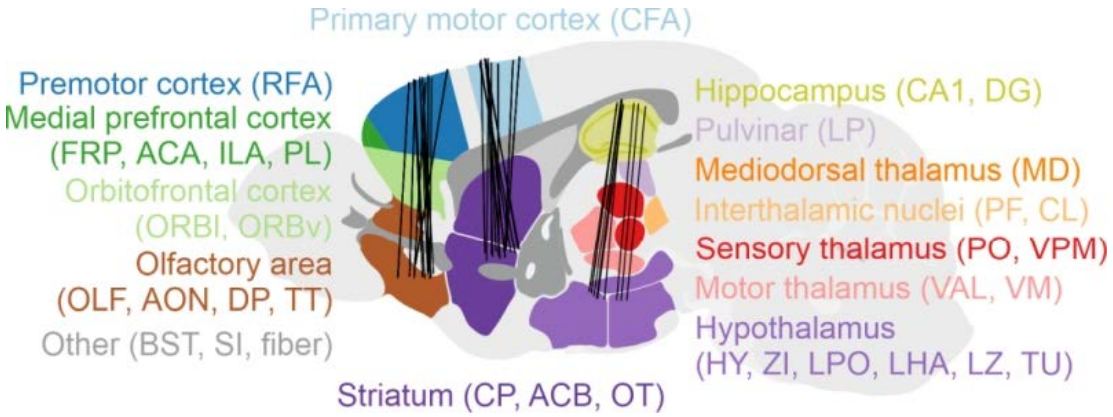
Large scale recordings have revealed that neurons encoding motor and non-motor variables are highly distributed across the brain. While these neurons generate population level dynamics during spontaneous behavior, it remains unclear how these latent subspaces relate to the simultaneous motor and cognitive demands during goal-directed behavior. Here, we show that continuously anticipated action outcome drives ubiquitous latent dynamics during goal-directed movements. We used multiple Neuropixels probes to simultaneously record spiking activity from cortical and subcortical regions during a reaching task in head-fixed mice. Task-related population dynamics was conserved across regions, recording days and animals and covaried within a common latent subspace, propagating from thalamic and cortical areas to other areas including basal ganglia, hippocampus, hypothalamus, and olfactory areas. These latent dynamics preceded movement onset and were modulated by reach distance and reward availability. Furthermore, their temporal progression continuously scaled with the timing of reward consumption and their activity decreased afterwards, despite ongoing stereotypical re-reaches. Our findings thus provide evidence for a brain-wide latent subspace for continuous representation of action-mediated proximity to goal, which could provide the basis for ubiquitous temporal difference learning based on predicted action outcome.



Schematic of sagittal brain atlas showing recorded region categories in colour. Black lines represent Neuropixels trajectories from different sessions approximating positions of active electrodes.

Descending control of walking direction in Drosophila

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To get from A to B, animals need to control the speed and direction of locomotion. A critical bottleneck in the neuronal circuits controlling walking are descending neurons (DNs), which connect higher-level control centers in the brain to lower-level motor circuits in the ventral nerve cord (VNC) or spinal cord. Here, we analyze how two DN populations contribute to the control of walking direction in Drosophila.

First, we focus on the well-described moonwalker DNs (MDNs). By combining in-vivo patch-clamp recordings in walking Drosophila with antennal mechanosensory stimulation and genetic silencing, we establish that MDNs contribute to antennal touch-mediated backward walking. Each of the four MDNs responds to bilateral antennal touch, and their activity is strongly correlated with backward walking speed, but not turning, in spontaneously walking flies. Hence, MDNs drive backward walking in the context of antennal touch, which could play a role in the negotiation of obstacles in the fly's walking path.

To broaden our understanding of the descending control of walking, we next investigated DNp17, a population of six DNs which has previously been implicated in driving fast forward walking (refs. 1 & 2). Bilateral activation of the DNp17 driver line drives forward walking with a strong turning component, leading to a meandering walking path. Recordings in behaving flies confirmed that the activity of neurons in the DNp17 driver line is strongly correlated with ipsiversive turning, and weakly correlated with forward speed. However, we also found that the DNp17 line labels a larger number of neurons than expected, and we currently investigate which of these neurons are responsible for the walking phenotype. Regardless, the neurons labelled by the DNp17 line and the MDN population control different aspects of forward walking, backward walking, and turning.

Finally, we investigated whether the two populations controlling walking are also active during flight. Remarkably, both populations of neurons controlling walking parameters were strongly inhibited during flight. This flight-dependent gating has the opposite sign to the gating observed in DNs controlling flight-related behaviors and peripheral visual circuits, suggesting that the differential gating of descending pathways enables adaptive behavior in different behavioral states.

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Analyzing individual locomotion behavior in Drosophila larvae

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Behavioural variability in genetically identical backgrounds is known for Drosophila adults where object responses in a Buridans paradigm correlate with asymmetric or symmetrical wiring in Dorsal Cluster Neurons (Linneweber et al., 2020). In addition, handedness in flies was shown to differ on the population level in Drosophila Genetic Reference Panel (DGRP) flies, where the activity of a subset of PFN neurons in the central complex modulates the more extreme handedness phenotypes (Buchanan et al., 2015). Curiously, these differences are not heritable, therefore not solely based on genetics and could not be explained by morphological differences.
Interestingly, also in Drosophila larvae there seems to be phenotypic variability in behavioral responses. Larval forward and backwards locomotion consists of peristaltic muscle contraction either from posterior to anterior (forwards movement) or anterior to posterior (backwards movement) (Heckscher et al., 2012). During reorientation, the larva stops while the head sweeps from left to right to sample sensory information in a temporal as well as spatial manner (Humberg et al., 2018). Looking not only at the locomotion but at the overall movement pattern, hence the trajectories of individual larvae, there seems to be variability in their overall locomotion. Trajectories of crawling Drosophila larvae are characterized by active movement and reorientation phases. But even in the absence of external stimulation, two strategies of reorientation can be observed. Firstly, a straight crawl - stop - head sweep - reorientation followed by a straight crawl. Secondly, a clockwise or counter-clockwise circling of the area. These strategies are observed for different individuals of the same isogenic background as well as well as for wild type strains like Canton S and Berlin K. Here we tested for individuality in larval locomotion behavior and aim to test if behavioral patterns persist after the metamorphosis.

Sleep Disruption Improves Performance in Simple Olfactory and Visual Decision-Making Tasks

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Sleep disruption is known to drastically affect cognitive function including decision-making and attention across many different species. In this study, we leveraged the small size and conserved brain structure of larval zebrafish to investigate the consequences of sleep disruption in the context of two well-described behaviors, a visual and an olfactory-based decision-making task. We find that in both paradigms, sleep disruption leads to an improvement in performance. Specifically, we show that sleep disruption increases reaction time and improves performance in a visual motion discrimination task, an effect that we attribute to longer integration periods in disturbed animals. With the use of a drift diffusion model we predict specific circuit changes underlying these effects. In olfactory decision making we find that sleep disruption leads to increased odor sensitivity, which we show is likely mediated by cortisol. Our findings set the groundwork for further investigation of the underlying circuit changes in the brain that occur as a result of sleep disturbance across different species.

Muscle control of multi-modal courtship signals in *Drosophila*

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The brain coordinates body movement by processing sensory cues, selecting from various motor programs. Muscle activity is therefore the ultimate output of neural computation. However, with fewer muscles than behaviors, it remains unclear how overlapping sets of muscles produce distinct motor outputs. Here, we use courtship signaling in *Drosophila* to study this issue. During courtship, males select between two signal types: air-borne song and substrate-borne vibrations. The song occurs in bouts and consists of two modes: Sine song corresponds to sustained tones. Pulse song consists of trains of short pulses produced at regular intervals (40ms). Substrate-borne vibrations are also pulsatile but have longer intervals (150ms). Song results from unilateral wing fluttering, controlled by well-understood mechanisms: indirect wing muscles create power, and direct wing muscles fine tune the song. Vibrations are not produced by wings and are correlated with abdominal movement. However, males without abdomen still vibrate. Therefore, how vibrations are produced remains unclear. We hypothesize that both song and vibration pulses originate from the thorax, with vibrations transmitted through the legs to the substrate. Due to the small number of thoracic muscles, overlapping muscles likely produce both signals. To test this, we deactivated wing muscles controlling song and examined changes in the amount and structure of vibrations. We find an extensive overlap between the muscles that drive song and vibration. Inactivating the indirect DLMs reduces the amplitude of both song and vibration. In addition, the direct wing muscle i2 shapes frequency and intervals of song and vibration pulses whereas the b2 muscle impacts vibration amplitude. We combine connectomics with genetic tools to identify networks controlling wing muscle patterns. In addition, we currently perform calcium imaging to assess the dynamical engagement of muscles throughout the body - for instance in legs and abdomen - during song and vibration.

Vocal Strategies for Territorial Defense and Mate Attraction in Nightingales

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The rapid and accurate interpretation of acoustic signals in different behavioral contexts is central to producing an appropriate behavioral response. Songbirds, for instance, demonstrate this capability by recognizing different behavioral contexts through vocalizations used for territorial defense, mate attraction, signaling danger, and individual identification. The common nightingale provides a compelling example of how a large repertoire of vocalizations can be strategically utilized in a vast number of different scenarios. During the breeding season in the European spring, male nightingales engage in intense nocturnal counter-singing duels with neighboring males to establish and defend territories after having migrated from Africa. Simultaneously, males attempt to attract females by singing their songs. Females search for potential mates during the darkness of the night after having completed their migration. Whereas females are incapable of singing, they can use vocal signals, for instance so called 'huit' calls, to indicate their presence to the singing males. These 'huit' calls have been suggested to be used as contact calls or even as luring calls. Differentiating the songs of rivalling males from 'huit' calls is of utmost relevance for male birds during the breeding season. Here, we investigated the influence of these two distinct acoustic contexts on vocal behavior in wild male nightingales, by exposing the birds to either male songs or 'huit' calls. During exposure to rival male song, male nightingales engaged in counter-singing and regularly exhibited their typical song matching behavior - listening to and repeating the same song that was just presented. During 'huit' call playback, male nightingales sang songs with a longer duration that were interspersed with shorter gaps compared to normal counter-singing. Males furthermore introduced new syllables into their vocal repertoire that were not observed during male-male song interactions. These new whistle-like elements that were produced in response to 'huit' calls are characterized by a distinctly high pitch that was found to be uncommon in the context of territorial defense. The ability to adjust temporal dynamics and spectral features depending on context indicates that nightingales are capable of categorizing auditory signals and in turn tailor their vocalizations accordingly.

Symmetry break and leg specific roles during curve walking in *Drosophila*

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To fulfill behavioral demands, walking animals must modify kinematic parameters and movement sequences of the limbs and joints. Insects, for example, adjust the step cycle period and stance duration of their legs to control speed during straight walking, and their leg coordination patterns change gradually with speed. During straight walking, contralateral pairs of legs in each body segment produce similar steps differing mostly in their phase relation. However, to modify the direction, on the contrary, the motor system must break symmetry and generate differences in stepping kinematics between left and right legs. We analyzed videos of free-walking flies with nonlinear trajectories and found three major strategies to produce curve walking: (i) adjustments (ADs), (ii) arcs (ARs), and (iii) turns (TUs) with increasing curvature from (i) to (iii). For analyzing the relationship between kinematic asymmetries and curve walking strategies, we implemented a visual paradigm to induce curves, and used optogenetics on a group of descending neurons known to promote changes in direction. ADs and ARs are usually performed at constant speed, comparable to straight walking, while before a TU, flies usually decrease speed. ADs are achieved as the result of fast kinematic changes occurring during one step of hind leg on the inner side of the curve, while ARs comprehend several steps with smaller stance amplitudes on all inner legs. We found that stance amplitude has a higher angular velocity dependence than other parameters. Interestingly, in ARs middle and hind inner legs also perform slightly shorter swings and longer stances, without strongly affecting step duration. In TUs, those changes on middle and hind leg kinematics become more pronounced, and front legs then exert a distinct role: during TUs, inner front legs execute longer swings and shorter stances than outside legs, while contrary hind and middle leg pairs perform longer swings and shorter stances on the outside of the curve. In both cases, kinematic changes promote differences in the temporal phase relationship between legs on the inner and the outer side of the curves. We found that curve walking not only requires breaking the symmetry between left and right side of the body, but in cases such as TUs, changing direction requires some pairs of legs to generate individually specific kinematics, which differ from the kinematics of the other ipsilateral and contralateral legs, indicating that curve walking kinematics do not just display side-specificity, but leg specificity in neural control.

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Auditory feedback influences syllable repetition in birdsong

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Many complex behaviors such as speech, dance or writing are composed of ordered sequences of simpler motor elements. One important model for a sequential motor skill is birdsong, which is composed of individual syllables. The song of Bengalese finches (*Lonchura striata domestica*) contains repeat phrases, in which the same syllable type is repeated a variable number of times, as in the syllable sequence “a-b-b-b-b-c”. Such repetitions are a common feature of many birdsongs and also occur in other types of human and animal communication.

Auditory feedback has been proposed as a key mechanism that controls syllable repetition. Theoretical work suggests that auditory feedback from the repeat syllable could lead to increased probability of repeating the same syllable, and adaptation to the feedback would be what eventually allows the system to move on to the next syllable. Here, we test this prediction by manipulating auditory feedback for specific syllables within repeat phrases. We use custom software for online recognition of target syllables while the bird is singing and song-triggered immediate playback of sounds. We played back either the repeat syllable, a different syllable from the bird’s own song, or other sounds, thus disturbing the auditory feedback of the repeat syllable. We find that different manipulations can either extend or shorten repeat phrases, indicating a more complex interaction between auditory feedback and motor control than previously assumed.

Using acoustic analyses, we show that subtle variations in the acoustic structure of repeat syllables contain information on the relative progress through the repeat phrase. We use low-dimensional embeddings of syllable spectrograms to follow trajectories associated with individual repeat phrases. Auditory feedback manipulations influence the progress along these trajectories, which we quantify using manifold learning methods. Future studies with parallel electrophysiological recordings will test whether deviations from these trajectories are associated with corresponding neuronal changes, indicating that such acoustic analyses could provide a complementary read-out of internal neuronal states.

Understanding how auditory feedback influences syllable repetition in birdsong may reveal general principles for the neuronal control of complex sequential behavior. This will shed light on the question how individual motor gestures are organized into sequences and how the nervous system selects between different possible courses of action.

Social context affects adaptive song sequence learning in songbirds

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Social interactions are crucial for imitative vocal learning such as human speech learning or song learning in songbirds. Recently, introducing specific learned modifications into adult song by experimenter-controlled reinforcement learning has emerged as a key protocol to study aspects of vocal learning in songbirds. This form of adult plasticity does not require conspecifics as a model for imitation or to provide social feedback on song performance. We therefore hypothesized that social interactions are irrelevant to, or even inhibit, song modification learning. We tested whether social context affects song sequence learning in adult male Bengalese finches (*Lonchura striata domestica*). We targeted specific syllable sequences in adult birds' songs with negative auditory feedback, which led the birds to reduce the targeted syllable sequence in favor of alternate sequences. Changes were apparent in catch trials without feedback, indicating a learning process. Each experiment was repeated within subjects with three different social contexts (male-male (MM), male-female (MF) and male-alone (MA)) in randomized order. We found robust learning performance in all three social contexts, with a nonsignificant trend toward facilitated learning with social company (MF, MM) compared to the single-housed (MA) condition. This effect could not be explained by the order of social contexts, nor by different singing rates across contexts. Our results demonstrate that social context can influence learning performance in adult birds even in experimenter-controlled reinforcement learning tasks, and therefore suggest that social interactions might facilitate song plasticity beyond their known role for imitation and social feedback.

Parallel sensorimotor pathways control landing in *Drosophila*

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Landing is the final and arguably most critical stage of flight. To avoid impact injuries, animals decelerate by modulating wing and body kinematics and extend their legs in a well-coordinated movement sequence before touchdown. This is a challenging motor task, which depends on the integration of different sensory modalities, in particular visual cues. How visual cues are integrated by peripheral and central networks in the brain and conveyed to lower-level motor networks in the ventral nerve cord (VNC), the fly's version of the spinal cord, to control landing is largely unknown. Here, we analyzed the distributed control of landing in the nervous system of *Drosophila*. Previous work has characterized early visual processing circuits, the landing behavior, and two descending neurons (DNs) controlling landing. Using a combination of light microscopy and connectomics approaches, we now identified complete neuronal pathways for landing from the sensory periphery of the brain to motor neurons in the VNC. By combining genetic activation and silencing with behavioral tracking, we then validated their functionality. We identified four classes of visual projection neurons (VPNs) that consistently drove landing upon optogenetic activation. Silencing three of these significantly impaired visually evoked landing responses. Hence, these VPNs are core components of the landing circuitry. The VPNs synapse directly onto a population of DNs which project to motor circuits in the VNC. Activating different VPNs and DNs drove landing responses with distinct leg, body, and wing kinematics. Finally, we used a connectome of the VNC to identify novel landing DNs based on their shared synaptic outputs in the VNC with previously described landing DNs. Different types of landing DNs recruit distinct sets of leg and wing motor neurons and drive different landing responses. In ongoing experiments, we characterize the sensory responses of different landing DNs to investigate how differential sensory tuning contributes to flexible motor output on the DN population level. Together, our findings elucidate parallel sensorimotor pathways from the brain to the VNC that enable flexible behavioral responses to visual cues involving coordinated movements of all body appendages.

Real-Time Segmentation and Classification of Birdsong Syllables for Learning Experiments

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Birdsong is an important model for learned vocalizations and skilled motor behavior more generally. Bengalese finch (Lonchura striata domestica) songs contain a limited number of distinct syllable types, which are combined into variable sequences. Many closed-loop experiments require the online recognition of a specific target syllable while the bird is singing. For example, adult songbirds can learn to modify aspects of their song through reinforcement learning. In this protocol, a target syllable in the song is covered with a short burst of aversive white noise, which masks auditory feedback of the bird's own song, and leads the bird to avoid the targeted syllable in future song renditions. Existing tools for this learning protocol, written in LabView, are cumbersome to adapt to new experiments and require manual creation of a spectral template, which the program uses to recognize the target syllable in real-time. In recent years, neural network solutions have led to dramatic improvements in the annotation of birdsong, but so far, there is no neural network-based out-of-the-box solution to reliably target syllables before their offset.

We here present Möve (Marking Online using Only the Onsets Of Vocal Elements), a novel approach to real-time birdsong syllable segmentation and classification. A convolutional-based audio encoder with a feedforward multi-layer perceptron is used to segment syllables in the raw audio signal of the acquisition system. Once a segment onset is detected, classification is performed by a standard CNN that gathers audio data only over the first part of a syllable to classify the spectral content before the syllable's offset. This two-stage architecture allows Möve to work with a lower audio chunk duration than other online annotation solutions, allowing fast and reliable syllable recognition. Its sparse architecture leads to an inference time of around one millisecond per stage on conventional CPUs. Crucially, this allows reinforcers or punishers to be applied with a high accuracy and a low latency, enabling effective operant conditioning experiments. Möve includes a GUI in which the networks can be trained and corresponding labeled datasets can be created effortlessly using unsupervised methods.

We conducted a learning experiment on one adult male Bengalese finch to validate the usability of this tool. The bird learned to avoid the targeted syllable sequence with comparable learning performance to the previous gold standard used in the lab. Our results show that Möve is able to correctly segment and classify Bengalese finch syllables in real-time and can be used effectively in learning experiments. Likewise, Möve could be used to detect target syllables for other closed-loop experiments, such as manipulation of auditory feedback, song-triggered neuronal microstimulation or reward delivery. Thus, this new tool represents a crucial building block for future studies on birdsong sequencing.

Kinematics of walking initiation in *Drosophila melanogaster*

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Behavioral repertoires in animals are large and comprise such diverse behaviors like locomotion, grooming, standing, courtship, or communication. Transitioning between behaviors and sub-parts of a single behavior poses a challenge for the nervous system: the kinematics of the body and extremities can be drastically different in two consecutive behavioral sequences and a motor output needs to be found that links them in a smooth and controllable manner. Generally, animals are able to do this effortlessly; exactly how this is done on a neuronal level is still largely unclear.

As a first step towards understanding this, we investigated the kinematics of the transition from standing to walking in the fruit fly *Drosophila melanogaster*. This transition is important for flies, as their walking behavior is segmented into short walking bouts interrupted by phases of standing; when a fly starts walking, it has to transition from its resting posture to a dynamical state in which all legs execute well-coordinated step cycles. Here, we characterized these transitions in a large number of flies, in terms of both high-level behaviour and leg kinematics. We show that walking initiation is highly variable in terms of frequency, speed, direction, and leg kinematics. However, all flies showed a preference to initiate walking with a front leg, in terms of the leg that first executed its swing phase (first leg moved, FLM). Furthermore, legs that were positioned more posteriorly or contributed less to stability at rest were preferred as FLM. The strength of these influences varied depending on the body segment: stability had a strong influence when a front leg was the FLM, but had no effect when walking was initiated with other legs. In contrast, the longitudinal position of a leg along the body axis had a large effect when the middle or hind legs were the FLM, but had little effect on the front legs. This suggests that the front legs are preferred as FLM, as this gives rise to higher stabilities, while the middle and hind legs are primarily used as FLM when they are positioned posteriorly. In this context, we hypothesize that the front legs might be preselected to ensure a stable transition to walking, when several options are equivalent. However, these influences were not entirely sufficient to explain all of the variability in FLM selection, indicating the existence of further mechanisms.

During the first step cycles after walking initiation it was observed that the legs did not strictly move in metachronal waves and directly neighbouring legs sometimes executed their swing phase at the same time; something that is never observed in the stable walking state. This indicates that interleg coordination mechanisms, and their neuronal mechanisms, are different directly after the onset of walking as compared to continuous walking. We hypothesize that this was necessary as the relative leg positioning in the stationary posture was different from the relative leg positioning in continuous walking. Accordingly, flies had to transition from one posture to another, presumably by adapting the stepping frequencies of individual legs.

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Kinematic synergies of leg stepping in walking fruit flies, *Drosophila melanogaster*

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Walking is the most common form of how animals move on land. When walking, animals can change their speed and heading direction, traverse diverse substrates, or can even compensate for the loss of a leg. This versatility arises from the numerous mechanical degrees of freedom (DOFs), i.e. independent directions of motion, inherent in biological limbs. However, there are usually more DOFs available than necessary for any given movement task. Based on the observation that joint movements are commonly coupled during execution of motor tasks, a possible solution to this redundancy problem could be that the nervous system does not control joint DOFs individually, but combines them into so-called synergies. Consequently, exploring the covariation in joint angle time courses, i.e. kinematic synergies, could shed light on the neural dynamics underlying motor control.

Drosophila melanogaster has emerged as a valuable model for studying the motor control of walking, due in part to its extensive genetic toolbox that enables the identification and manipulation of individual neurons. Here, we aimed to identify kinematic synergies for stepping in forward-walking fruit flies by exploiting the covariation between the angle time courses of the leg joints. For this, we employed a kinematic leg model that we recently developed (Haustein et al., 2024; doi: 10.3389/fbioe.2024.1357598). In brief, flies walked on a spherical treadmill and leg movements were recorded with six synchronized high-speed cameras (400 Hz). We used the convolutional neural network toolbox DeepLabCut to track the positions of the leg joint in the videos and derived their 3D positions by triangulation from the various views captured. Afterwards, we fitted our kinematic leg model, which is based on micro-computed tomography scans, to the recorded leg postures of the flies to extract accurate joint angles. Moreover, we used our model to reconstruct leg postures from kinematic synergies.

To identify kinematic synergies, we performed principal component analyses (PCAs) on average joint DOF angle time courses for each leg pair of individual flies (n=12). We found that stepping kinematics can be captured by the first three PCs which cumulatively explained over 97% of variance for the front, middle, and hind leg pairs in all flies. The scores' time courses of these PCs were highly consistent between animals, and their coefficients for many PCs exhibited broad similarity across flies. Strikingly, the first three PCs were sufficient to accurately reconstruct the movements of the tarsus tip. For the front and hind legs, PC1 predominantly captured protraction/retraction movements of the tarsus tip, while combining PC2 and PC3 resulted in leg levation/depression. For the middle legs, although there was greater variability in the individual PCs between animals, each PC primarily reflected either protraction/retraction, levation/depression, or both combined.

In conclusion, our findings show that the inter-joint coordination during forward walking in fruit flies can be effectively represented by only three linear kinematic synergies. The similarity of these synergies between flies and across leg pairs suggests that stepping behavior in *Drosophila* may be rooted in generic motor patterns.

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Speed-related changes in kinematic variability in walking *Drosophila* in the context of stability and interleg coordination

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Walking is a behavior commonly found in most terrestrial animals. During walking, each leg has to produce a basic rhythmic motor output consisting of swing and stance phase, on the one hand, while all legs have to be well-coordinated with each other to produce an overall pattern that efficiently and reliably propels the animal forward, on the other hand. In insects, which have six ambulatory legs, much is known about how individual legs function in this context and the rules governing interleg coordination. Like in other species, the most influential factor that will affect the specifics of kinematics and coordination in insects is walking speed. In the fruit fly *Drosophila melanogaster*, walking speed has been shown to correlate with interleg coordination and the duration of stance phases, for instance, while other kinematic aspects, such as swing duration and stance amplitude, are largely unchanged (Wosnitza et al., 2013). Previous studies as well as anecdotal observations suggested that also kinematic variability changes with walking speed: the faster a fly walks the stricter its motor output becomes, on the single leg level as well as on the level of interleg coordination (Berendes et al., 2016). However, a systematic characterization of this observation and its putative causes is still missing.

Here, we investigated this issue in *Drosophila*. Based on a large corpus of low-level walking data in many individuals, we explored the basic qualitative observation that walking behavior becomes stricter at higher walking speeds in a systematic and quantitative way. We show that tarsal trajectories, as well as the positions of lift-offs and touchdowns become generally less variable the faster flies walk. Analogously, the phase-relationships between legs become stricter at higher walking speeds. We then evaluated two putative causes for this speed-dependent variability: the requirements of static stability (Szczecinski et al., 2018) as well as the temporal coherence of interleg coordination rules (*sensu* Cruse, 1990). Previous studies have shown that stability is an important factor for interleg coordination; it could be a factor that is optimized at low walking speeds on a step-by-step basis, thus increasing variability. On the other hand, the behavioral coordination rules postulated for insect walking might interfere with each other in a walking speed-dependent manner, which might explain higher variability particularly at low walking speeds at which these rules tend to be less coherent. We show that while both of these measures, stability and temporal coherence, can predict walking speed-dependent variability, temporal coherence is more strongly related to these changes. Consequently, we argue that that variability during walking and its speed-dependence is largely due to how well and coherently individual legs can influence each other during rhythmic motor output.

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Structure-function analysis of cell types mediating corollary discharge signaling in larval zebrafish

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The importance of corollary discharge signals for distinguishing between sensory input coming from external sources and that caused by self-motion has been shown in several animal species, but the neuronal elements of the underlying circuitry remain unclear. Recently, we discovered an inhibitory synaptic signal which is temporally locked to spontaneous and visually driven swim patterns (Ali*, Lischka* et al., Nat Commun 2023). Using high-resolution calcium imaging, we identified motor-related signals in the most superficial, marginal layer of the tectal neuropil, suggesting that corollary discharge enters the tectum via this route. A glutamatergic population of torus longitudinalis (TL) neurons is known to project its axons to the marginal layer of the ipsilateral tectal hemisphere. We hypothesize that these TL neurons may play a role in relaying motor-related neural activity to the intratectal circuitry. For glutamatergic TL-derived inputs to be converted into an inhibitory synaptic signal, we propose the existence of a sign-converting tectal interneuron as the interface between TL excitatory input and motor-related inhibition in a subpopulation of tectal neurons. Using calcium imaging with high temporal resolution and electrophysiological recordings, we investigate the structure and function of neural cell types in this corollary discharge circuit implicated in modulating visuo-motor processing in the context of self-motion, such as saccadic suppression and adaptation of perceptual sensitivity.

Analysis of the local search behavior in Drosophila melanogaster larvae

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The ability to navigate and orientate oneself in the environment is a crucial characteristic of animals. Usually, animals utilize allothetic cues such as landmarks or odor gradients, which they perceived while moving, to reach their navigational targets. In environments with few or no external stimuli animals can use self-motion cues to keep track of these targets. These idiothetic cues have been shown to be processed in the central complex of adult Drosophila melanogaster. Since Drosophila larvae possess only non-functional parts of the developing central complex it is unclear whether they be able to navigate to a known goal in the absence of external stimuli.
Recently we developed a method to study the search behavior of Drosophila larvae in absence of external stimuli – the larval local search paradigm. Initially larvae leave the center of the search arena after being placed and remain there if no further stimuli are presented. After the contact with a food-source-containing container and its removing larvae perform a centralized search. Experiments with naïve animals indicate that potentially remaining stimulus residues are not the cause of this behavior. The aim of our study is the investigation of neuronal networks that enable Drosophila larvae to perform a local search.

Poster Topic

T22: Homeostatic and Neuriendocrine Systems, Stress Response

T22-1A Neurophysiological mechanisms of bad food decisions.
Samantha Aurich, Ulrike S. Franke, Nikita Komarov, Simon Sprecher, Peter Kovacs, Dennis Pauls

T22-2A Impaired Satiety Mechanisms in Obesity:
Disrupted PVHMC4R Neuron Activity During Feeding and Fasting
Marta Porniece, Jessica Baker, Charlotte Ausfahl, Stephen X. Zhang, Mark L. Andermann

T22-3A Neural substrates in the postpartum brain for flexible maternal care
Mingyu Yang, Silvana Valtcheva

T22-4A Impact of thyroid hormone transporters Mct8/Oatp1c1 on hippocampal neurotransmission and seizure susceptibility
Andrea Alcaide Martin, Steffen Mayerl

T22-5A A role of prefrontal inputs to lateral hypothalamus and their noradrenergic modulation in coping with stress
Alisa Bakhareva, Anne Petzold, Tatiana Korotkova

T22-1B Leptin receptor-expressing cells of the lateral hypothalamus regulate adaptive behaviors under anxiogenic conditions
Rebecca Figge-Schlensok, Anne Petzold, Nele Hugger, Alisa Bakhareva, Chantal Wissing, Tatiana Korotkova

T22-2B Stress, Gender and Prolactin – Immunofluorescence Differences in Rat Lactrotrophs.
Zuraiha Waffa, Abeer El Emmam Dief, Elena V. Sivukhina, Antje Prohaska, Gustav F. Jirikowski, Veronika M. Gebhart

T22-3B Neuron type specific noradrenergic modulation in the paraventricular nucleus of the hypothalamus
Debora Fusca, Andreas C. Klein, Jon M. Resch, Henning Fenselau, Peter Kloppenburg

T22-4B The proteomic profile of the midbrain periaqueductal gray: impact of sex and social context
Elena Kutsarova, Kristina Desch, Petros Chalas, Imke Wüllenweber, Jakob Meier-Credo, Eloah de Biasi, Genesis Rosiles, Julian D. Langer, A. Vanessa Stempel

T22-5B Hypothalamic-thalamic pathways enable leptin-sensitive regulation of social and sexual behaviours
Anne Petzold, Rebecca Figge-Schlensok, Deema Awad, Tatiana Korotkova

T22-1C Experience- and state-dependent adaptation of eating behavior by BDNF-expressing lateral hypothalamic populations
Carolin Schumacher, Mingyu Yang, Silvana Valtcheva, Tatiana Korotkova, Anne Petzold

T22-2C New methods to measure risk of perinatal depression
Allison Eriksson, Andreas Frick, Marcus Grueschow, Emma Fransson

T22-3C Contribution of leptin signaling to the sex- and estrous cycle-dependent regulation of adaptive behaviors
Deema Awad, Rebecca Figge-Schlensok, Tatiana Korotkova, Anne Petzold

T22-4C Regulation of thyroid hormone gatekeeper genes on tanycytes by modulating hormones of the HPT axis
Akila Chandrasekar, Paula Marie Schmidtlein, Lena Kleindienst, Sebastian Abele, Frauke Spiecker, Markus Schwaninger, Helge Müller-Fielitz

T22-1D Neuronal circuitries underlying sepsis induced adaptation of feeding behavior and locomotion in *Drosophila melanogaster*
Thomas Dieter Riemensperger, Fabienne Reh, Lennart Baumeister, Torben Gläser, Kei Ito

T22-2D Coordinated control of feeding and metabolism through reciprocal activity of AgRP and POMC neurons
Jan E. Radermacher, Fynn R. Eggersmann, Alain J. de Solís, Almudena del Río-Martín, Weiyi Chen, Lukas Steuernagel, Corinna Bauder, Donald A. Morgan, Anna-Lena Cremer, Michael Sué, Maximilian Germer, Christian Kukat, Stefan Vollmar, Heiko Backes, Kamal Rahmouni, Jens C. Brüning, Peter Kloppenburg

T22-3D Understanding the role of insulin signaling in the choroid plexus
Marleen Trapp, Aurica Ritter, Annarita Patrizi

T22-4D Investigating Galanin function in Stress and Anxiety regulation
Purba Kashyap, Laura Corradi, Suphansa Sawamiphak, Alessandro Filosa

Neurophysiological mechanisms of bad food decisions.

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Hunger or malnutrition causes insufficient caloric intake which leads to extensive physiological changes up to organ damage and death. Thus, at a certain point, a progressive malnutrition evokes the individual ´s inevitable decision to intake food of bad quality to ensure survival. The underlying mechanisms of such a vital decision are, however, barely understood. Our project now aims to gain a holistic understanding of changes that occur in brain-body communication to induce the intake of bad quality food, which is normally avoided. We aim to identify the metabolic signatures, endocrine and neurophysiological mechanisms that trigger bad food decisions. To test feeding decisions in *Drosophila*, individual larvae are exposed to liquid yeast-rich food as reference. On top, the larva´s decision to intake bad quality food is challenged by high amount of bitter or salt added to the yeast solution. During each experiment under certain conditions, the number of mouth hook contractions per minute is analyzed which reflects food intake behaviour in *Drosophila*. Accompanying behavioural studies, we used calcium imaging to investigate response properties of target neurons to stimuli such as quinine (bitter) or NaCl (salt).
Our data provide evidence that individuals intuitively avoid the intake of bad quality food. However, this active decision is dependent on the physiological state and thus animals reconsider their decision under starvation ensuring survival. Further, our data suggest that neuromodulation through octopaminergic neurons, along with adipokinetic hormone (AKH) plays an essential role in triggering physiological and behavioural changes to regulate food intake context-dependently in *Drosophila*.

Impaired Satiety Mechanisms in Obesity: Disrupted PVHMC4R Neuron Activity During Feeding and Fasting

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The melanocortin pathway is central to the homeostatic regulation of feeding, energy expenditure, and body weight. Several hunger-suppressing and hunger-promoting signals are integrated by the melanocortin 4 receptor (MC4R)-expressing neurons of the paraventricular nucleus of the hypothalamus (PVH). In lean animals, we confirmed our previous finding of a food consumption-evoked response in PVH-MC4R neurons that increases gradually during meal consumption to promote satiety. However, consuming calorie-rich diets can lead to obesity, which in turn results in hypothalamic injury and diminished neuronal sensitivity to melanocortins. Moreover, in general obesity, the activation of PVHMC4R neurons fails to reduce body weight and thereby is biased toward protection from weight loss. In this study we track PVH-MC4R neuron activity in high-fat-diet-induced obese animals employing fiber photometry, both after obesity was established and during its reversal, to investigate the extent of impairment of state-dependent integration of hunger and satiety signals in PVH-MC4R neurons during food consumption. We demonstrate that, compared to lean animals, obese animals have reduced motivation to consume highly palatable milkshakes. We hypothesize that this is driven, in part, by stronger-than-expected excitatory inputs to satiety-promoting PVH-MC4R neurons at early stages of meal consumption. Further fasting motivates licking and food consumption, but in contrast to lean animals, in obese animals, PVH-MC4R neurons don't display gradual potentiation of excitatory inputs throughout meal consumption. Lastly, switching HFD-fed obese animals to normal chow diet re-engages the motivation to consume milkshake, and partially reinstates the gradual potentiation of responses to food consumption in PVH-MC4R neurons in the fasted condition. These findings highlight functional changes in hypothalamic satiety-promoting neurons in obesity.

Neural substrates in the postpartum brain for flexible maternal care

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Flexible infant-oriented maternal behavior is driven by multisensory cues from the offspring. An example of such behavioral flexibility in maternal mice (dams) is the rapid transition from nursing in the nest to exiting the nest in search of misplaced pups. This involves a sensory competition between distress calls, emitted by pups outside the nest, and somatosensory contact with pups inside the nest. Inflexible maternal behavior can be a symptom of postpartum depression. While selective serotonin reuptake inhibitors are a common treatment, it remains unclear how serotonin regulates responses to infant cues in maternal brain networks. Therefore, understanding the neural mechanisms responsible for processing competing infant cues and modulating flexible maternal behavior is essential for improving maternal care. Here, we investigated the neural substrates involved in the processing of competing infant cues, and their modulation by serotonin. We identified a neural hub for infant cue processing: calbindin-expressing neurons in the posterior intralaminar thalamus (PIL^{CB}). Using channelrhodopsin-assisted circuit mapping, we found that PIL^{CB} neurons receive input from primary sensory nuclei and send output to the paraventricular nucleus to control oxytocin release and maternal behavior. PIL^{CB} neurons were more excitable in dams than in virgins and showed a particular preference for input frequencies consistent with the frequency range of pup calls. We observed a dense distribution of serotonergic fibers in PIL, and bath application of serotonin significantly increased the resting membrane potential of PIL^{CB} neurons. Calcium imaging revealed that both auditory and tactile stimuli activated PIL^{CB} neurons. Finally, using the $GRAB_{5\text{-}HT}$ sensor, we found that serotonin levels in PIL are modulated by pup calls. Our findings establish PIL as a bottleneck station, uniquely positioned for processing of competing multisensory infant cues, potentially modulated by serotonin.

Impact of thyroid hormone transporters Mct8/Oatp1c1 on hippocampal neurotransmission and seizure susceptibility

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The monocarboxylate transporter 8 (Mct8) and the organic anion transporting polypeptide 1c1 (Oatp1c1) are key transporters that facilitate the passage of thyroid hormones (THs) across brain barriers and into target cells in the murine CNS. Inside target cells, THs bind to nuclear receptors that regulate gene transcription, influencing critical processes of brain development such as neurogenesis, progenitor migration, differentiation and synaptogenesis. Inactivation of both TH transporters in mice (Mct8/Oatp1c1 dKO mice, dKO) results in CNS alterations including reduced thickness of the cerebral cortex, hypomyelination and impaired development of the cortical GABAergic system. However, little is known about the hippocampus in these animals, a structure particularly sensitive to TH and with a key role in seizure pathology. Here, we investigated the impact of Mct8/Oatp1c1 deficiency on murine hippocampal development and function. We first assessed seizure susceptibility in dKO mice using the pilocarpine model and found a significantly reduced seizure threshold and a stronger response to seizure induction. Analysis of hippocampi 12 h post-seizure revealed strong expression of the neuronal activation marker cFos together with ectopic somatostatin expression in dKO animals. To identify potential underlying alterations, we studied the expression pattern of inhibitory and excitatory neuronal components using immunofluorescence, in situ hybridization and qPCR during early postnatal development (P12) and in adulthood (P120). Our analysis revealed an abnormal development of the inhibitory GABAergic (e.g. GABAA receptors), the excitatory glutamatergic (e.g. Nr1, GluN2B) and cholinergic system in the hippocampus of dKO animals. Additionally, LC-MS/MS analysis revealed altered expression of proteins involved in ion and glutamate homeostasis, such as Kir4.1 in hippocampi of adult dKO mice. Preliminary spatial metabolomic results further indicated increased glutamate levels in dKO hippocampi. Altogether, our findings demonstrate that absence of TH transporters Mct8 and Oatp1c1 disrupts the development of neurotransmitter systems, resulting in an imbalance between excitatory and inhibitory neurotransmission and increased seizure susceptibility.

A role of prefrontal inputs to lateral hypothalamus and their noradrenergic modulation in coping with stress

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Animals experience various forms of stress - such as hunger, thirst, social isolation and aggression - throughout their lifetime. To successfully cope with such stressors, animals need to flexibly adapt behaviour. The impact of stress on behavioural adaptation depends on the type and duration of stress, as well as on the sex and age of the animal, and individual vulnerability.

The medial prefrontal cortex (mPFC) is involved in the stress response and in adapting behaviour to a certain context. One of the output targets of the mPFC is the lateral hypothalamus (LH), a brain region that regulates innate behaviours. Yet, little is known about whether and how the prefrontal-hypothalamic circuit mediates the influence of stress on innate behaviours. Both norepinephrine (NE) and dopamine (DA) dynamics respond to stressful experiences and strongly modulate neuronal activity in mPFC and LH. However, the dynamics of neuromodulator release in mPFC and LH during stress experiences is still elusive.

To address these questions, we first optogenetically stimulated mPFC inputs to LH and analysed innate behaviours of mice following physical, metabolic or social stress. We found that this circuit promotes behaviours that alleviate stress, such as food seeking following fasting or seeking out conspecifics following restraint. Further, we employed dual-site, dual-colour fibre photometry of neurotransmitter sensors for NE and DA in mPFC and LH of freely-moving mice. We identified the release patterns of these neuromodulators during innate and anxiety-related behaviours in response to different stressors.

Taken together, our data highlights the role of the mPFC-LH circuit and its neuromodulation in state- and context-dependent behavioural adaptation to stress.

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Leptin receptor-expressing cells of the lateral hypothalamus regulate adaptive behaviors under anxiogenic conditions

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Sensing anxiogenic conditions and being able to adapt behavior in order to eat are crucial for survival. Malfunction of these adaptive mechanisms often underlie anxiety- and eating disorders. A reduction of food intake – and concurrent loss of body weight – in combination with physical hyperactivity are main symptoms of the eating disorder anorexia nervosa. Continuous loss of fat mass results in hypoleptinemia, which initiates multiple starvation-related adaptive functions. Although leptin administration showed beneficial effects in treatment of symptoms of anorexia nervosa, including anxiety, neuronal mechanisms of possible anxiety-relieving action of leptin remains incompletely understood.

Here, combining calcium imaging and opto- or chemogenetics in freely behaving mice, we investigated functions of leptin receptor-expressing neurons in the lateral hypothalamus (LepR-LH), the brain region crucial for the regulation of feeding. We found that this cell population signals anxiogenic stimuli. Activation of LepR-LH cells in multiple behavioral arrays reduced anxiety-related behaviors and thereby enabled exploration of new terrain as well as eating despite an anxiogenic environment.

The activity-based anorexia model is a well-established protocol to mimic anorexia nervosa, as mice typically display maladaptive excessive locomotion, despite loss of body weight. Mice undergoing the protocol of time-restricted feeding and free access to running wheels displayed a reliable increase of running wheel activity. Using calcium imaging, we found that LepR-LH cells encoded running wheel activity. Furthermore, chemogenetic activation of LepR-LH neurons limited the development of anorexia-like behavior.

This study suggests a role of LepR-LH neurons in regulation of anxiety-related behaviors in health and identifies neuronal circuit mechanisms, which process adaptive behaviors in a model of anorexia nervosa.

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Stress, Gender and Prolactin – Immunofluorescence Differences in Rat Lactrotrophs.

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Acute stress is known to activate a cascade of neuroendocrine pathways, including the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system, to maintain homeostasis and survival. While actions of cortisol and epinephrine are well characterised in this context, the role of the anterior pituitary lobe peptide hormone prolactin (PRL) in stress response is understudied. PRL is traditionally known for its role in lactation and reproduction. PRL's involvement in stress adaptation and recovery due to its anti-apoptotic and anti-inflammatory protective effects is evident in previous studies. Recently we found evidence for a novel role of PRL in acute stress response. This study focuses on the cellular distribution of PRL immunofluorescence (IF) in rat lactotrops during acute stress and explore the potential sex differences. Adult Wistar rats (n=5 females and n=5 males for each stressor) were subjected to either 2 hours of restrain stress or 15 minutes of forced swimming stress. Control groups (n=3 females and n=3 males) remained in standard conditions without exposure to either restrain or forced swimming. Following the acute stress experiments, the rats were anaesthetised and sacrificed by decapitation. Pituitary glands were dissected and fixed embedded in resin. Semi-thin sections were immunostained for PRL and examined with fluorescence microscopy. IF was analysed qualitatively and quantitatively to assess PRL distribution patterns and vesicle intensity differences in the stress versus control groups. PRL distributions and intensities in rat lactotrophs were increased in response to acute stress. Female rats, particularly in the forced swimming stress group exhibiting the highest IF intensity of PRL vesicles followed by the restrain stress group, manifested more widely scattered and abundant PRL distribution, in comparison to the control group. PRL *de novo* synthesis and secretion may be stimulated by acute stress in a sex specific manner. Apparently, PRL is not merely secreted during lactation but also functions as a stress modulator, which may be among the functional properties of PRL in males. Male rats under restraint stress showed slight increase in PRL IF intensity, whereas the male forced swimming stress group showed the lowest PRL IF intensity amongst all groups. This can be attributed to the sex differences in PRL expression and cellular intolerance to acute stress, as well as variations in hypothalamic regulation. Control groups manifested fewer yet more compact PRL vesicles, primarily localised in the perinuclear cytoplasm, indicating that PRL could be stored within the lactotrophs. Our findings highly suggest that PRL is a further factor involved in the neuroendocrine stress response with remarkable sex differences.

Neuron type specific noradrenergic modulation in the paraventricular nucleus of the hypothalamus

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The paraventricular hypothalamus (PVH) is a central regulator of neuroendocrine and autonomic functions, integrating signals from various pathways, including noradrenergic inputs, to maintain processes like energy balance. It receives input from various modulatory centers, including the arcuate nucleus and locus coeruleus. Our long-term goal is to understand how the noradrenergic system influences feeding behavior by investigating the properties of PVH neurons and their modulation by noradrenaline (NA).

We first characterized the intrinsic electrophysiological properties of PVH neurons using perforated patch-clamp recordings in mice, aligning our findings with previous studies in rats. Three distinct neuron types were identified: Type I neurons, corresponding to magnocellular neurosecretory neurons, displayed delayed spike onset after hyperpolarization and slow spike-frequency adaptation. Type II neurons (putative parvocellular neurosecretory) fired repetitive spikes without delay, while Type III neurons (putative preautonomic) showed spike bursts after hyperpolarization and rapid adaptation. Morphologically, Type III neurons had more complex dendritic structures, while Type I had the largest somata. These distinct neuron types were distributed differently across PVH regions, with Type I neurons located anteriorly and Type III neurons more posteriorly.

NA differentially modulated the activity of these neuron types. In Type I neurons, around 80% showed increased firing in response to NA, while the remaining 20% were inhibited. Type II neurons mainly decreased their firing (60%), although 35% showed excitation. In Type III neurons, 65% were inhibited, and 17% were excited by NA. This demonstrates that NA modulates PVH neurons in a cell-type-specific manner.

To explore the molecular basis of these effects, we conducted transcriptomic analyses to assess adrenergic receptor (AR) expression in PVH neuron populations. Oxytocin (Oxt)-neurons expressed predominantly α1a-ARs, while arginine-vasopressin (AVP)-neurons showed high α1a- and α2c-AR expression. Thyrotropin-releasing hormone (TRH)-neurons were characterized by α2a-AR dominance, and corticotropin-releasing hormone (CRH)-neurons expressed α1b-, α2a-, α2c-, and β1-ARs. These findings indicate that different neuron populations in the PVH have distinct AR expression profiles.

We confirmed the functional relevance of these AR subtypes using pharmacological methods. In Oxt-neurons (predominantly Type I), NA-induced excitation was blocked by the α1a-AR antagonist WB4101, while the α2a-AR antagonist BRL44408 blocked inhibition. In AVP-neurons (predominantly Type I), WB4101 blocked excitation, and JP1302, an α2c-AR antagonist, blocked inhibition. TRH-neurons' (predominantly Type II) inhibition by NA was blocked by BRL44408 in 75% of neurons and by JP1302 in the remaining 25%. Finally, in CRH-neurons (constituted by all three types), NA-induced excitation was blocked by the α1b,d-AR antagonist CEC and β-AR antagonist propranolol, while inhibition was prevented by BRL44408 and JP1302.

In conclusion, our findings reveal that NA differentially modulates PVH neurons through distinct AR subtypes, suggesting a complex regulatory mechanism for autonomic processes such as energy balance.

The proteomic profile of the midbrain periaqueductal gray: impact of sex and social context

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Instinctive behaviours such as defence, feeding and reproduction have evolved across animal phyla and ensure survival of both the individual and its kin. A brainstem region central to the initiation of virtually all instinctive behaviours is the periaqueductal gray (PAG). Although stereotyped to some degree, the expression of instinctive behaviours exhibits significant variability within and across individuals. Previous work has mostly focussed on behavioural variability arising from plasticity mechanisms occurring in forebrain regions projecting to the PAG, including the hypothalamus,
amygdala and cortex. Here, we asked whether the PAG itself possesses the molecular machinery to support synaptic plasticity and neuromodulation. We used a label-free LCMS-based proteomics approach with data-independent acquisition to compare tissue from the PAG and two other highly plastic brain regions: neocortex and hippocampus. We analysed differences in the PAG proteome of male and female mice in two housing conditions (single- versus group-housed) as a means to induce plasticity. This comprehensive proteomic dataset, containing ~10,000 proteins per sample, allows us to posit that 1) the PAG expresses all proteins critical for postsynaptic plasticity and 2) changes in the social environment lead to significant differential expression of the majority of the PAG proteome. Third, we identified specific protease inhibitors, linked to the extracellular matrix, that are strongly upregulated in males throughout the brain. These proteins have been associated with Alzheimer's disease (AD) and may point to a molecular mechanism of sex-specific AD vulnerability. In summary, we reveal how sex and social environment influence the proteome of the PAG.

Hypothalamic-thalamic pathways enable leptin-sensitive regulation of social and sexual behaviours

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Animals continuously weigh nutritional needs against competing drives such as mating according to state and opportunity. However, the neuronal mechanisms of sensing and resisting metabolic pressure such as hunger or thirst remain poorly understood. Hypothalamic circuits are sensitive to peripheral signals indicative of the physiological need state. An important signal is leptin, an anorectic adipocyte-derived hormone secreted in proportion to fat stores. We previously observed that leptin treatment in male mice not only reduced food intake, but increased social motivation. We furthermore identified leptin receptor-expressing (LepRLH) neurons of the lateral hypothalamus which convey, in male mice, resistance to hunger and thirst to support the pursuit of social needs.

In this study, we used a combination of pharmacological interventions, single-cell calcium imaging in freely moving animals, and LepR-specific neural activity manipulations to investigate the contribution of leptin signaling to the expression of sociosexual behavior both in males and naturally cycling females.

Using single-cell calcium imaging in freely socially interactive animals, we found that LepRLH neurons not only preferentially encoded conspecifics of the opposite sex – potential mates – but specifically encoded sexual behaviors. Optogenetic activation of LepRLH neurons increased social exploration in a sex-specific manner: activation of LepRLH neurons in females increased preference for familiar females, while activation in males increased preference for novel females. In a two choice paradigm, optogenetic activation of LepRLH neurons increased preference for social exploration over food.

We analyzed the projection profile of LepRLH neurons in detail and determined specific thalamic targets. Activation of LepRLH inputs into midline thalamic nuclei increased sociality akin to somatic LepRLH stimulation, indicating that this nucleus constitutes a thalamic output node for the leptin-mediated control over social behaviors.

In summary, our results demonstrate that leptin signaling mediates the expression of essential innate behaviors in a sex- and cycle-dependent manner, at least in part through leptin receptor-expressing populations located in the lateral hypothalamus.

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Experience- and state-dependent adaptation of eating behavior by BDNF-expressing lateral hypothalamic populations

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In modern societies, we live in environments that are highly conducive to eating – with high food palatability, easy food availability, and omnipresence of food cues – leading to increased caloric intake, weight gain and associated health disorders. Therefore, the identification of anorectic mechanisms is of paramount importance for public health. Eating behaviour is regulated through neurochemically distinct neural subpopulations of the lateral hypothalamus (LH). In this study, we focused on an LH subpopulation that secretes the neurotrophin brain-derived neurotrophic factor (BDNF) and may thereby influence the formation of eating patterns over time through synaptic plasticity mechanisms.

Using single-cell Ca^{2+} imaging in freely moving mice with free access to nutritional and social rewards, we found that $BDNF^{LH}$ neurons are excited by food more than by any other rewards. The prevalence of food-encoding among $BDNF^{LH}$ neurons increased over time. To investigate whether the activity of $BDNF^{LH}$ cells affects feeding behaviour, we selectively activated $BDNF^{LH}$ cells in freely behaving mice using a chemogenetic approach. Chemogenetic activation of $BDNF^{LH}$ neurons acutely decreased the consumption of unhealthy, but palatable, high fat food after three days of activation of $BDNF^{LH}$ neurons, without affecting food intake over 24 h, body weight or glucose tolerance compared to control group. Conversely, the activation of $BDNF^{LH}$ neurons did not impair homeostatic re-feeding after overnight food deprivation. Thus, chemogenetic activation of $BDNF^{LH}$ neurons acutely affects food intake in a state-dependent manner without shifting the homeostatic body weight setpoint. To test whether $BDNF^{LH}$ neurons are involved in the experience-dependent adaptation of eating behavior, we performed a context-conditioned overconsumption task. Here, hungry animals are allowed to feed in one particular context, leading to overconsumption of sated animals when presented again with the conditioned context previously associated with satiation. We found that chemogenetic activation of $BDNF^{LH}$ neurons during consolidation of the conditioned context led to overconsumption across contexts. Satiation is mediated by peripheral hormones such as leptin, which is released by adipose tissue in response to a meal and reaches hypothalamic control centers through the brain's ventricular system. To evaluate the degree of leptin sensitivity among $BDNF^{LH}$ neurons, we performed calcium imaging and patch clamp experiments and found that $BDNF^{LH}$ neurons tend to be activated by leptin. Overall, our findings suggest an important role of $BDNF^{LH}$ neurons in the experience- and state-dependent adaptation of eating behavior.
Thereby, we provide an entry point for the identification of neural mechanisms that shape eating patterns and that could potentially be harnessed to limit food overconsumption in the modern obesogenic environment.

New methods to measure risk of perinatal depression

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Perinatal depression (PND), i.e. depression during pregnancy and after childbirth is a common and potentially fatal condition affecting up to 20 percent of childbearing women. There are preventive interventions for PND, but effective methods to identify individuals at risk are lacking. Pregnancy can be considered a specific highly emotional state and a physiological strain, constituting a potentially potent stressor. This project aims to apply knowledge of the biological and psychological stressors of pregnancy to develop a physiological risk assessment tool that identifies women at high risk of PND.
One measure that we test within this project is Pupil Dilation (PD) – a measure of the arousal system (sympathetic) responsivity. The pupil's response during affective picture viewing has been shown to reflect emotional arousal, associated with stress, i.e. increased sympathetic activity. During stress, norepinephrine (NE) is released in both the brain and the periphery. The major source of NE to the brain is the locus coeruleus (LC), a small structure in the brainstem. The LC-NE system can modulate cognition, behavior and autonomic tone. Pathological changes in this arousal system can lead to behavioral and mood alterations and hyperarousal is associated with increased symptoms of depression. PD has been developed as a proxy for LC-NE arousal and has been used as a marker of stress vulnerability. We will present preliminary results from our ongoing data collection with pregnant participants who report on mental and pregnancy health at two laboratory visits during pregnancy and in a follow-up interview postpartum. At the laboratory visits, PD is measured during a version of the Emotional Stroop Task. We expect that the results of this project will improve the precision and timing of preventive interventions and treatment to the right individuals in good time, with the overall purpose of limiting individual suffering, neonatal risks, and societal costs.

Contribution of leptin signaling to the sex- and estrous cycle-dependent regulation of adaptive behaviors

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Mammalian reproduction is highly energy intensive, and requires an animal to prioritize feeding or sociosexual interaction, depending on need and opportunity. Such innate behaviors are regulated by hypothalamic circuits which are sensitive to peripheral signals indicative of the physiological need state. An important signal is leptin, an anorectic adipocyte-derived hormone secreted in proportion to fat stores. Although leptin levels strongly differ in males and females, little is known about sex-specific differences in leptin signaling.

In this study, we used pharmacological interventions to investigate the sex-dependent role of leptin signaling for the expression of innate behavior - feeding behavior, social and sexual behaviors, as well as exploration under anxiogenic conditions - both in males and naturally cycling females.

We first evaluated the effects of leptin treatment on the expression of feeding behavior. Leptin treatment reduced food intake in males and in females in the non-receptive estrus cycle stage, but increased food intake in females in the receptive cycle stage, in comparison to animals treated with the control substance (PBS). To test the effect of leptin treatment on sociosexual behavior, we treated mice with leptin or control substance and measured the expression of social and sexual behaviors in pairs of freely interacting mice of opposite sex. In males, leptin treatment enhanced exploratory behavior, but did not strongly affect social or sexual behavior. The same treatment decreased sociosexual behavior in non-receptive females, while increasing sociosexual behavior in receptive females.

In summary, our results demonstrate that leptin signaling mediates the expression of essential innate behaviors in a sex- and cycle-dependent manner.

We gratefully acknowledge support by the ERC Consolidator Grant (772994, FeedHypNet, to T.K.) and DFG (Project-ID 431549029 – SFB 1451, to T.K., EXC2030 CECAD, to T.K., EXC 2030 – 390661388, to A.P., 233886668-GRK1960, to R.F.).

Regulation of thyroid hormone gatekeeper genes on tanycytes by modulating hormones of the HPT axis

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Thyroid hormones (TH) play an important role in brain development, central nervous system functions and energy metabolism. In order to mediate these effects in the brain, TH are actively transported through the blood-brain barrier from the periphery to the brain. Tanycytes are specialized ependymal cells that line the wall and the base of the third ventricle in the mediobasal hypothalamus. They can be broadly classified into α-tanycytes, which send their processes to hypothalamic nuclei, and β-tanycytes which are in contact with the fenestrated vessels in the median eminence (ME). They are renowned for their role in hormone (eg: gonadotropin releasing hormone [GnRH] and thyrotropin releasing hormone [TRH]) and nutrient sensing and transport of substances to and from the hypothalamus (eg: leptin and ghrelin). Tanycytes are important in the context of thyroid hormone regulation and transport, since they express the necessary repertoire in terms of expression of deiodinases (*Dio2*, *Dio3*), thyroid hormone transporters (*Slc16a2*, *Slco1c1*) and thyroid receptors including *Thra*, *Thrb* and *Tshr*. However, the precise mechanism of how TH and their receptors modulate tanycytic functions and the role tanycytes in turn play in hypothalamic TH availability is unclear. In this project, we would like to investigate the role of the hormones of the hypothalamus-pituitary-thyroid (HPT) axis, namely TRH, thyroid stimulating hormone (TSH), thyroxine (T4) and tri-iodothyronine (T3), in modulating TH gatekeeper genes on tanycytes. Primary tanycytes were treated with either TRH, TSH, T3 or T4 and qPCR was performed to identify changes in gatekeeper gene regulation. We used genetic tools to modulate thyroid hormone receptors (THRs) specifically on tanycytes. We inhibit or activate TH receptor functions by either overexpressing a dominant negative mutant ($TR\alpha1^{DN}$) or a dominant positive mutant ($TR\alpha1^{VP16}$) of the thyroid hormone receptor α (TRα1) in tanycytes. Using qPCR, RNAscope, ELISA and calcium imaging we identified changes in the hormone axes and changes in gene expression in both tanycytes and hypothalamus when the THRs on tanycytes were modulated. It has previously been shown that treatment of mice with the TRH analog, taltirelin, led to an increase in the size of the endfeet of tanycytes. Therefore, to identify morphological changes of tanycytes due to TH, we performed scratch assays to track the migration of primary tanycytes. Further, we treated primary tanycytes with thyroid hormones and monitored the changes in actin regulation by measuring the F/G actin ratio using western blot. Overall, we hypothesize that the inhibition or activation of TRα1 specifically in tanycytes plays an important role in TH gatekeeper gene regulation in tanycytes. This in turn could modulate TH related genes in the hypothalamus giving us an insight into TH availability in the hypothalamus. Understanding the role of tanycytes in modulating TH functions and vice versa could be key to improving treatment options in central TH resistance.

Neuronal circuitries underlying sepsis induced adaptation of feeding behavior and locomotion in *Drosophila melanogaster*

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Microbiotic hazards and potential contamination through pathogens are omnipresent in the environment and have resulted in the evolution of multi-layered defense mechanisms. These can range from physical impediments, to rapid and unspecific, as well as specific immune defense mechanisms up to the adaptation of innate behavioral routines in response to a potential micro-biological hazard, known as behavioral immunity or sickness behavior.

For concise behavior modulation in the case of infection, the nervous system needs to receive and evaluate information about an acute infection and integrate this information in the equation of internal and external stimuli to modulate behavior as an adequate response to the animal's situation. Whereas much is known about the perception and evaluation of stimuli from the environment, our knowledge about how the nervous system receives and evaluates information about the acute state of the immune system and the neuronal circuits underlying an infection induced modulation of behavior is scarce.

We found that female *Drosophila* respond to acute sepsis-like bacterial infection through *Erwinia carotovora carotovora* by anorexia and hyperlocomotion. Using opto-physiological methods we have identified a group leucokinin / dopamine co-expressing neurons in the abdominal ganglion of the adult female fly that display increased neuronal activity in presence of a sepsis-like bacterial infection. Reduction of dopamine expression through expression of dsRNA specifically leucokinin neurons in the abdominal ganglion counteracts infection induced anorexia and hyper-locomotion. We further found that both Dop1R1 and Dop2R receptor activity is involved in sustained inhibition of feeding after infection on the second and third day after infection but not during the acute response during first 24 h. This gives an insight into the complex signaling dynamics required for the performance of appropriate sickness behaviour in response to infection with Ecc.

Coordinated control of feeding and metabolism through reciprocal activity of AgRP and POMC neurons

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Agouti-related peptide (AgRP) and proopiomelanocortin (POMC) neurons in the arcuate nucleus (ARC) of the hypothalamus play crucial, opposing roles in energy homeostasis. AgRP neurons stimulate food intake and inhibit anorexigenic pathways through neuropeptides like GABA and AgRP, while POMC neurons suppress feeding by releasing α-MSH to activate melanocortin receptors. These populations integrate hormonal signals like leptin and ghrelin to regulate energy balance. This study employed a novel transgenic approach using non-interacting recombinases to express DREADD receptors in these two populations simultaneously. This approach enabled selective modulation of AgRP and POMC neurons, allowing to investigate their reciprocal effects on feeding behavior and metabolic regulation.

To assess AgRP and POMC neuron activity in response to clozapine N-oxide (CNO), we used perforated patch-clamp electrophysiology in acute brain slices. These neurons were genetically labeled via stereotaxic injection of Cre- and Dre-dependent adeno-associated viruses expressing fluorescent reporters, enabling visualization and projections. Electrophysiological recordings demonstrated CNO-induced depolarization and increased firing in AgRP neurons expressing hM3Dq receptors. Concurrently, POMC neurons with hM4Di receptors exhibited hyperpolarization and reduced firing, confirming the selective effect of chemogenetic manipulation. We compared electrophysiological responses under different conditions to dissect interactions between these circuits. When CNO was applied, 83% of AgRP neurons showed a clear excitatory response, while the same percentage of POMC neurons displayed significant inhibition. Notably, the inhibition of POMC neurons was more pronounced when both AgRP and POMC neurons were manipulated compared to when AgRP neurons were activated alone, indicating a synergistic interaction. Further investigation revealed that downstream areas, including the paraventricular nucleus of the hypothalamus (PVH) and the nucleus tractus solitarii (NTS), receive concurrent projections from AgRP and POMC neurons. In particular, Npy1R-expressing PVH neurons and tyrosine hydroxylase-positive (Th^+) neurons in the NTS were activated by the simultaneous manipulation of AgRP and POMC neurons, highlighting a complex neural network integrating signals from these opposing circuits to regulate feeding behavior and metabolic adaptations.

Our findings suggest that AgRP and POMC neuron interplay involves direct and indirect mechanisms, modulating downstream activity and leading to differential effects on food intake, insulin sensitivity, and gluconeogenesis. While AgRP neuron activation primarily promotes short-term feeding and reduces insulin sensitivity, POMC neuron inhibition affects glucose homeostasis, particularly when combined with AgRP activation. These results underscore the importance of bidirectional modulation within these circuits for maintaining energy balance and provide insights into the neural regulation of metabolism. This study establishes a powerful transgenic platform for probing the roles of AgRP and POMC neurons in metabolism, offering insights into how these opposing populations contribute to feeding behavior and systemic metabolic processes.

Understanding the role of insulin signaling in the choroid plexus

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The choroid plexus (ChP) is a specialized membrane mainly composed of multiciliated epithelial cells that are tightly interconnected to form the blood-cerebrospinal fluid (CSF) barrier. ChP is located in all ventricles of the brain and it is known for producing the CSF. The ChP also secrets various active molecules into the CSF including growth factors as insulin, EGF and FGF. Recent data have suggested that ChP can sense and react to internal and external stimuli leading to changes in its function. However, only a few selective stimuli have been identified and little is known on how the ChP can sense and respond to them. To understand how stimuli can modulate the ChP function, we first developed a ChP epithelial cells (CPEC) primary in vitro model. By pairing this model with a multi-tiered approach, ranging from biochemistry to confocal microscopy, we analysed how selective growth factors can influence CPEC formation and maturation. We demonstrated that all tested growth factors significantly increased the percentage of multiciliated CPEC in vitro. Interestingly, only the hormone insulin significantly regulated cilia maturation by elongating their length. We, then, mapped the development of the insulin signalling pathway in mouse ChP from embryonic stages (E) throughout adulthood. By combining confocal microscopy, western blotting, and qRT-PCR, we demonstrated that the expression level of insulin receptors in the ChP commenced at E16, right after ChP epithelial cells are fully differentiated and at the end of multiciliogenesis. The expression of insulin receptors is maintained at a stable level into adulthood. Surprisingly, insulin receptors were mainly located on the apical CSF-facing side of the cells rather than directly at the cilium. Finally, we demonstrated that insulin influenced cilia rearrangement by modulating intraflagellar transport protein (IFT) 20 selectively in CPEC in vitro model. In conclusion we demonstrated that the ChP responds to various growth factors especially insulin by modulating ChP cilia maintenance. These observations could have important implications for neurological disorders associated with ciliary and insulin dysfunction.

Investigating Galanin function in Stress and Anxiety regulation

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Stress and anxiety are crucial processes evolved to help animals adapt to environmental threats. However, chronic activation and dysregulation of these processes, as seen in conditions such as post-traumatic stress disorder (PTSD), anxiety disorders, and burnout, contribute significantly to the burden on modern society. Understanding the neurobiological mechanisms underlying stress and anxiety, including their overlap and divergence, is imperative. Galanin is a neuropeptide expressed in neurons regulating activation of the hypothalamus-pituitary-adrenal (HPA) axis, a neuroendocrine system responsible for stress and anxiety responses. Although Galanin has been implicated in the regulation of the HPA axis, the mechanisms mediating its action are still unclear. We have used zebrafish as a model organism to dissect the role of Galanin in stress and anxiety at molecular, cellular, and circuit levels, employing behavioral assays and live imaging methods.

Poster Topic

T23: Neural Networks and Rhythm Generators

T23-1A Interhemispheric synaptic inputs to neocortical pyramidal cells with dendritic versus somatic axon origin
Martin Both, Aline Pannier, Tina Sackmann, Andreas Draguhn

T23-2A Exploring neuronal organisation in the posterior slope neuropil of the *Drosophila Melanogaster* brain
Hannah Jones, Sandor Kovacs, Kei Ito

T23-3A Network integration of neurons with different (somatic vs. dendritic) axon origin: a computational modelling approach
Livia Marina Klostermann, Andreas Draguhn, Martin Both

T23-4A Motor control of multi-modal courtship signals in *Drosophila*
Bjarne Luca Schultze, Melanie Stenger, Kimia Alizadeh, Jan Clemens

T23-5A A New Approach to Phase-Amplitude Coupling (PAC) Measurement: Distinguishing Phase and Temporal Dispersion
Marjan Nosouhi, Moein Esghaei, Stefan Treue

T23-6A Identification and characterization of brain and descending neurons controlling adaptive walking in *Drosophila*
Fathima Mukthar Iqbal, Federico Cascino-Milani, Jens Goldammer, Hannah Volk, Chris J. Dallmann, Kei Ito, Jan M. Ache

T23-7A Role of the $P2Y_1$ receptor in processing of olfactory signals
Shiva Shahmorad, Christian Lohr, Daniela Hirnet

T23-1B Sensory filtering during sleep regulation
Davide Raccuglia, Cedric Beat Brodersen, Johannes Wibroe, Raquel Suárez-Grimalt, Jörg Geiger, Lorna May Shakespeare, David Owald

T23-2B Synchronization between the hippocampus and the thalamic nucleus reuniens accompanies spatial decision making
Tristan Baumann, Oxana Eschenko

T23-3B Chronic optogenetic stimulation has the potential to shape the collective activity of neuronal cell cultures
Cyprian Sebastian Adler, Friedrich Schwarz, Julian Vogel, Christine Stadelmann, Fred Wolf, Manuel Schottdorf, Andreas Neef

T23-4B Induced respiratory dysfunction by focal stimulation of specific brain areas - implications for SUDEP
Moritz Jung, Jennifer Bauer, Henner Koch, Yvonne Weber, Markus Rothermel

T23-6B Neuronal circuits for flexible visuomotor transformations in the fly brain
Mert Erginkaya, Chris J. Dallmann, Sander Liessem, Jan M. Ache

T23-1C Lactate utilization alters sharp wave-ripple networks activity in mouse hippocampal slices
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T23-1D Prefrontal-hippocampal neural dynamics as useful biomarkers of cognitive impairment and rescue in schizophrenia: Role of serotonin receptors
M. Victoria Puig, Thomas Gener, Cristina López-Cabezón, Sara Hidalgo-Nieves

T23-2D Gamma frequency tunes Na^+ channel availability and thereby increases dendritic excitability in cortical pyramidal neurons
Michael Gutnick, Nadav Astman, Oron Kotler, Yana Khrapunsky, Ilya Fleidervish

T23-3D De novo assembly of a functional neuronal circuit in embryos of an ancestral metazoan
Christopher Noack, Sebastian Jenderny, Jörg Wittlieb, Lisa-Marie Hofacker, Ornina Merza, Christoph Giez, Urska Repnik, Marc Bramkamp, Karlheinz Ochs, Thomas C. G. Bosch

T23-4D Distinct connectivity patterns along the anterior-posterior axis of the piriform cortex
Saule Nabiyeva, Sebastian H. Bitzenhofer

T23-5D A distinct hypothalamus-habenula circuit governs risk preference
Dominik Groos, Anna Maria Reuss, Peter Rupprecht, Tevye Stachniak, Christopher Lewis,

Shuting Han, Adrian Roggenbach, Oliver Sturman, Yaroslav Sych, Martin Wieckhorst, Johannes Bohacek, Theofanis Karayannis, Adriano Aguzzi, Fritjof Helmchen

T23-6D Cell type and Molecular Architecture of the Pigeon Brain
Thamari Neranjana Kapuruge, Gregory C. Nordmann, Spencer Balay, Siebe van Manen, David Anthony Keays

T23-7D Interactions of a sleep-control centre with a neural circuit used for navigation in Drosophila
Lea Kristin Ballenberger, Gero Miesenböck

Interhemispheric synaptic inputs to neocortical pyramidal cells with dendritic versus somatic axon origin

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Recent data show that a significant fraction of cortical pyramidal neurons has an axon originating from a basal dendrite (axon-carrying dendrite, AcD) rather than from the soma. In the rodent hippocampus, these AcD cells convey privileged synaptic excitation especially in network states with strong perisomatic inhibition (Hodapp et al., 2022). We have recently shown that hippocampal AcD cells receive stronger commissural input than neurons with somatic axon origin (non-AcD) (Stevens et al., 2023).
AcD cells are also present in the neocortex, though seemingly less frequent than in the hippocampus (~20% compared to ~30-50%; Thome et al., 2014, Wahle et al., 2022). However, nothing is known about the network integration of neocortical AcD versus non-AcD cells. We therefore studied commissural inputs in the mouse primary motor cortex (M1), an area with strong interhemispheric connections. Channelrhodopsin 2 was expressed in presynaptic neurons of adult mice by viral injection into the contralateral M1 and whole-cell patch clamp recordings were performed in coronal slices of the M1. The vast majority of all recorded cells responded with strong inward currents to optogenetic activation of presynaptic inputs. Repetitive activation of commissural inputs at 20Hz showed prominent paired-pulse depression, without overt differences between both morphological subtypes. Reconstruction of recorded neurons revealed that 16/64 (i.e. 25%) were AcD neurons, while 42 had their axon emerging from the soma and six neurons showed a shared root of a basal dendrite together with the axon. Further analysis is required to uncover potential functional or structural asymmetries between both cell types.

Exploring neuronal organisation in the posterior slope neuropil of the *Drosophila Melanogaster* brain

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Over a century of research on the *Drosophila melanogaster* brain has primarily focused on specific brain regions involved in sensory processing, learning, memory, and motor control. However, much of the brain, which we refer to as the "terra incognita" regions, remains poorly understood. One such region is the posterior slope (PS). This region includes 621 PS-named neurons, as detailed in the Janelia hemibrain FlyEM connectome dataset. It is also innervated by nearly 1,000 projection neurons from the visual systems, over 100 descending neurons, and approximately 4,000 other associated interneurons.
To understand how these neurons are organised within the three-dimensional space of the neuropil, and to enable unambiguous annotation of the detailed locations in the neuropil that reflects the underlying neuronal organization , we conducted systematic mapping of neuronal structures and identified specific volumes that are contributed by certain groups of neurons, such as the bundle of fibres as well as the mass of interconnected neurite branches. We refer to these volumes as microcompartments and gave them names that are easy to remember and pronounce .
We used two approaches to cluster all the neurons in the PS into groups. Firstly, we hierarchically categorised neurons into types, families, orders, and classes based on their projection pattern similarities. Secondly, we employed a random forest-based computational classification model, based on dividing the neuropil into volumetric layers. This model quantifies the innervation patterns of associated neurons within the PS, with key features including the level of innervation and geometric measurements (e.g., bifurcation points, endpoints, and Euclidian distance inside the neuropil).
So far, we have identified 20 distinct microcompartments within the PS and 9 in neighbouring neuropils, with further identification ongoing. This comprehensive mapping of microcompartments will be crucial for detailed analysis of region-specific connectome data, much like the comprehensive mapping of larger compartments, called neuropils, is helping the analysis of brain-wide connectome map of *Drosophila*.

Network integration of neurons with different (somatic vs. dendritic) axon origin: a computational modelling approach

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Cortical principal neurons receive multiple synaptic inputs via their dendrites, integrate them in their soma and once a threshold is reached, generate action potentials. Dendritic branches express multiple mechanisms for modulation and integration of signals, depending on the spatiotemporal pattern of arriving inputs. Recently, we have reported a new structural feature of pyramidal neurons affecting neuronal signal processing: axon localisation. In about 50% of CA1 hippocampal pyramidal cells, the axon emerges from a basal dendrite instead of the soma. Input to the axon-carrying dendrite (AcD) is privileged to trigger action potentials, especially during strong perisomatic inhibition particularly pronounced during network oscillations. Therefore, we hypothesized that 'AcD cells' play a specific role in network oscillations and their synchronisation.

To analyse this, we implemented a multi-compartment model of pyramidal cells using the NEURON simulation environment in Python. We then constructed networks of either AcD or non-AcD cells and added inhibitory cells to simulate perisomatic inhibition. In particular, the network contained excitatory (E) and inhibitory (I) neurons in a 10:1 ratio with 50% I->E, 25% E->I, and 3% E->E connectivity. We simulated two different scenarios: 1) One network constructed as described above, 2) two independently initialized networks sparsely connected to each other via pyramidal cells. The individual networks expressed stable gamma-oscillations (~40 Hz) upon constant random noise input in all cases. In scenario 2), neuronal activity was synchronised between connected networks after an asynchronous activation onset. Preliminary results suggest that AcD cells foster stronger synchronisation between two connected, remotely localized networks while decreasing synchrony within a single network.

Motor control of multi-modal courtship signals in *Drosophila*

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Insects do not only produce air-borne songs to communicate with conspecifics in calling or courtship, many of them also communicate via substrate-borne vibrations. While vibrations receive much less attention than song, vibrations are abundant and important for many insect species, including the well-studied fruit fly *Drosophila melanogaster*. Males of *D. melanogaster* produce an air-borne courtship song as part of their courtship repertoire which was extensively classified in the past. Recent studies identified neuronal circuits in the ventral nerve cord (VNC) which drive and pattern song. Besides this courtship song, male flies also produce substrate-borne vibrations during courtship. While the sets of muscles for vibration and song production overlap (unpublished results), nothing is known about the pre-motor networks in the VNC that drive these muscles to produce vibrations.

Using an activation screen combined with connectome analyses, we here identified neurons at the interface between the pre-motor networks for song and vibration. We find several types of interactions between song and vibration when activating candidate neurons known to affect singing: The activation of the neuron types TN1A and vPR13 decreased the amount of vibrations while promoting song. The vMS12-SS3 neurons on the other hand suppressed song but did not affect vibrations. We also tested the pIP10 and pMP2 neurons which descend from the brain to the VNC and were shown to provide input to the song network. Both neuron types only elicited song upon activation but no vibrations, suggesting that dedicated descending neurons drive vibration.

TN1A and vMS12 are members of the fly's song network in the VNC. However, different subsets of vMS12 neurons showed different effects on song and an analysis of the connectome reveals heterogeneous connectivity among the vMS12 neurons. This suggests that a subset of the vMS12 neurons is part of the vibration network. The vPR13 neurons were not proposed as part of a song circuit. While they make most of their direct connections in the leg neuropils, they are indirectly connected to the song circuit and the wing motor neurons which would be necessary for the observed effects.

These results provide first insights into the pre-motor networks that drive multi-modal signaling in *Drosophila*. The neurons TN1A, vPR13, and vMS12-SS3 all influenced both song and vibrations, making them likely members of a pre-motor circuit in the VNC that drives and coordinates song and vibration.

A New Approach to Phase-Amplitude Coupling (PAC) Measurement: Distinguishing Phase and Temporal Dispersion

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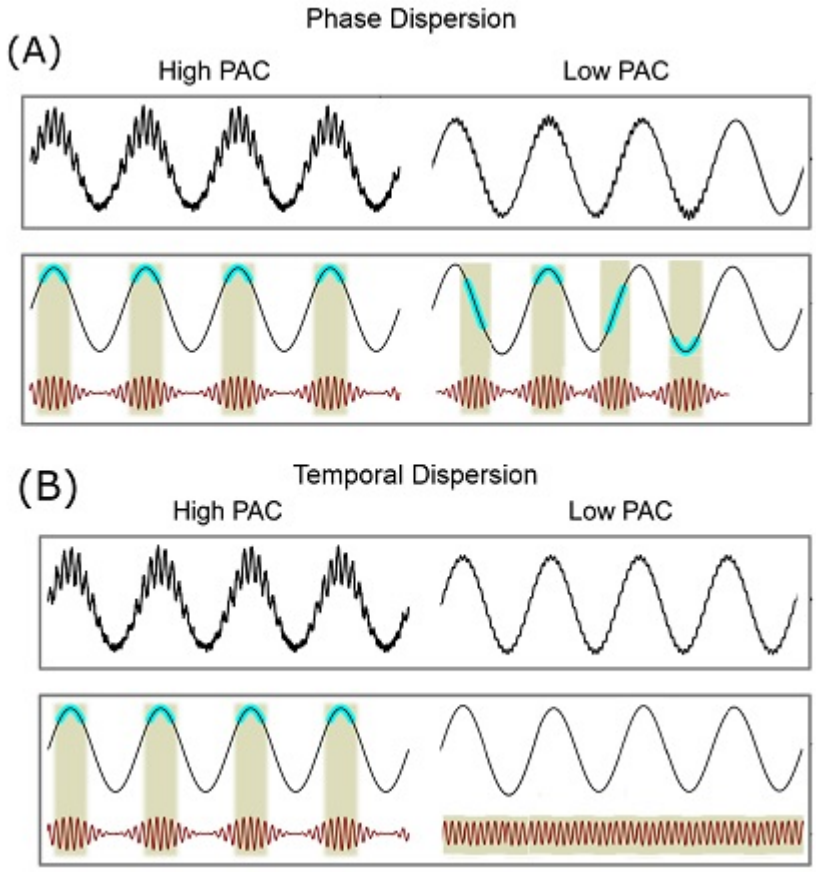
Phase-Amplitude Coupling (PAC) is a critical neural phenomenon in which the phase of a low-frequency oscillation modulates the amplitude of a higher-frequency oscillation. Traditional PAC measurement methods often focus on a single coupling mechanism, potentially overlooking the distinct dynamics involved in PAC modulation. In this study, we identify two different mechanisms underlying PAC modulation: phase dispersion, where gamma bursts lock to the same or different low-frequency phases, and temporal dispersion, where gamma oscillations are spread or localized over time.

We propose a novel method to distinguish between these two mechanisms. Phase dispersion is quantified using circular variance, capturing the variability in phases to which gamma oscillations are locked across multiple cycles. Temporal dispersion is measured using an entropy-based approach, which assesses how gamma oscillations are distributed over time. These complementary approaches enable more precise PAC measurements by accounting for the dynamic nature of neural oscillations.

Through simulations, we show that traditional PAC metrics may either underestimate or overestimate PAC strength depending on the presence of phase or temporal dispersion. Our method provides researchers with a more accurate way to detect PAC by adapting the metric to the underlying mechanism. This refinement enhances our understanding of the role PAC plays in cognitive processes and brain function.

We also show how our method enhances the interpretation of Modulation index (MI) results. In cases of weak MI coupling, it distinguishes whether the weakness is due to high phase dispersion or broad temporal distribution. For example, a low MI score in recordings with high temporal dispersion but low phase dispersion reveals how MI alone can miss key details. Integrating our method with MI analysis provides deeper insights into PAC dynamics and neural communication patterns.

This work offers a refined perspective on PAC analysis, emphasizing the importance of adaptive metrics to capture the complexity of PAC dynamics. By distinguishing between phase and temporal dispersion, our method provides a more robust framework for studying the neural processes underlying PAC.



Two different mechanisms underlying PAC modulation: A) phase and B) temporal dispersion

Identification and characterization of brain and descending neurons controlling adaptive walking in *Drosophila*

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Animals need to flexibly adjust their walking behavior to efficiently negotiate complex, dynamic environments. Although they are capable of flying, *Drosophila* spend a large proportion of their active periods walking. In flies, environmental sensory cues are integrated by higher-level brain interneurons (BNs) and conveyed to lower-level motor centers in the ventral nerve cord via descending neurons (DNs). DNs dynamically modulate ongoing motor activity to match the task at hand. The precise organization of these sensorimotor pathways, how BNs are recruited, and how they engage different DN populations for different tasks, is poorly understood. Here, we combine optogenetics with behavioural analyses to identify and map sensorimotor pathways involved in the control of adaptive walking.

First, we performed an optogenetic activation screen of 131 split-GAL4 driver lines targeting different BN types and 46 split-GAL4 driver lines targeting DNs using a free walking assay. The DNs were pre-selected based on prior studies that identified their involvement in walking control[1,2]. Basic walking parameters of individual flies were quantified using 'TRex'[3] a multi-animal tracking software. Among the lines we screened, 12 BNs and 13 DNs had strong effects on walking.

Our findings highlight five BNs that control key aspects of adaptive walking, including walking initiation, termination, speed, and direction. Additionally, we identified BNs that drove complex behavioral sequences, such as combinations of backward, curve, and forward walking, as well as simpler behaviours, like leg extensions. Silencing experiments revealed that some of the identified BNs are required for specific aspects of adaptive walking. For example, the BN 'Roadrunner' initiates walking, controls walking speed, and is required for flies to increase their walking speed upon starvation – a hallmark of foraging.

Through our experimental and behavioral analysis, we also uncovered new aspects of previously known phenotypes. For example, the DN population DNp17 was originally thought to drive fast forward walking[2]. Our findings reveal that it also drives turning upon activation. The locomotor phenotypes obtained from these screens will be further examined using behavioral set ups and quantitative approaches that allow for detailed kinematic analyses. Moreover, we traced the connectivity of core identified neurons of interest in a fly brain EM volume to reveal their inputs and outputs and understand the circuit architecture underlying the central control of locomotion. The most interesting candidates identified in our screen are being further characterized by in-vivo patch clamp recordings in behaving flies to determine which sensory cues they integrate to control adaptive walking. Combining these approaches will enable a mechanistic understanding of central neuronal pathways that control fundamental aspects of adaptive locomotion.

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Role of the $P2Y_1$ receptor in processing of olfactory signals

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Adenosine 5'-triphosphate (ATP) plays an important role as a co-transmitter in different parts of the nervous system. Being released from synaptic vesicles or through alternative pathways such Pannexin or Connexin channels in the extracellular space, ATP activates purinergic signaling by binding to P2 receptors. P2 receptors are divided into two subgroups, P2X and P2Y. While P2X receptors work as ionotropic receptors and are activated exclusively by ATP, P2Y receptors are metabotropic receptors which, depending on the subtype, respond to various nucleotides such as ADP, UTP, UDP, ATP, UDP-glucose.

In the central nervous system, purinergic signaling modulates neuronal communication in a multitude of brain circuits such as motor control and sensory pathways. Here, we want to investigate the role of purinergic neuromodulation for the processing of olfactory signals.

Olfactory receptor neurons (ORNs) in the olfactory epithelium detect odors and project to the olfactory bulb, the first relay station in the olfactory pathway. There, within separate processing units called glomeruli, the ORN axons release glutamate and ATP exciting mitral cells (MCs) which integrate excitatory input from ORNs and inhibiting interneurons in this processing circuit and serve as the output neurons of the olfactory bulb.

Using patch-clamp recording of MCs in acute brain slices revealed that the uncaging of caged ATP in the glomerulus significantly depolarizes MCs, with the selective $P2Y_1$ antagonist MRS2179 reducing strongly the uncaging response. Therefore, mitral cells MCs in the olfactory bulb are modulated by ATP/ADP through a P2Y1 receptor-dependent pathway. Furthermore, approximately 50% of ATP-induced excitation was mediated by glutamate, since the ATP- effect was reduced in the presence of glutamate receptor blockers, suggesting a feed-forward excitation induced by purinergic signaling. Application of TTX suppressed the glutamatergic feed-forward mechanism whereas a small mitral cell depolarization persisted. Importantly, the astrocytic network, examined by confocal Ca^{2+} imaging in parallel with MCs recording in astrocyte-specific $P2Y_1$ knockout mice, was found not to reduce the effects of MRS2179 on MCs ATP-response.

In conclusion, this pathway highlights a modulatory role of ATP in olfactory processing, involving both purinergic receptor-mediated excitation and an indirect glutamatergic feed-forward mechanism in MCs.

Sensory filtering during sleep regulation

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All animals require undisturbed periods of rest in which they undergo recuperative processes. However, it is currently unclear how quiescent brain states arise that are able to dissociate an animal from its external world, while retaining vigilance to salient sensory cues.
Here, we describe a neural mechanism in Drosophila that creates a visual filter that engenders a quiescent brain state by generating coherent slow-wave activity between sleep-need- and locomotion-promoting neural networks. On a circuit level, we find that these networks can regulate behavioral responsiveness by providing synchronous antagonistic inputs to downstream head direction neurons, thus reducing the functional connectivity between locomotion-gating and navigational networks. Moreover, we show that the same locomotion-promoting network interacts with other sleep-promoting neurons to form an inhibitory feedback loop that regulates the transition from quiescence to falling asleep via specific inhibitory channels. These networks and inhibitory channels are also involved in forming an auditory filter which regulate behavioral responses to courtship sounds depending on the relative tiredness of the animal.
We propose that the temporal pattern of slow-wave activity and functional interactions with inhibitory networks provide the neural architecture to create a ‘breakable’ filter for different sensory modalities and thus permit the animal to enter a quiescent state and fall asleep.

Synchronization between the hippocampus and the thalamic nucleus reuniens accompanies spatial decision making

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The reciprocal connectivity of the nucleus reuniens (RE) of the ventral midline thalamus with the prefrontal cortex (PFC) and hippocampus (HC) suggests that RE may be a critical node within a large-scale brain network underlying spatial cognition. RE activity has been implicated in navigation, decision making, and spatial memory consolidation. We have previously shown that RE activity is critical for the retrieval of spatial memory. In the present study we explored the contribution of RE to the extended prefrontal-thalamo-hippocampal circuit. Specifically, we hypothesized that RE facilitates cross-regional communication by synchronizing the PFC-HC network during spatial task performance. To test this hypothesis, we simultaneously recorded local field potentials (LFPs) from rat RE, PFC, and HC while the rats learned a spatial task in a complex crossword maze. Analysis of the LFP signal on cross-regional interactions revealed strong mPFC-RE synchronization at the theta (7-12 Hz), beta (20-30 Hz) and low gamma (30-60 Hz) ranges regardless of the learning dynamics. In contrast, the HC-RE and HC-mPFC synchrony in the theta and beta bands was learning-dependent. Besides, we observed that both navigational errors (incorrect turn at the maze intersection) and “vicarious trial-and-error” (VTE, a behavioral marker of uncertainty or deliberation) were associated with a transient reduction of HC-RE and HC-mPFC beta coherence. Our findings further support the role HC-mPFC beta coherence in spatial cognition and provide a new evidence for the role of HC-RE synchronization in successful navigation and decision making.

Chronic optogenetic stimulation has the potential to shape the collective activity of neuronal cell cultures

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Neuronal cultures and human stem-cell-derived organoids are fundamental building blocks of neuroscientific research and future personalized medicine. However, in-vitro networks show considerably more synchronous bursting activity than networks in-vivo [1].

One leading theory proposes that under a lack of external input, maladapted network's plasticity leads to a pathological collective activity pattern: long periods of quiescence are interspersed with bursts of strong, synchronous activity [2, 3]. In theoretical simulations, weak external input can reduce this bursting behavior. However, experimental proof is still missing.

To address the challenge of missing external input, we developed a system providing month-long arbitrary 2D light stimulations with a 16x16 LED array to optogenetically light-sensitized neurons whilst enabling a simultaneous redout with an incubator-resistant multi-electrode setup (MEA2100-Mini, MultiChannelSystems) [4]. Custom made protocols enable us to perform an extensive range of theory-driven experiments, including detecting receptive fields via spike-triggered averages, pattern completion experiments after prolonged stimulus exposure, or measuring the cell culture's cell composition after the experiments.

With this platform we exposed neuronal cell cultures (N~15) to temporally and spatially-uncorrelated stimulation (Poisson Noise) of varying input strength for a period of one week and compared their activity patterns with that of unstimulated control cultures (N~15).

Preliminary results indicate that external input does indeed change the collective activity regime. Inter-spike interval distributions shifted from multi-modal to gamma-like, reflecting the reduction of bursts and a transition to Poisson-like firing. Correlations in the activity between units markedly decreased compared to unstimulated controls. These preliminary results suggest that chronic stimulation can indeed harness the intrinsic plasticity and homeostasis to shape the functional connectivity within the cultures. The cultures conditioned in this way are promising model systems for all research areas from computational neuroscience to high-throughput screening.

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Induced respiratory dysfunction by focal stimulation of specific brain areas - implications for SUDEP

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Sudden unexplained death in epilepsy patients (SUDEP) is a common cause of death in patients with severe, medication-resistant epilepsy. While the precise neural mechanisms of SUDEP are still unknown, we hypothesize, that focal epileptic seizures in higher brain areas might disrupt cardiorespiratory brainstem function leading to a postictal collapse of respiratory and cardiac function. To test this hypothesis, we performed electrical stimulations in the amygdala, hippocampus, somatosensory and insular cortex of anesthetized mice to mimic “seizure-like” activity. In all tested regions, this induced acute and transient respiratory depression. Interestingly, stimulation effects varied in amplitude and time course between the different brain regions. Pulse frequency clearly affected the strength of the evoked respiratory depression, while stimulation strength had little effect. In the future, we plan to perform extracellular recordings in brainstem nuclei, while optogenetically stimulating selective cell populations in higher brain regions to determine how the disruption of respiration is mediated at a systemic level. Additionally, we plan to apply the same stimulations to *Scn1a* mice, an epilepsy model that mimics Dravet syndrome, to see how this result changes in an organism that is actually at risk of experiencing SUDEP.

Neuronal circuits for flexible visuomotor transformations in the fly brain

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Survival often demands precise and quick adjustments to movement based on self-generated and external cues. Behaviors such as evading predators, pursuing food, or finding a potential mate require not only speed but also flexibility. How do neural circuits enable reliable integration of complex sensory cues, while allowing flexibility in rapid behavioral responses?

We tackle this question in fruit flies—*Drosophila melanogaster*—which can execute fast, complex maneuvers both in flight and on the ground. As the largest species with a fully mapped connectome of its central nervous system, an exquisite genetic toolkit, and the amenability for physiological recordings, Drosophila offers a unique opportunity to study complete sensorimotor circuits.

Given the high visual dependence of *Drosophila*, with 63% of its brain neurons located in the optic lobes, we asked how visual information is relayed to motor centers. We first focused on the direct connections between visual projection neurons (VPNs) and descending neurons, the critical bottleneck connecting the brain to motor circuits in the ventral nerve cord.

We surveyed previously characterized descending neurons involved in collision detection behaviors such as take-off or landing, and descending neurons involved in walking-related behaviors such as turning or halting. We find that descending neurons involved in rapid, collision detection behaviors tend to directly connect to VPNs, receiving information from different parts of the visual field, depending on the task. Descending neurons that control turning often receive global self-motion input, hinting at a potential role in course correction. In contrast, neurons involved in halting do not receive direct VPN input, suggesting they integrate information from other modalities or central commands.

We are currently investigating the intermediate steps between visual inputs and descending motor commands to identify circuit motifs that allow context-dependent behavior. For example, how does the brain adjust posture based on the landing surface, or decide between fight or flight when facing a looming threat? These insights will help us reveal how much of this sensorimotor flexibility is driven by central brain computations and how much is autonomously governed by the VNC.

Lactate utilization alters sharp wave-ripple networks activity in mouse hippocampal slices

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Lactate, once considered merely a metabolic waste byproduct, has emerged as a multifaceted player in neuronal function, serving both as an alternative energy source and a signaling molecule with diverse effects. Brain lactate levels can raise in various physiological and pathological contexts such as increased neuronal activity, physical exercise, ischemia, and neuroinflammation (e.g., multiple sclerosis and Alzheimer's disease). These changes can affect neuronal energy states, network excitability, and synaptic properties, as reflected in key hippocampal network patterns such as sharp wave-ripples (SPW-R) that are crucial for memory consolidation. Our study seeks to elucidate how lactate fuel affects network activity, synaptic transmission and intrinsic neuronal properties.

To assess the impact of lactate on neuronal energy metabolism and network function, we used acute hippocampal slices of mice (4-6 weeks old), and varied the concentrations of glucose and lactate in the recording solution. Local field potential (LFP) recordings were performed in the CA3 and CA1 regions to investigate spontaneous SPW-Rs that associate with intermediate energy demand. Synaptic transmission was assessed by electrical stimulation of Schaffer collaterals or by monitoring baseline field activities in CA1. Simultaneously, sharp microelectrode recordings were used to evaluate the intrinsic properties of CA1 pyramidal cells.

Our results revealed that 20 mM lactate was insufficient to substitute for 10 mM glucose as a sole energy source because it led to a reduction in both incidence and amplitude of SPW-Rs in CA3 and CA1, but it did not affect ripple frequency (>180 Hz). Interestingly, SPW-R incidence was already reduced by partial replacement of glucose with lactate (lactate/glucose ratio of >1:1). We also added the monocarboxylate transporter (MCT1/2) blocker AR-C155858 to 10 mM glucose, which led to a reduction in SPW-R incidence, without affecting SPW-R amplitude or ripple frequency. When AR-C155858 was added to 20 mM lactate, it strongly reduced SPW-R amplitude, frequency as well as incidence. Synaptic transmission at CA1 synapses showed a marked reduction when substituting 10 mM glucose with 20 mM lactate. However, intracellular recordings revealed only moderate changes in intrinsic firing properties of CA1 pyramidal cells under these conditions.

In summary, lactate as a sole energy substrate is insufficient for maintaining SPW-R network activity and synaptic transmission in mouse hippocampal slices. However, MCT1/2 expression is important for metabolic flexibility of neuronal networks.

Transcriptionfactors CLK and CYC differentially participate in the circadian clock of the Madeira cockroach *Rhyparobia maderae*

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Geophysical rhythms such as the daily night-dark cycle shape life on earth. To anticipate favorable times for rest and activity, organisms evolved endogenous circadian clocks that generate a rhythm of ~24 hrs. Circadian clock cells comprise a molecular clockwork of transcriptional-translational feedback loops (TTFLs) that generate endogenous oscillations of ~24 h in clock gene expression. Closely connected to the visual system a neuronal network of circadian clock neurons with TTFL-based clockworks in the brain of mammals and insects alike, orchestrate circadian rhythms in physiology and behavior via mostly unknown mechanisms. In the fruitfly Drosophila melanogaster, the TTFL clockwork is described best. It comprises a positive feed forward loop of transcription factors CLOCK (CLK) and CYCLE (CYC). They heterodimerize and initiate the transcription of the negative feedback loop elements PERIOD (PER) and TIMELESS (TIM), which in turn inhibit their own transcription. It is generally hypothesized that the resulting oscillations of clock gene expression drive all other physiological and behavioral circadian rhythms in clock cells. However, it is not understood, how the TTFL-based oscillations can drive circadian membrane potential oscillations and circadian neurotransmitter release to orchestrate neuronal circuits controlling sleep-wake cycles in physiology and behavior.

As an alternative hypothesis to the master-slave model of hierarchical clock structure, we propose that the TTFL oscillation is one among many endogenous physiological oscillations in single circadian clock cells that are interconnected and coupled via coupling factors. We propose that plasma membrane-associated post-translational feedback loops (PTFLs) generate endogenous circadian oscillations in membrane potential and second messenger levels. These PTFLs are connected to, but not directly driven by, TTFL oscillations.

Employing RNAi induced knockdown of CLK and/or CYC combined with behavioral analysis in the Madeira cockroach we challenge our hypothesis of PTFL clockworks also participating in controlling circadian rest-activity patterns. While CLK knockdown disrupts behavioral rhythms within 2 weeks, CYC knockdown caused only a lengthening in the period. Possibly, cockroach's CLK contains a nuclear localization sequence, while CYC may have none. Additionally, PTFLs may couple mostly to CLK, maintaining a robust rhythm, also in the absence of CYC. The initial lengthening of the period before complete breakdown of rhythmicity in *clk* knockdowns, we attribute to a slow desynchronization amongst the clock cells and amongst TTFL and PTFL oscillations, rather than a slow acting downregulation of the target protein. Additionally, we found that rhythmic behavior reoccurs after different time spans in *clk* knockdowns, as well as in *clk/cyc* double knockdowns. We currently test with qPCR whether knockdowns were reversible or not. Since previous experiments showed that the negative elements of the TTFL do not result in arrhythmicity, we hypothesize that there are different clock neurons that comprise different sets of clock proteins to generate TTFL-based oscillations, while all of them might depend on the same transcription factors. Now, we focus on single cell transcriptome analysis, besides behavioral analysis, and the assembly and annotation of the cockroach´s genome to further challenge our hypotheses of different clock neurons and PTFL and TTFL coupling for robust rhythms.

Large scale remodeling of the *Drosophila* nociceptive circuit during metamorphosis

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Different developmental stages, changing physiological needs and the demand for refinement, challenge the plasticity of the nervous system. In order to adapt to such changes, all animals can remodel their nervous systems using mechanisms such as apoptosis or neurite and synapse pruning. While the cell biological mechanisms of remodeling are becoming increasingly clear, its effects at the circuit level still remain poorly understood.

The nociceptive circuit of *Drosophila melanogaster* mediates crucial escape responses in larvae. In this circuit, the peripheral class IV dendritic arborization (c4da) neurons provide key sensory input to different layers of interneurons which compute the appropriate defensive behaviors. C4da neurons prune and regrow their dendrites and pre-synapses during metamorphosis, but little is known about the structure and function of the nociceptive circuit in the adult.

We have started to elucidate the development and structure of the nociceptive system in the adult. By tracing cell fates during metamorphosis, we show that at least two major types of larval interneurons are removed from the circuit through a) apoptosis, and b) neurite pruning and repurposing. Using connectomics, we next identify interneurons in the adult nociceptive circuit and show that these neurons are recruited from other larval circuits.

Thus, our results suggest that repurposing is a pervasive mechanism of developmental circuit remodeling.

Neonatal prefrontal efferent is behaviorally relevant but show differential developmental trajectories

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The prefrontal cortex (PFC) plays a crucial role in cognitive processing. It receives inputs from various regions, including the hippocampus and lateral entorhinal cortex, which are known to shape PFC activity during neonatal development, leaving a lasting impact on cognition. However, the extent to which PFC outputs influence the development of downstream brain areas, such as the striatum (Str) and thalamus (TH), remains largely unexplored. Our study addresses this gap through both structural and functional investigations. Using CTB retrograde tracing, we found that by postnatal day 5, the Str preferentially receives projections from the superficial layers of the PFC, while the TH is almost exclusively targeted by the deep layers. However, functional assessment using in vivo electrophysiological recordings reveals a different scenario: the PFC communicates strongly with the Str across all its layers, whereas its functional interactions with the TH are minimal, if present at all. These findings are further supported by layer-specific optogenetic activation of the PFC. Notably, this three-site network shows high activity approximately 500 milliseconds before neonatal ultrasonic vocalizations (USVs) with heightened synchrony particularly between PFC and Str. Our results suggest that the anatomical and functional development of prefrontal efferents occurs at different dynamics, with the thalamic connections lagging behind the striatal ones. Moreover, we provide the first evidence that the PFC and its efferents are critical for behavioral abilities already at this early developmental stage.

Functions of the neuropeptide PDF in the cockroach circadian clock network

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In brains of mammals and insects alike, networks of neuropeptidergic circadian clock neurons, which are entrained to the 24 h light-dark cycle, orchestrate daily rhythms in behavior and physiology. Since isolated clock neurons continue to generate physiological oscillations with a period of about 24 h in constant conditions, these individual neurons are endogenous clocks. Besides the well-studied circadian oscillations in clock gene expression, which is based on transcriptional-translational feedback loops (TTFL), the electrical activity of clock neurons shows plasma membrane-dependent circadian oscillations. While the current view is that the TTFL molecular clockwork drives all other cellular circadian oscillations, we hypothesize that the clock cell's membrane is an endogenous multiscale oscillator. The membrane clock is based on posttranslational feedback loops (PTFL) that are linked to, but not driven by, the nuclear TTFL clock. We challenge our hypothesis with electrophysiological methods in circadian clock neurons of the large, long-lived Madeira cockroach.

In the Madeira cockroach, the circadian clock is located the accessory medulla (AME) of the optic lobes and comprises pigment dispersing factor (PDF) expressing neurons. Depending on the time of day, clock neurons are recruited by neuropeptide release into ensembles to promote specific physiological setpoints such as sleep or activity.

We hypothesize that PDF release, under control of the membrane PTFL clock, triggers the formation of a clock neuron ensemble which promotes sleep. We use patch clamp recordings of primary cell cultures of AME clock neurons in combination with pharmacology to investigate which ion channels and second messenger cascades of the PTFL membrane clock are targeted by PDF. Furthermore, we knock down TTFL components with RNAi and challenge our hypothesis that a system of coupled PTFL and TTFL oscillators controls physiological and behavioral rhythms via neuropeptides as coupling factors *in vivo* and *in vitro*.

Brain circuits that control walking speed and halting in *Drosophila*

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The brain controls high-level aspects of movement including the initiation, speed, and halting of locomotion. These control signals are generated by central brain neurons and communicated to the spinal cord (in vertebrates) or ventral nerve cord (in invertebrates) by descending neurons. The identity of these neurons, their organization into circuits, and their recruitment during locomotion remains unclear. Here, we used an optogenetic activation screen to identify specific neurons in the central brain of *Drosophila* that control the speed and halting of walking. Selective activation of the speed neurons increased walking speed as a function of activation intensity, whereas selective activation of the halting neurons halted walking in a naturalistic manner. Using single-cell patch clamp recordings, we found that the speed neurons are active during walking, whereas the halting neurons are activated by mechanosensors on the antennae, suggesting they can induce halting in response to specific sensory inputs. Using connectome-informed computational models, we predict that the speed neurons excite parallel descending pathways known to promote walking, whereas the halting neurons have inhibitory effects on these pathways. In ongoing experiments, we are studying how the descending signals are integrated in low-level motor circuits of the ventral nerve cord. Together, our findings start delineating a multi-layer circuit for walking control in *Drosophila*.

Electrophysiological characterization of central brain neurons controlling walking in *Drosophila*

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Walking is a fundamental, yet surprisingly complex behavior many animal species rely on. The initiation, direction, and speed of walking must be finely orchestrated for an animal to successfully negotiate complex, dynamic environments. This requires continuous adjustments based on sensory cues. Despite their importance, the neuronal pathways enabling animals to adjust their walking behavior to changing environmental demands are not fully understood.

The fruit fly, *Drosophila melanogaster*, can serve as an excellent model organism for studying the neural circuits that govern locomotion. Despite their name, flies spend a large portion of their lives walking through complex environments. The combination of their compact nervous system, with only approximately 130.000 neurons in the brain and 15.000 neurons in the ventral nerve cord (the fly's version of the spinal cord), an extensive genetic toolkit, and available connectomes, offers the opportunity to systematically investigate the role of different neuronal populations in the control of walking.

We performed an optogenetic activation screen to identify central brain neurons involved in specific aspects of walking, and identified several cell types involved in forward walking, backward walking, and turning. Using intracellular recordings from individual neurons in tethered flies, we measured the neuronal activity of identified neurons during spontaneous locomotion. Thus, we were able to correlate the activity of individual neurons with distinct phases of spontaneous locomotion. Walking-related activity in these neurons aligned with their optogenetic activation phenotypes, and often preceded the behavior. Additionally, we examined how sensory inputs from different modalities shape neuronal activity and, ultimately, the corresponding motor outputs.

In summary, our detailed characterization of neurons involved in walking direction and speed control provides novel insights into the neuronal circuits underlying adaptive locomotion. This work offers an entry point for understanding how sensorimotor pathways control adaptive walking in complex environments.

Prefrontal-hippocampal neural dynamics as useful biomarkers of cognitive impairment and rescue in schizophrenia: Role of serotonin receptors

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We have investigated dysfunctional neural networks and circuits underlying intellectual disability in a pharmacological model of schizophrenia. We focus on prefrontal and hippocampal neural activities, paying special attention to the circuit's connectivity. Recent studies by the group unravelled a functional disconnection between the medial prefrontal cortex and the dorsal hippocampus during psychosis and memory impairment associated with NMDAR hypofunction (Delgado-Sallent C et al., 2022, 2023), a pathophysiological mechanism relevant for schizophrenia. More specifically, during psychosis induced by the NMDAR antagonist phencyclidine, aberrant gamma (~60 Hz) and high frequency (~160 Hz) bands emerged within prefrontal microcircuits whereas overall activity in the dorsal hippocampus decreased. We are currently mapping the emergence of these abnormal neural networks within the distinct areas of the medial prefrontal cortex and investigating the underlying cellular mechanisms via chemogenetics. The prefrontal-hippocampal disconnection also correlated with short- and long-term memory impairment assessed by the novel object recognition test following subchronic administration of phencyclidine. Together, our results suggest that dysfunctional prefrontal-hippocampal communication plays critical roles in cognitive impairment observed in schizophrenia. Another main aim of the laboratory is to find novel therapeutical targets for cognitive amelioration produced by new generation antipsychotic drugs. Interestingly, among highly prescribed antipsychotic medication, the ones showing the most pro-cognitive abilities bind strongly to one or more serotonin receptors. To gain further insight into the distinct roles of serotonin receptors in cognition and intellectual disability, we have investigated at a cellular and functional levels the contribution of serotonin 5-HT1A, 5-HT2A, 5-HT4 and 5-HT7 receptors to the modulation of prefrontal-hippocampal circuits and to the pro-cognitive actions of several antipsychotic compounds, including risperidone and lurasidone. Finally, the group also contributes to the development of state-of-the-art neurotechnologies to advance personalised treatment for brain disorders. These include the in vivo testing of new generation neural probes based on graphene (Viana D et al., 2024), that allow simultaneous recording and stimulation of brain tissue, and novel photoswitchable neuroinhibitors that restrain pathological hyperexcitability with light illumination on demand (Matera C et al., 2022).

Gamma frequency tunes Na^+ channel availability and thereby increases dendritic excitability in cortical pyramidal neurons

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Cortical waves in the gamma frequency range are associated with cognitive functioning, learning, memory, and information processing. Because gamma reflects oscillation of neuronal membrane potential, we asked how it relates to availability of voltage-dependent Na^+ channels, which play a key role in determining cortical neuronal excitablity. In models, the amplitude of the Hodgkin-Huxley inactivation parameter (h) is influenced by both the amplitude of the V_m oscillation and the local steepness of the h_∞ - V_m relationship. If the time constant of channel inactivation (T_h) is very fast, the value of h will closely follow the oscillating V_m waveform, with a 180° phase shift. However, measured values of T_h are shown to be relatively slow. In this case, Na^+ channel availability is expected to become sensitive to the frequency of voltage oscillations, particularly when the oscillation period approaches the duration of T_h, since the instantaneous h value asymptotically approaches the steady-state h_∞ value corresponding to the midpoint of the voltage oscillation, and channel availability is increased at the positive peak of the wave.

To experimentally test the functional consequences of these theoretical predictions, we used the cell-attached configuration of the patch-in-slice technique to measure the relevant Na^+ channel parameters in somata of neocortical Layer 5 cells. The number of “ready-to-open” channels during oscillation was measured by applying sine wave voltage commands of constant amplitude (±5 mV from V_{rest}) and varying frequencies (5-100 Hz), superimposed by brief depolarizing test pulses. At physiological temperature, Na^+ channel availability was lowest at 5 Hz, and it increased sharply as a function of frequency, reaching a maximum at ~ 40 Hz. This frequency dependence of channel availability was associated with an increase in backpropagation of action potentials (AP) into dendrites, as measured by applying brief antidromic stimuli near the axon during the positive peaks of the oscillations. We found that Ca^{2+} transients in the apical dendrite were significantly enhanced (~54%) during 40 Hz oscillations as compared to 5 Hz. We conclude that enhanced membrane excitability associated with the high-pass filtering properties of Na^+ channels renders the gamma frequency optimal for AP backpropagation.

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De novo assembly of a functional neuronal circuit in embryos of an ancestral metazoan

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In recent decades, the complexity, function and development of the nervous system have aroused attention in the scientific community and focused on understanding its origin and function by observing a variety of different model organisms. Over the past years, *Hydra* has demonstrated its high potential for a fundamental understanding of an evolutionarily ancient nervous system. Its evolutionary position, a small number of neurons, a continuously self-renewing nervous system in adult polyps and the accessibility to transgenic lines offer considerable potential for addressing and elucidating fundamental questions. Despite the increase of neurobiological papers on *Hydra*, the de novo formation of neuronal circuits in embryos remains unexplored. For the first time, we present the emergence and population-specific formation of a connecting and growing nerve net in transgenic Hydra embryos, including the impact of the environmental factors temperature and microbiome on the nerve net plasticity as well as the implementation of an artificial electro engineering approach to predict specific network features like pruning. Single-cell tracking of first active neurons revealed an increase in synchronization levels and varying community sizes in different morphological stages during long-term GCaMP6s recordings. Additionally, Immunohistochemistry and high-resolution imaging support a timepoint-specific nerve net topology in late embryogenesis and early hatchlings. This all contributes to understanding the initial formation of the nerve net in *Hydra*, an emerging model organism for neurobiology.

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Distinct connectivity patterns along the anterior-posterior axis of the piriform cortex

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The piriform cortex (PC), a key brain region involved in the processing of olfactory information, has been linked to a variety of functions, from odor identification to more complex processes such as odor-value associations and context-related odor memory. To support this wide range of functions, the PC maintains connections with multiple brain areas. However, the connectivity of the PC is not uniform across its entirety but varies along the anterior-posterior axis of this elongated brain region. Previous research has delineated two main subdivisions of the PC – the anterior and posterior piriform cortex (aPC and pPC). In addition to being anatomically distinct, aPC and pPC exhibit different electrophysiological properties, which in turn contribute to their specific roles in olfactory-related processing. Another distinguishing factor between these two regions is their connectivity patterns. Despite these insights, key questions remain: How do the input and output connections align at specific locations within the PC? Is the transition in connectivity patterns along the anterior-posterior axis gradual or abrupt? To address these questions, we employed anatomical tracing of axonal projections and conducted detailed reconstructions of injection sites to investigate the connectivity patterns along the anterior-posterior axis of the PC. Specifically, we used and adjusted the QUINT workflow to register microscopy images with anatomical reference atlases, create segmentations of labelled regions, and quantify the distribution and intensity of tracer signals across the entire brain. This comprehensive approach allowed for a precise reconstruction of the injection sites and an in-depth examination of connectivity patterns. By targeting different regions of the PC during tracer injections and performing subsequent mapping and quantification analyses, we were able to distinguish the projection patterns along the anterior-posterior axis. This method allows to ensure consistency in injection site targeting which is critical for the comparison of results across animals. Our findings provide new insights into the structural differences along the anterior-posterior axis of the PC. These results lay the groundwork for future investigations into the functional distinctions between aPC and pPC, shedding light on their specific roles in olfactory processing.

A distinct hypothalamus-habenula circuit governs risk preference

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Appropriate risk evaluation is essential for survival in complex, uncertain environments. Confronted with choosing between certain (safe) and uncertain (risky) options, animals show strong preference for either option consistently across extended time periods. How such risk preference is encoded in the brain remains elusive. A candidate region is the lateral habenula (LHb), which is prominently involved in value-guided behavior. Here, using a balanced two-alternative choice task and longitudinal two-photon calcium imaging in mice, we identify LHb neurons risk-preference-selective activity reflecting individual risk preference prior to action selection. By employing whole-brain anatomical tracing, multi-fiber photometry, and projection- and cell-type-specific optogenetics, we find glutamatergic LHb projections from the medial (MH) but not lateral (LH) hypothalamus providing behavior-relevant synaptic input before action selection. Optogenetic stimulation of MH->LHb axons evoked excitatory and inhibitory postsynaptic responses, whereas LH->LHb projections were excitatory. We thus reveal functionally distinct hypothalamus-habenula circuits for risk preference in habitual economic decision-making.

Cell type and Molecular Architecture of the Pigeon Brain

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Birds have intricate neural systems that drive advanced sensorimotor and cognitive abilities. The neuronal repertoire underlying their sophisticated behaviours are still largely unexplored. Therefore, we aimed to establish a molecular cell type atlas of the pigeon brain at unprecedented resolution, comprising a million single nuclei from 21 major neuroanatomical areas, using single nucleus RNA sequencing and fluorescent in situ hybridisation. This resource will shed light on conserved brain regions, neuronal classes, and circuits within the avian brain, facilitating evolutionary analysis and functional studies.

Interactions of a sleep-control centre with a neural circuit used for navigation in Drosophila

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Sleep is a crucial part of everyday life, but the neural networks that regulate behavioural manifestations of sleep are not fully understood. In the fruit fly Drosophila melanogaster dorsal fan-shaped body neurons (dFBNs) sit at the centre of homeostatic sleep control: the neurons' excitability increases with sleep drive, and their artificial activation induces sleep. Despite significant advances in understanding of the molecular events that regulate the activity of dFBNs, insight into how they impose sleep on the organism is still lagging. By combining optogenetics and in vivo patch-clamp recordings in the brain of the fly, we have identified two neuronal groups that show functional monosynaptic connections with dFBNs. These cells reside in the fan-shaped body, an area involved in navigation, which allows us to study the interaction between the encoding of sleep drive and cognitive computations in a tractable system. Understanding the behavioural relevance of these neurons, the computations that underly their role in behaviour, and the influence of dFBNs on these computations will enhance our understanding of the sleep control imposed by dFBNs, as well as form a crucial step towards a systems view of sleep.

Poster Topic

T24: Attention, Motivation, Emotion and Cognition

T24-1A Top-down control of dopamine learning signals in the amygdala
Wulf Haubensak, Lars Kopel, Dominic Kargl, Dow Glikman

T24-2A Developmental trajectories of prefrontal activity patterns during working-memory performance in mice
Jastyn A. Pöpplau, Johanna K. Kostka, Ileana L. Hanganu-Opatz

T24-3A Projection specific information coding in frontal cortical networks
Irene Lenzi, Alice Despatin, Nilufar Nojavan Lahiji, Moritz Nesseler, Luca Koenig, Marc Spehr, Björn Kampa, Simon Musall

T24-4A Anatomical organization of genetically-defined prefrontal projections to sensory cortices
Felix Jung, Loran Heymans, Xiao Cao, Marie Carlén

T24-5A Lateral septal neuronal populations play complementary roles in regulating social and feeding behaviors
David Keller, Francisco J. de los Santos, Robson Scheffer Teixeira, Letizia Moscato, Hanna Elin van den Munkhof, Haena Choi, Tatiana Korotkova

T24-6A Characterization of Oxytocin Receptor-expressing Neurons in the Medial Septum of Mice
Laura Stangl, Inga D. Neumann, Rohit Menon

T24-7A Number selective sensorimotor neurons in monkey prefrontal and intraparietal cortices
Laura Elisa Seidler, Stephanie Westendorff, Andreas Nieder

T24-1B Electrophysiological correlates of selective auditory spatial attention: Effects of intranasal oxytocin
Michael-Christian Schlüter, Marlies Pinnow, Martin Brüne, Jörg Lewald

T24-2B Determinants of the explore-vs-exploit courting strategies of the Drosophila males
Madhura D. Ketkar, Jan Clemens

T24-3B Encoding of basic visual features across the field of view in the crow nidopallium caudolaterale
Linus Hahner, Lena Hoeppe, Andreas Nieder

T24-4B Spontaneous and sensory-evoked arousal fluctuations engage a specific brain activity wave
José-Maria Martinez de Paz, Johanna Luise Mayer, Paulina Gabriele Wanken, Beatriz Rodrigues Apgaua, Emilie Macé

T24-5B Cytoarchitectonical mapping and analysis of the human temporal pole
Carla Hogrebe, Sebastian Bludau, Hartmut Mohlberg, Katrin Amunts

T24-6B Multisensory integration and modality-specific decision-making in frontal cortex and superior colliculus
Alice Despatin, Irene Lenzi, Peter Severin Graff, Gerion Nabbefeld, Kerstin Cohlst, Daniel Gerber, Luca König, María Laura Pérez, Anoushka Jain, Sonja Grün, Björn Kampa, Simon Musall

T24-1C Cognitive flexibility training facilitates fear extinction in C57BL/6J and 129/S1 mice
Markus Fendt, Mei Ling Iu, Laura de los Ángeles Molano Moreno, Iris Müller, Daniela C. Dieterich

T24-2C Lateral hypothalamic neuronal dynamics command behavioral transitions and coordinate different stages of feeding
Mahsa Altafi, Changwan Chen, Mihaela-Anca Corbu, Aleksandra Trenk, Hanna Elin van den Munkhof, Kristin Weineck, Franziska Bender, Marta Carus-Cadavieco, Alisa Bakhareva, Tatiana Korotkova, Alexey Ponomarenko

T24-3C Diverse representation of various rewards in the dopaminergic neurons of the ventral tegmental area
Kamil Pradel, Katharina Wolff, Robson Sheffer-Teixeira, Vasyl Mykytiuk, Tatiana Korotkova

T24-4C Crows recognize geometric regularity
Philipp Schmidbauer, Madita Hahn, Andreas Nieder

T24-5C The role of dopamine receptors in semantic associations between signs and quantity categories in primate prefrontal neurons
Saskia Erdle, Laura E. Seidler, Andreas Nieder

T24-6C Linking Attentional States and Neuronal Dynamics in the Locus Coeruleus During a Decision-Making Task
Ananya Joshi, Csilla Novák, Rafael Parker, Matthias Prigge

T24-7C Effect of chemogenetic manipulations of the orexin system on cognitive flexibility and working memory in mice
Niki Panagiotou, Markus Fendt

T24-1D Dissecting the multisensory dimensions of the social brain in mice
Johanna Luise Mayer, Emilie Macé

T24-2D Opposite coding of competing rewards by VTA dopamine neurons
Hanna Elin van den Munkhof, Vasyl Mykytiuk, Tatiana Korotkova

T24-3D Whole-brain activity patterns underlying uninstructed behavioral switching in mice
Paulina Gabriele Wanken, Bradley Jay Edelman, Leafy Behera, José-Maria Martinez de Paz, Emilie Macé

T24-4D Social context shapes behavioral and neural dynamics of foraging and decision-making in freely moving rhesus macaques
Ayuno Nakahashi, Jessica Grunwald, Zurna Ahmed, Irene Lacal, Alexander Gail

T24-5D Neural Dynamics of Context, Cue and Rule Encoding: The Role of PV- and SOM-Interneurons in the mPFC
Florian Steenbergen, Brice De La Crompe, Julian Ammer, Ilka Diester

T24-6D Gaze following is not grounded in the perception of implied motion
Masih Shafiei, Marius Görner, Peter Thier

T24-7D Cognitive biases influence numerosity judgments in macaques and crows
Lena Jannasch, Julia Grub, Andreas Nieder

Top-down control of dopamine learning signals in the amygdala

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Adaptive reactions to salient stimuli in the form of affective states are a hallmark of emotions and critical for survival in ever-changing environments. Extensive research identified the amygdala and especially the central amygdala (CE) as a key hub for affective response selection. Throughout the course of learning, salience shifts to predictive cues and, depending on the cue, drives the appropriate affective state (fear-, reward-states). To ensure flexible and informed response selection, the CE is receiving strong neuromodulatory inputs, and instructions from cortical areas. We hypothesized that dopaminergic (DA) neurons, originating from the ventral periaqueductal gray (vPAG) , induces plasticity in the CE during affective learning, while a feedback loop via the cortex recruits affective models to facilitate appropriate response selection by top-down signals.
In this study, we examined this circuit module in vivo during valence specific conditioning (fear and reward), by recording DA related activity in the CE using DA sensors and axonal Calcium (Ca2+) sensors in combination with chemogenetic inhibition of cortical inputs to the vPAG. We found that DA release in the CE shifted to predictive cues in both valences, resembling an unsigned prediction error (PE). CE DA signaled CS properties and was related to both, the presence of an affective model as well as correct behavioral responses. These signals were gated by vPAG interaction with the cortex, which operate as top-down switch to couple or decouple DA activity to learning progress and behavioral output.
This work provides a model for top-down control over DAergic learning signals in the amygdala, elucidating the intricate interplay between cortical and subcortical structures in affective learning and response selection.

Developmental trajectories of prefrontal activity patterns during working-memory performance in mice

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Cognitive flexibility allows individuals to continuously adapt and modify their strategies in response to changing environmental conditions. Within the spectrum of cognitive flexibility, working memory (WM) plays a crucial role. As a prerequisite for planning, WM is the cognitive ability to temporarily store and manipulate information. While multiple brain regions contribute and interact in the execution of WM, the prefrontal cortex (PFC) is widely recognized as a central hub. The PFC exerts “top-down” control by processing incoming information and directing behavior towards specific goals. WM abilities typically emerge towards the end of the juvenile period, coinciding with the structural and functional maturation of the PFC. Notably, several mental disorders, such as schizophrenia, tend to manifest during or towards the end of this developmental period. However, little is known about how the maturing PFC facilitates the development of adult-like WM capacities. Here, we investigated WM abilities across different developmental stages - pre-juvenile, juvenile, and young adult - using a delayed non-match to sample task, while monitoring local field potentials and single-unit activity in the medial PFC of mice. Our findings showed that decision speed increased with age, with pre-juvenile mice spending the most time in the decision zone. WM task induced prefrontal gamma power and firing rates of single units increased with age. Moreover, only adult mice showed a decision preceding peak in theta power. Overall, our data provide first insights into the neuronal mechanisms within the PFC that support the age-dependent emergence of WM abilities.

Projection specific information coding in frontal cortical networks

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The anterior lateral motor cortex (ALM) is crucial for integrating sensory information to drive associated behaviors. This process critically relies on long-range projections to subcortical regions, in particular the striatum which receives robust projections from cortico-striatal projecting neurons (CStr). A potential role for this prominent projection pathway is the formation of new sensorimotor associations when learning new behaviors. However, the specific role of CStr neurons during learning remains unclear.
To address this issue, we anatomically characterized the distribution of ALM terminals in the striatum and found a gradient along the anterioposterior axis. This pattern was preserved for ipsi- versus contralateral projections, with contralateral being ~35% weaker compared to ipsilateral expression. Next, we retrogradely labeled CStr neurons in ALM and performed 2-photon imaging over a series of behavioral tasks with increasing cognitive demand. First, mice underwent an innate motor behavior, collecting a reward from two available water spouts, requiring no novel stimulus-response associations. Second, mice learned to associate the location of click sounds to the side of a water reward, and lastly had to retain their choice for a short delay period.
Across sessions, we observed a clear increase in choice selectivity for all ALM neurons, starting with the introduction of the non-innate auditory task. CStr neurons were more selective as non-CStr neurons, although this effect was subtle compared to the overall learning-related changes. These changes persisted when repeating the innate task, with a 2-fold increase of choice selective neurons after learning, pointing to a general restructuring of choice-related circuitry. To causally confirm these results, we optogenetically inactivated either all or only CStr neurons in ALM during the innate and auditory tasks. Inhibiting CStr neurons during the innate task strongly impaired performance before, but not after training. To further isolate this effect, we used the synaptic silencer eOPN3 to specifically inactivate ALM projections to either the striatum, the thalamus or the superior colliculus (SC) during task learning. In agreement with our earlier results, we found that inhibiting CStr projections mostly reduced task performance early in training, whereas inhibiting projections to SC and thalamus became more relevant when further increasing cognitive demands. Lastly, to better understand the neural underpinnings of these behavioral effects, we used fiber photometry to measure the activity of ALM neurons as well as their respective axonal projection in each of the ALM target regions. Here, we found distinct signals for each axonal projection pathway, indicating area-dependent changes in choice selectivity over the course of learning.
Overall, our results demonstrate that task learning induces long-lasting changes in choice-related circuitry in ALM with distinct subcortical projection pathways performing different roles in the acquisition of new behaviors.

Anatomical organization of genetically-defined prefrontal projections to sensory cortices

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The mouse prefrontal cortex (PFC) is an umbrella term for rostrally located neocortical brain regions. The anterior cingulate, pre- and infralimbic, orbital, agranular insular and secondary motor cortices are generally included in the PFC. The PFC is critically involved in the acquisition and expression of flexible behavioral responses to changing sensory information, and prefrontal signaling to (neocortical) sensory regions are assumed to be essential to these processes.

In the present study, we characterized the anatomical organization of excitatory, prefrontal projections to neocortical sensory (i.e. somatosensory, auditory, visual) regions using retrograde viral tracing in transgenic mouse lines identifying three main classes of pyramidal neurons (i.e. layer 2/3 IT, layer 5 IT, layer 5 IT/PT). As part of this work, we developed an open-source software platform (DMC-BrainMap) for analysis of (whole-brain) anatomical data.

Our results reveal profound differences in the organization of PFC projections to the sensory cortices. Only the visual and auditory cortices receive predominant input from the orbital subregions of the PFC. Interestingly, we found a dorsoventral gradient of the different classes of pyramidal neurons projecting to both cortices, respectively. In contrast, the somatosensory cortices predominantly receive input from the secondary motor cortices (irrespective of PFC pyramidal class). Additionally, the somatosensory cortices receive moderate input from the orbital and agranular insular cortices displaying stark differences between pyramidal cell classes.

In conclusion, our results provide further insights into the anatomical organization of sensory-prefrontal circuits opening the possibility for its functional interrogation by future research.

Lateral septal neuronal populations play complementary roles in regulating social and feeding behaviors

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Social behaviors, whether conflictual or cooperative, are essential for survival and reproduction. However, the neural circuit mechanisms that regulate different social behaviors are still not well understood. Additionally, there is limited knowledge about how the brain processes decisions when faced with competing stimuli that drive mutually exclusive behaviors. The lateral septum (LS), a key brain region, plays a role in regulating aggression and feeding behaviors through its connections with the hypothalamus, prefrontal cortex, and hippocampus. We previously showed that somatostatin-expressing (Sst) neurons in the LS promote food-seeking (Carus-Cadavieco *et al.*, *Nature* 2017). Here we investigated functions of two cell populations in the LS, Sst- and neurotensin-expressing (NT) cells, in social and feeding-related behaviors.

We combined opto-, chemogenetics and calcium imaging in freely behaving mice, to characterize to role of Sst- and NT-expressing cells in social- and feeding related behaviors.

We observed distinct patterns of neuronal activity in the LS that selectively varied across different stages of social behaviors. Optogenetic activation of NT cells resulted in increased social interaction, accompanied by decreased dominant behavior towards conspecifics. At the same time, opto- or chemogenetic activation of NT cells in the LS decreased food intake. Conversely, optogenetic activation of Sst cells in LS decreased social interactions. Both NT and Sst cells exhibited positional firing, whereas Sst cells had higher place field stability and mutual information. Optostimulation of hippocampal inputs to the LS-NT and LS-Sst cells changed activity of those cell populations.

In summary, our findings indicate that Sst- and NT-expressing cell populations in the LS work in a complementary manner to regulate various aspects of innate behaviors.

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Characterization of Oxytocin Receptor-expressing Neurons in the Medial Septum of Mice

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Oxytocin (OXT) is a highly conserved neuropeptide involved in regulating various social behaviors. It is primarily, but not exclusively, synthesized in the paraventricular (PVN) and supraoptic (SON) hypothalamic nuclei and mediates its various functions via its singular receptor (OXTR). The OXTR is expressed in several brain regions, including the medial septum (MS), a structure of the limbic system known for regulating locomotion and forming social memory by influencing hippocampal theta oscillation. The OXTR-expressing neurons of the MS (MS^{OXTR}) pose as a putative regulator of social behaviors due to their ideal location in the basal forebrain and the proximity to the adjacent lateral septum, which is a key modulator of social behavior. However, the function of the MS^{OXTR} subpopulation has not been investigated so far. To this end, we first characterized the MS^{OXTR} on a biochemical level, before we conducted tracing studies to elucidate the underlying circuitry. With this, we could show that the MS^{OXTR} neurons are predominantly GABAergic (~ 80%). However, we still need to identify the subtype of these inhibitory neurons. Furthermore, we revealed several downstream targets of MS^{OXTR} neurons, including the prefrontal cortex and the hippocampus by infusing a cre-dependent reporter virus into the MS of OXTR-Cre mice (AAV9-CMV-Flex-Synaptophysin-mCherry). Additionally, we performed a retrograde tracing approach using a combination of viral (AAVrg-hSyn1-EGFP2A-iCRE-WPRE) and immunohistochemistry approaches to investigate the source of OXT transported to the MS in WT CD1 mice. However, we could not reveal OXT synthesizing cells that directly project to the MS, but we could show OXT^{+} fibers in proximity to the MS, suggesting an innervation of OXT-producing neurons. In conclusion, this study set the groundwork for further characterization of the OXT system within the MS. Ongoing experiments aim to identify behaviors associated with the MS^{OXTR} by screening the activity of MS^{OXTR} neurons across a range of potential behaviors using calcium imaging.

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Number selective sensorimotor neurons in monkey prefrontal and intraparietal cortices

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Humans and animals are often required not only to estimate the number of external elements but also to translate this information into a matching number of actions in order to make adaptive decisions. This process is referred to as sensorimotor transformation, where sensory input is converted into precise motor responses. We examined this link in the counting domain by training two macaque monkeys on a numerical sensorimotor transformation task and recorded single cell activity in the intraparietal sulcus and the prefrontal cortex. The task consisted of numerical stimuli from quantity one to five in form of dot displays or signs (Arabic numbers). The monkeys were trained to assess the displayed number and flexibly plan a corresponding number of self-performed movements, which in this case was the release of a handle the monkeys were holding onto. We found number selective sensorimotor neurons both in the monkeys' prefrontal cortex as well as the intraparietal sulcus maintaining the numerical information and preparing the impending motor response. The activity of these neurons was significantly reduced during error trials. Training and testing of a statistical support vector machine classifier showed that the neuronal population activity during the sensorimotor transformation period encodes the number of planned actions. Population responses also predicted incorrect trials, where the monkeys intend to release the handle fewer or more times. Overall these findings are in a strong support of an abstract neuronal code of a sensorimotor number system found in two connected areas of the primate brain.

Electrophysiological correlates of selective auditory spatial attention: Effects of intranasal oxytocin

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Oxytocin (OT) is a peptide hormone known to play an important role in human social behavior and communication [1,2]. As sexual steroid hormones have been suggested to modulate functions of selective auditory attention [3,4], here we aimed to investigate related effects of OT. A double-blind, randomized, placebo-controlled study design was employed, with intranasal administration of OT (24 IU) or placebo in men, women using hormonal contraception (HC), and women using no hormonal contraception (NHC); overall number of participants: n = 100. Auditory event-related potentials (ERPs) were recorded in a “cocktail-party” task [4,5,6], where participants were required to localize a target sound source presented among three distractors (one-syllable German numerals [7]) at different locations. Only correct trials were included in the analysis. Data were collected in three blocks: immediately before, 20 min and 60 min after OT application. NHC women were tested at three points in their menstrual cycle and HC women at three corresponding points in time; men were tested once. The analyses focused on the N2 component of the ERP, which has been shown to be a correlate of selective auditory spatial attention [6]. In NHC women, OT administration led to a significant reduction in N2 amplitude during the follicular phase (d = -0.88). In HC women, after OT administration a longer N2 latency was observed in the phase 4-8 days after the start of contraceptive use (d = 1.0). No significant effects were found for women at other points in time, or for men. These findings suggest that intranasal OT influences brain processes underlying selective auditory spatial attention in women, with effects seemingly modulated by natural or drug-induced monthly hormonal fluctuations. Supported by the Wilhelm und Günter Esser Foundation.

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Determinants of the explore-vs-exploit courting strategies of the Drosophila males

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From acquiring food to persuading a mating partner, animals engage in activities that require persistent efforts. For example, courtship drive in various species must persist for several minutes or even hours until their courtship ritual convinces a conspecific of mating. However, not every courtship attempt culminates in mating, and mechanisms to assess the mating likelihood should be in place to minimize the costs towards fruitless courtship. On the one hand, cues from conspecifics indicate their likelihood of mating, and the chance to court a high-likelihood conspecific should be exploited by being more persistent. On the other hand, long futile courtship of any conspecific can be viewed as sunk costs and should be discontinued in favor of an exploratory behavioral switch. How is such 'explore-vs-exploit' trade-off settled in social behaviors? To gain insights into this question, we study the courtship of fruit flies, *Drosophila melanogaster*.

Male fruit flies engage in enduring pursuit of females. We find that, when no mating occurs, some males adopt a progressively explorative courting strategy reflected in their declining courtship index, whereas other males' courtship remains persistent over several tens of minutes. This variability in courtship strategy across males could be driven by factors external to the male, such as female feedback cues, or factors internal to the male brain that interact with an autonomous, time-dependent decline of persistence. Among the female rejection behaviors apparent during courtship, we observe that the female escape frequency changes with the time into being courted. These escape attempts often discourage the courtship, thus potentially influencing the male courting strategy. However, the female escapes prompt some males to change the strategy much more strongly than others, perhaps owing to the males' differential internal states. These findings suggest a role of both female feedback cues and male-intrinsic factors in determining the male courting strategy over time. By manipulating the female and male factors in our courtship assay, for example by using optogenetics to evoke the female behaviors, we now continue to examine how they influence the male courtship.

Altogether, our approaches will reveal the principles of explore-vs-exploit decision making in a social context. Elucidating the determinants underlying this trade-off will allow us to understand how the fluctuating estimates of expected outcome modulate persistent internal states.

Encoding of basic visual features across the field of view in the crow nidopallium caudolaterale

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The avian nidopallium integrates visual inputs from both the tectofugal and the thalamofugal pathway that can be distinguished at the retinal level, corresponding to a differential mapping of monocular and binocular stimuli. In corvid songbirds, the nidopallium caudolaterale (NCL) has been shown to flexibly represent stimulus features relevant to current goals, but it is unclear how sensory information is encoded across the frontal and lateral visual fields. We recorded single-cell activity in the left-hemispheric NCL of 2 awake carrion crows (*Corvus corone*) while presenting visual stimuli in the frontal binocular as well as both peripheral monocular visual fields. Crows were required to maintain head position to avoid shifts of retinal stimulation. Stimuli consisted of three types: Gabor gratings of different spatial frequencies and orientation that were either moving or stationary, as well as distinct colorful images. Across subjects, we found that almost half of all recorded units selectively encoded stimulus position, i.e. whether stimuli were presented in the left, middle, or right visual field. Among position-selective units, significant proportions preferentially encoded either stimuli in the binocular field visible to both eyes, or stimuli in the monocular right field covered by the right eye. This indicates both contralateral processing and integration of ipsilateral information. Around a third of all units responded selectively to colorful images, stationary or moving Gabor patches. The vast majority of such selective units distinguished Gabor gratings from complex images. By contrast, moving gratings were not well-distinguished from stationary gratings. Different feature dimensions including spatial frequency, edge orientation and motion direction were encoded by only a small amount of units. Our findings demonstrate that sensory information from distinct visual fields is integrated in the crow NCL and represented by distinct cell populations. They extend our knowledge about how the visual environment is represented at different stages along the visual paths of the avian brain.

Spontaneous and sensory-evoked arousal fluctuations engage a specific brain activity wave

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Arousal state has a major impact on perceptual ability, task performance, and diverse aspects of physiology and behavior. During wakefulness, spontaneous fluctuations in arousal state strongly modulate neural activity in numerous brain regions in mice, but the lack of large-scale, deep imaging methods has prevented testing whether these fluctuations affect the entire brain uniformly. Moreover, it remains unclear whether spontaneous and sensory-evoked fluctuations in arousal state engage the same brain circuits or are fundamentally distinct processes. To address this gap, we used functional ultrasound imaging in awake, head-fixed mice and correlated the recorded whole-brain activity with pupil size fluctuations, known to track arousal. We characterized a large-scale arousal 'wave' of activity from specific brain regions showing a distinct temporal dynamic. Next, we compared this spontaneous arousal wave to the brain-wide wave elicited by arousing stimuli (mild air-puffs) and found a large overlap between the two, the main difference being that external stimuli also activate sensory areas. Finally, we assessed how the arousal wave is affected by manipulations in the tonic levels of noradrenaline, a well-established regulator of arousal, using optogenetics and pharmacology. We found that the cortical component, but not the subcortical component, of the arousal pattern was sensitive to the noradrenergic tone. Our work refines the role of arousal as a global modulator of neural activity by identifying a specific brain network that responds to spontaneous and evoked fluctuations in arousal, and by characterizing its constitutive components across sustained arousal states.

Cytoarchitectonical mapping and analysis of the human temporal pole

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Introduction

The temporal pole (TP) is involved in face (Pisoni et al., 2020) and object recognition (Nilakantan et al., 2017), verbal semantic memory (Mehta et al., 2016), language processing (Mesulam et al., 2013) and social behavior (Thompson et al., 2003). Its many functions are not yet fully understood, and not well linked to their microstructural correlates. Moreover, the extent and number of areas of the TP differ between existing cortical maps (Brodmann, 1909; von Economo and Koskinas, 1925; Hopf, 1954). The pattern of gyri and sulci varies between brains making the definition of the TP areas even more difficult (Insausti, 2013). The aim of this study was therefore to provide a detailed, three-dimensional microanatomical map of the TP in a sample of ten brains.

Methods

We delineated four areas on every 60th, cell-body-stained section in 5 male and 5 female brains including the BigBrain (Amunts et al., 2013). Statistics and image analysis were used to detect borders in an observer-independent approach (Schleicher et al., 1999). The volumes of the new areas were analyzed regarding hemispheric and sex differences. Cell density profiles were extracted and a hierarchical cluster analysis was used to describe cytoarchitectonic differences between areas. The areas were 3D-reconstructed and superimposed in two MNI reference spaces (MNI "Colin 27", Holmes et al., 1998; ICBM152casym, Evans et al., 2012) resulting in probability maps for each area in stereotaxic space (Amunts et al., 2020). In addition, areas were 3D reconstructed in the BigBrain with a resolution of 20 micrometer (Amunts et al., 2013) using a deep-learning based tool (Schiffer et al., 2021).

Results

Areas Tp1, Tp2, Tp3 in the TP and a new temporo-piriform transitioning area TPir were identified. Areas Tp1 and Tp3 are located ventrolateral in the TP; areas Tp2 and TPir mediodorsal. Area Tp1 extends until the superior temporal sulcus (STS). Inferior to it area Tp3 extends into the STS or inferior temporal sulcus. Area Tp2 is located superior to area TPir, both reach the Limen insulae. Volumes of the areas did not differ between hemispheres or sex. The new areas are bordering previously mapped areas in the temporal lobe Te3 (Morosan et al., 2005), areas STS2, TI, TeI (Zachlod et al., 2020) and the piriform cortex (Kedo et al., 2024), as well as not yet mapped areas of the middle and inferior temporal gyrus. Areas Tp1 and Tp3 are cytoarchitectonically most similar to each other and to STS1 of the STS (Zachlod et al., 2020), while Tp2 is more similar to area TPir. Interindividual variability in location and extent of the areas is represented in probability maps (Figure 1). They will be available as part of the Julich-Brain Atlas (Amunts et al., 2020). The reconstruction of the areas in the BigBrain (Amunts et al., 2013) shows their complex topography in 3D space.

Conclusion

The new segregation of the TP into four areas is finer-grained than shown in previous maps. Two of them were more similar to lateral temporal areas, while the more mesial areas show properties that bring

them closer to periallocortical areas. The probability maps provide a microanatomical reference map for functional studies to better understand the functional organization and connectivity of the TP.

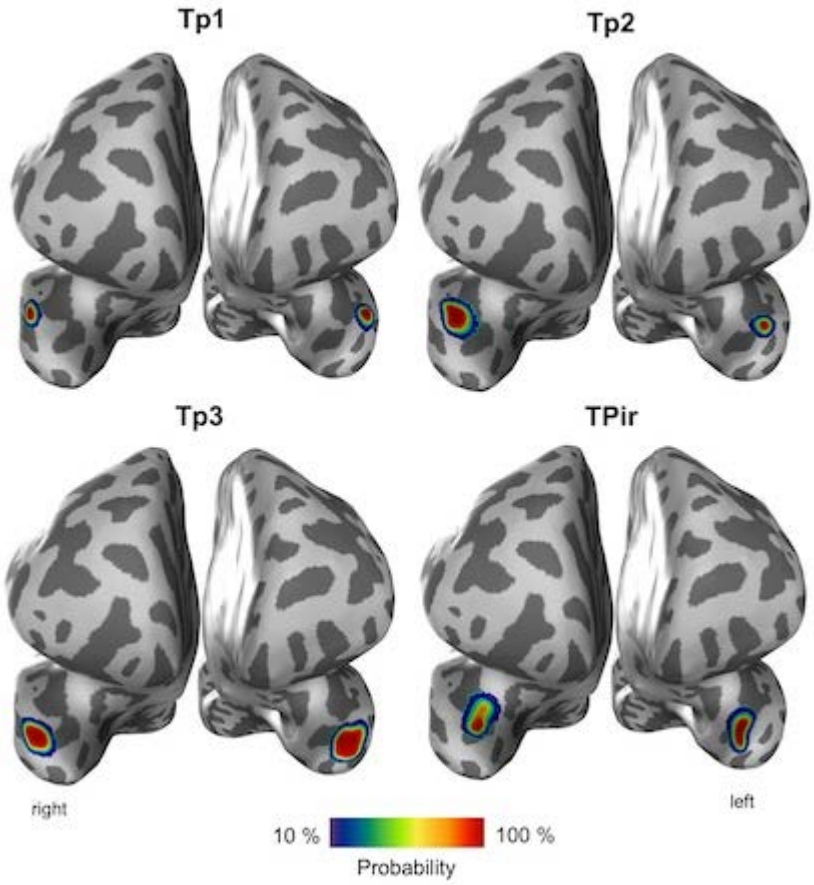

Figure 1: Probability maps of the new areas

Multisensory integration and modality-specific decision-making in frontal cortex and superior colliculus

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The integration of sensory inputs from different senses is a crucial aspect of sensory perception and the generation of corresponding behavioral decisions. However, whether such multisensory integration occurs at specific stages of neural processing, for example after the initial processing of unisensory information but preceding the formation of a behavioral choice, remains unclear. Two brain regions, the anterolateral motor cortex (ALM) and the superior colliculus (SC), have been implicated as particularly important structures for both multisensory integration and decision-making, suggesting that they are part of a cortico-subcortical loop that transforms multisensory inputs into behavioral decisions. To study the role of these areas in multisensory integration and decision-making, we trained mice in a multisensory discrimination task, where animals had to integrate visual and tactile information over time to identify the target stimulus side. We then performed simultaneous neural recordings in ALM and SC, using high-density Neuropixels probes, in task-performing animals. We found robust visual and tactile responses in ALM and SC, with a clear separation of modalities between superficial and deep SC layers (dSC). To ensure that multisensory responses were not driven by correlated movements, we used a generalized linear model that included rich behavioral information to separate stimulus-related from movement-related neural activity. Aside from sensory responses, both ALM and dSC showed strong choice-predictive activity during stimulus presentation and a subsequent delay period. Interestingly, visual and tactile choices were encoded in ALM through different neuronal populations. Moreover, neurons encoding multisensory choices were not simply a combination of these populations. In contrast, choice signals in dSC neurons were largely independent of the sensory modality. ALM thus showed modality-specific choice-tuning, possibly contributing to the transformation of unisensory information into modality-independent choices. To causally confirm these results, we performed optogenetic inactivation in each area during simultaneous Neuropixels recordings. Optogenetic inactivation of both ALM and dSC strongly reduced animals' choice performance during the stimulus and delay period and disrupted choice-related dynamics in both regions. This suggests a hierarchical transformation of multisensory information into behavioral decisions, where the SC sends multisensory information to ALM, which creates modality-specific decisions that are then returned to the SC to create motor outputs.

Cognitive flexibility training facilitates fear extinction in C57BL/6J and 129/S1 mice

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The 129/S1 mouse line is a well-established model for impaired fear learning. Compared to C57BL/6J mice, 129/S1 mice exhibit exaggerated conditioned fear responses and deficits in fear extinction, though their safety learning remains intact. However, it remains unclear whether 129/S1 mice also display abnormalities in latent inhibition of conditioned fear—the process by which a familiar, previously neutral stimulus comes to predict something fearful, often considered the inverse of fear extinction. In our first experiment, we tested latent inhibition in both 129/S1 and C57BL/6J mice. While 129/S1 mice displayed enhanced conditioned fear, their latent inhibition appeared normal. Building on this, our second experiment hypothesized that fear extinction could be viewed as a form of reversal learning, requiring cognitive flexibility. To test this, we assessed cognitive flexibility in both mouse strains using the attentional set-shifting task (ASST), which challenges animals with training phases involving reversals, intra- and extra-dimensional shifts. Following the ASST, we exposed both ASST-trained and untrained (control) mice to a combined fear and safety conditioning protocol, followed by fear extinction training. Lastly, we evaluated the recall of fear extinction and conditioned safety. Compared to C57BL/6J mice, 129/S1 mice required more trials across the ASST phases to meet performance criteria, indicating impaired cognitive flexibility. However, ASST-trained 129/S1 mice showed significantly improved fear extinction recall compared to untrained 129/S1 mice. A similar effect of ASST training was observed in C57BL/6J mice, which, overall, demonstrated better fear extinction relative to 129/S1 mice. Of note, conditioned safety was neither impaired in 129/S1 mice nor affected by ASST training. These findings suggest that cognitive flexibility training enhances fear extinction in both 129/S1 and C57BL/6J mice. Future research should explore the longevity of this effect and whether further cognitive flexibility training can amplify these benefits.

Lateral hypothalamic neuronal dynamics command behavioral transitions and coordinate different stages of feeding

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Innate behaviors such as food intake, social interaction, and novel object exploration fulfill adaptive needs in a sequential manner, suggesting an underlying brain dynamic that regulate these behaviors and transitions between them. To investigate this, we combined electrophysiological neuronal recordings in freely behaving mice with machine learning and multicolor optogenetics. We identified distinct populations of lateral hypothalamic (LH) neurons that are sequentially activated during feeding, while additional populations remain active during exploration and social interaction. LH slow gamma oscillations (30-60 Hz) promote the assembly of feeding-related neurons, whereas fast gamma oscillations (60-90 Hz) coordinate multiple behavioral populations across different innate behaviors. These findings suggest that appetitive behaviors and phases of consummatory acts are supported by distinct LH populations, organized in a temporal sequence. We further found that LH neuronal populations encode transitions between innate behaviors via peak-phase signatures during beta oscillations (15-30 Hz). Optogenetic manipulation of intrahypothalamic inhibition, particularly from the lateral preoptic area (LPO) at these phases, disrupted behavioral transitions and prolonged the duration of ongoing behaviors. These transitions are driven by beta-rhythmic inputs from the medial prefrontal cortex, which synchronize with LH "transition cells" encoding potential future behaviors. Disruptions in these oscillatory interactions may impair the LH's ability to regulate behavioral transitions, with implications for understanding eating disorders and other behavioral dysregulations. Together, our findings reveal a hypothalamic temporal dynamics that signals alternative future behaviors and coordinates their organization during individual behaviors, such as feeding, with potential impairments contributing to eating disorders.

References:
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Diverse representation of various rewards in the dopaminergic neurons of the ventral tegmental area

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The ventral tegmental area (VTA) encompassing dopaminergic (DA) neurons, is a brain region crucial for processing information about various rewards and guiding appetitive behaviours directed towards them. Importantly, how the VTA DA neurons encode different rewards and to what extent this information overlaps within different DA cells is poorly understood.

For that reason, we recorded the electrophysiological activity of VTA neurons in freely-behaving male mice using silicon probes while the animals were free to spontaneously explore different rewards. The arena contained several natural rewards including water, food, a toy, a female conspecific and a running wheel.

The activity of a majority of putative DA neurons was modulated by at least one reward. Despite the heterogeneity of responses, some patterns could be observed. For example, two subpopulations with different firing patterns were modulated by different types of rewards – one of them responded more strongly to the running wheel while the other to food. Encoding of speed in the running wheel and during free locomotion was uncorrelated, suggesting that voluntary exercise is represented differently in the VTA DA neurons than the spontaneous exploratory behaviour.

Overall, our results show that the putative VTA DA neurons code different natural rewards heterogeneously. Some encoding patterns were present as, for example, we observed that separate subpopulations of DA neurons represent food and voluntary exercise. This suggests that VTA DA neurons are able to distinguish between different rewards, including the competing ones.

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Crows recognize geometric regularity

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The perception of geometric regularity in shapes, a form of elementary Euclidean geometry, is a fundamental mathematical intuition in humans. We demonstrate this geometric understanding in an animal, the carrion crow. Crows were trained to detect a visually distinct intruder shape among six concurrent arbitrary shapes. The crows were able to immediately apply this intruder concept to quadrilaterals, identifying the one that exhibited differing geometric properties compared to the others in the set. The crows exhibited a geometric regularity effect, showing better performance with shapes featuring right angles, parallel lines, or symmetry over more irregular shapes. This performance advantage did not require learning. Our findings suggest that geometric intuitions are not specific to humans but are deeply rooted in biological evolution.

The role of dopamine receptors in semantic associations between signs and quantity categories in primate prefrontal neurons

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The prefrontal cortex, known as an important center for cognitive processing in primates, is modulated and regulated by the neuromodulator dopamine, released by neurons reaching from the midbrain into the PFC and acting via the dopamine D1 (D1R) and the D2 receptor (D2R) families. Dopamine is crucial for reinforcement learning, which is the process of learning from rewards and feedback. We therefore hypothesize that dopamine is also involved when human and nonhuman primates learn to associate a sign (e.g., an arbitrary visual shape such as an Arabic numeral) with a specific meaning, such as a numerical value of a set of dots. We trained macaque monkeys to associate visual shapes with different quantities in a delayed response task. After this extended learning period, we observed that many prefrontal neurons responded to the visual shapes in a manner that reflected the corresponding numerical values, aligning with the monkeys' behavior. To directly evaluate the effects of dopamine receptor-targeting agents, we conducted simultaneous neuronal recordings alongside micro-iontophoretic drug application. In each recording session, we alternated between control conditions without drug application and conditions in which the drug was applied, allowing for a clear comparison of neuronal responses. We found that the drug targeting D1R reduced baseline firing, whereas the D2R agonist showed an increase in the firing rate and enhanced selective tuning. Both D1R and D2R agonists changed the strength with which association neurons mapped numerical values onto Arabic numerals. These findings highlight the crucial role of prefrontal dopamine in forming semantic associations between signs and abstract categories, such as numbers. This process serves as a foundational cognitive step that may eventually contribute to the development of symbolic thinking, a hallmark of language in humans.

Linking Attentional States and Neuronal Dynamics in the Locus Coeruleus During a Decision-Making Task

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The network reset theory (Bouret & Sara, 2005) suggests that phasic firing of the locus coeruleus (LC) enables the reorganization of functional cortical networks. To test some of the assumptions of this theory, we trained animals in a head-fixed binary perceptual decision-making task standardised by the International Brain Laboratory (IBL et al., 2021). Using a previously published probabilistic model (Ashwood et al., 2022), we identified discrete latent states based on the performance of the mice – each state represents a distinct decision-making strategy. We used fiber photometry to record the physiological activity of the LC in this task, and to identify LC activity in each state. In the future, our aim is to apply phasic optogenetic stimulation to achieve a switch between latent states.

Effect of chemogenetic manipulations of the orexin system on cognitive flexibility and working memory in mice

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The orexin system is implicated in feeding behavior, the sleep/wake cycle, reward-driven behavior, as well as cognition. Altered levels of orexin may be associated with neurodegenerative and neuropsychiatric disorders such as dementia, schizophrenia, and depression, in which impairments in cognitive flexibility and working memory are often observed. Thus, the aim of this work is to examine whether chemogenetic stimulation of the orexin system improves cognitive flexibility in mice. Adeno-associated viral vectors carrying Cre-dependent stimulatory designer receptors exclusively activated by designer drugs (DREADDs) were injected in the lateral hypothalamus of orexin-Cre mice to target orexinergic neurons. After at least two weeks for recovery and DREADD expression, the attentional set-shifting task (ASST), an established measure of cognitive flexibility, was performed following intraperitoneal injections of either clozapine-N oxide or saline to test whether stimulation of the orexin neurons and thus elevated orexin levels lead to better performance across the ASST phases. The same mice also underwent the Y-maze test twice - before and after ASST testing - to assess spatial working memory. The experiments were performed under blinded conditions to the treatment groups. The project is still underway and results are pending until the final analysis can be performed.

Dissecting the multisensory dimensions of the social brain in mice

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One main function of the brain is initiating social interactions with conspecifics. Social behaviors are guided by diverse and rich sensory inputs: scent, vocalization, touch and posture contribute to social interactions in mice, a model of choice for neural circuit analysis. While the processing of social cues has been studied for individual senses, the integration of the sensory domains for social processing remains elusive, due to the difficulty to image brain-wide multisensory responses. With this work, we will present an experimental approach to identify the social sensory processing areas by presenting social and non-social olfactory, visual and auditory cues during functional ultrasound imaging in a head-fixed paradigm.

Opposite coding of competing rewards by VTA dopamine neurons

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Adequate regulation of innate behaviours is crucial for survival. The ventral tegmental area (VTA) forms the core of the neural circuitry driving these motivated behaviours. VTA dopamine (VTA-DA) neurons encode reward and reward-predicting cues heterogeneously. Moreover, individual neurons respond to different behavioural and kinematic variables. However, it remains unclear whether individual dopamine neurons respond to competing rewards like feeding and voluntary exercise heterogeneously. Additionally, it is unknown how motivational state changes, such as hunger or enhanced exercise drive, influence these responses. The aim of this study was therefore to uncover how individual VTA-DA neurons encode competing rewards, and how motivational state affects their function.

We performed 1-photon calcium imaging in DAT-cre mice expressing GCaMP6m in the VTA, while they freely explored an arena with multiple rewards, including food, water and a running wheel. To study the effects of increased drive for feeding and voluntary exercise, calcium activity of single neurons was recorded during food restriction and after repeated running wheel exposure. To assess the contribution of anatomically different subpopulations on food intake and locomotion, we recorded and optogenetically activated projection-specific VTA-DA neurons.

We found that VTA-DA neurons encode food and voluntary exercise in an opposite manner, but not food and water. Similarly, projections to different targets controlled feeding and locomotion in an opposite manner. During food restriction and enhanced exercise drive, the responses of VTA-DA neurons became tuned to the prioritized reward. These results suggest that VTA-DA neuron subpopulations distinguish between competing rewards and shift their responses according to motivational state changes.

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Whole-brain activity patterns underlying uninstructed behavioral switching in mice

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The ability to switch between different behaviors is essential to all animals' survival. Behavior selection is guided by multiple factors, such as sensory inputs, internal states, and memory, which suggests that many regions across the brain are involved in the decision to switch. While whole-brain information is necessary to investigate the neural basis of behavioral switching, brain-wide imaging in behaving mice has proved challenging. We employed functional ultrasound imaging (fUS) to record large-scale neural dynamics in head-fixed mice while simultaneously tracking their behavioral state. Our aim was to identify brain regions that predict self-initiated transitions of behavior occurring in the absence of external triggers. Accordingly, we utilized the virtual burrow assay in which head-fixed mice are placed in an air-floating tube, from which they can voluntarily egress. We found that mice (N = 11, 60 sessions) robustly exhibit distinct, uninstructed behavioral states in this assay, including egress, whisking, inactivity, and grooming. Utilizing brain-wide fUS, we subsequently observed activity patterns associated with these distinct behavioral states and performed whole-brain time-resolved decoding around behavioral transitions. Remarkably, our results revealed that whole-brain activity can predict a spontaneous egress event seconds before its onset, indicating that a change in uninstructed behavior is preceded by a detectable change in brain state. Furthermore, region-wise decoding revealed specific brain areas driving the prediction of behavioral transitions. Utilizing an optogenetic approach we find that inhibiting these regions increases the probability of being in an active state as observed before behavior transitions. Through this unbiased approach, our work sheds light on the neural dynamics preceding uninstructed transitions of behavioral state.

Social context shapes behavioral and neural dynamics of foraging and decision-making in freely moving rhesus macaques

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Foraging is a central aspect of daily life. Making ideal foraging decisions is influenced by many different factors, such as the availability and abundance of food sources but also the immediate actions and movements of conspecifics. Previous studies have shown the effects of such factors at the behavioral and neuronal level. However, the majority of our knowledge of foraging decisions in primates, especially of the neural mechanisms, come from experiments in laboratory environments which prevent full-body search behaviors. The question remains how rhesus macaques (macaca mulatta) integrate information about a conspecific's actions and intentions into their own action planning to maximize foraging success in a freely moving context.

To investigate free-moving foraging behavior and the neural decision making processes involved, we developed an experimentally controlled 'Playground Experiment' in an Exploration Room with enhanced ecological validity. In a large enclosure, freely moving monkeys foraged for food or fluid rewards from three different types of foraging sources: wall-mounted touchscreen-based kiosk systems with fluid rewards, ceiling-mounted elastic strings with grapes, and litter piles on the floor with vegetable pieces hidden underneath. To investigate how the presence of a social partner influences foraging behavior, monkeys foraged either alone (solo) or with a partner (dyadic). Neural activity was wirelessly recorded from primary motor cortex and dorsal premotor cortex.

Preliminary analyses suggest that the presence of a second monkey in this shared space has a prominent effect on the displayed foraging and movement behaviors. In a dyadic context, monkeys spent less time foraging at one food source and switched sources more often. The attractiveness and attendance at a specific food source also increased. Observations suggest that they developed preferences for different food sources across sessions, potentially to avoid conflicts in a shared space. Building on this, a change in the pattern of the walking trajectories between solo and dyadic context could also reflect altered foraging decisions (regarding when and where to forage), influenced by the presence of a potential competitive conspecific. We hypothesize that the proximity of a partner will influence and predict foraging decisions, such as the choice to leave a particular foraging station. Future analysis will investigate how different foraging parameters are represented in sensorimotor cortices, such as the availability of different foraging sources. Additionally, the presence and proximity of the second monkey may modulate neural activity, potentially influencing the decision-making process of whether to leave a foraging station or continue foraging.

Our preliminary results show that rhesus macaques changed how they forage in a dyadic context compared to being alone. The emergence of non-overlapping preference for certain food sources

suggests a possible strategy to share limited resources without a conflict, suggesting the integration of others' intentions based on their inferred preferences. Avoiding conflict by territory formation during social foraging might be less likely to surface in a classic economic game-style paradigm, afforded here by the rich yet strategically designed nature of our Playground Experiment and higher risk associated with conflict.

Neural Dynamics of Context, Cue and Rule Encoding: The Role of PV- and SOM-Interneurons in the mPFC

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The prefrontal cortex plays a pivotal role in representing and integrating various cognitive features. This includes task-relevant sensory information, decision-making processes such as action selection, and representation of spatial contexts. It has been proposed that parvalbumin-expressing (PVI) and somatostatin-expressing (SOMI) interneurons may be involved in context- and rule encoding, although the precise extent of their contribution remains unclear. To investigate this, we designed a contextual-auditory 2-alternative forced choice task paradigm. It allows us to clearly separate auditory cues, spatial contexts in the form of different task boxes and rule components. In combination with calcium imaging it helps us to understand the role of PVIs and SOMIs in encoding these task components.

The apparatus is composed of a linear tract, divided into two distinct sections: the trial initiation and the task area. To gain access to the task area, the mice are required to initiate a trial via a nosepoke port. The nosepoke triggers one of two auditory cues and opens a gate. Behind the gate, one of the two task boxes is presented, in which the mice must react by licking one of two spouts. After each trial, the gate is closed and a new task chamber and auditory cue is selected at random. The mice are trained under varying conditions, where either no cues, the auditory cue, the spatial context, or both provide information about the reward location. This specific design allows us to distinguish the neuronal representation of spatial context, sensory cues and rule encoding and allows us to mimic it in a virtual reality (VR) setup.

Mice were able to discriminate the reward location based on auditory or contextual cues, however a rule based on spatial contexts was easier for mice to learn compared to a rule based on pure tones. To shed light on the interneuron dynamics in context, tone and rule encoding, we perform calcium imaging in task performing mice using the UCLA miniscope V4. Activity of SOMIs and PVIs expressing GCaMP8m was recorded across training stages. Dimensionality reduction analysis of neural activity of fully trained mice revealed a separation of auditory and contextual stimuli. These results suggest an important role for interneurons in encoding task relevant information in the prefrontal cortex and help to understand the underlying interplay of different neuron types.

Gaze following is not grounded in the perception of implied motion

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The "gaze beam hypothesis (GBH)" of gaze following posits that the other´s eyes emit imaginary beams of moving energy travelling to the other´s object of attention (Guterstam et. al., 2020), drawing the observer´s attention to the same object. This idea was initially supported by behavioral experiments showing a motion aftereffect (MAE), indicated by longer reaction times in detecting motion direction after viewing a cartoon face looking at an object in the same direction (Guterstam et. al., 2020). However, this effect could also be expected if the observer used gaze direction to assume an intentional link between the looker and the object, envisioning directed actions toward the latter (Görner et. al., 2020). To critically compare the two hypotheses, we tested whether an MAE could be induced by having human subjects detect motion direction after viewing various cue images, designed to differentiate the explanatory power of the two. Cues either suggested a connection between an agent and an object through the agent's gaze or an object-oriented intention by the presence of equipment in the agent's hand, without the agent directly looking at the object. Using Bayesian statistics, our findings provided strong evidence against both hypotheses at the population level, as reaction time modulations did not align with the MAE, leading us to reject motion adaptation as the underlying mechanism for gaze following but also intention attribution. As on an individual level we observed highly diverse effects, with some compatible with one or the other hypothesis, we assume that individual subjects may resort to different perceptual strategies based on different scene interpretations.

Cognitive biases influence numerosity judgments in macaques and crows

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Our judgments are influenced by prior outcomes or extreme values, to which our cognitive processes tend to adjust or normalize over time. Two examples of such cognitive biases are a "regression to the mean" and a "serial effect". Regression to the mean refers to a statistical tendency for extreme values to move closer to the average of the tested range, whereas serial effects occur when previous judgments influence subsequent ones, creating a systematic bias toward similarity between successive estimates. In human estimation tasks, both regression to the mean and serial effects impact individual estimates. We hypothesized that animals might also be subject to the same cognitive biases during numerosity judgments. We trained two rhesus macaques and two carrion crows – both species known for their advanced numerical abilities – to discriminate the number of dots (numerosities) in visual displays. By presenting a wide range of target numerosities and analyzing detailed behavioral performance functions, we investigated potential deviations in performance caused by cognitive biases. We found that both macaques and crows exhibited systematic biases in their responses that had previously gone undetected. The animals demonstrated a regression to the mean effect by biasing their responses toward the numerical center of the tested range. Additionally, both species displayed a serial effect: when subjects had previously seen a large numerosity, they tended to overestimate the current numerosity, and vice versa. This strong relationship between quantity judgement of the current trial and the magnitude of the previous trials diminished over time as a function of task history. Our results indicate that cognitive biases in magnitude estimations, previously demonstrated in humans, extend to numerosity judgments in nonhuman primates and corvid songbirds – two species with entirely different endbrain structures. The underlying biasing processes resemble active inference or predictive coding mechanisms, which may offer an efficient solution to the common computational challenge posed by sensory and cognitive noise. Simulating such behavior with a Bayesian model that incorporates task history as a prior may explain the observed signatures of both effects. An integrative approach that combines internal representations with context-dependent predictions could also help resolve the long-standing debate in numerical cognition regarding the scaling scheme of internal numerosity representations.

Poster Topic

T25: Learning and Memory

T25-1A Dopamine modulates the excitabilty of dopaminergic neurons involved in feeding in *Drosophila*.
Michael-Marcel Heim, David Owald

T25-2A The role of recurrent long- and short-range connections in experience-dependent modulation in *Drosophila*
Sayantani Biswas, Julio Antonio Otarola-Jimenez, Bill S. Hansson, Markus Knaden, Silke Sachse

T25-3A Memory induction in drosophila using a virtual olfactory arena
Sridhar rajan Jagannathan, Tania Fernandez d.V. Alquicira, David Owald

T25-4A Maturation of decision making across adolescence in mice
Amelie Hagelüken, Anne Günther, Johanna K. Kostka, Ileana L. Hanganu-Opatz

T25-5A Memory Patterns across Synaptic Boutons: Compartmentalized Dopamine Effects along the Mushroom Body Gamma Lobe

Philip Baxter Aßmann, Ibrahim Alperen Tunc, Martin Paul Nawrot

T25-6A Determinants of trace- delay- and relief conditioning in fruit flies
Edanur Sen, Christian König, Thomas Niewalda, Fatima Amin, Sevval Demirci, Melissa Comstock, Bertram Gerber

T25-7A Open plasticity window during memory consolidation in *Drosophila melanogaster*
Tania Fernandez del Valle Alquicira, Lisa Schuenemann, Desiree Laber, Marine Balcou, David Owald

T25-8A Cortex and hippocampus differentially contribute to spatial coding in subiculum
Oliver Barnstedt, Dennis Dalügge, Hiroshi Kaneko, Liudmila Sosulina, Silvia Vieweg, Kimia Farghadayn, Stefan Remy

T25-9A Dissection of neuronal circuits underlying olfactory sensitization – The role of a neuronal circuit in the mushroom body calyx
Lisa Epple, Hannah Mariedele Luksch, André Fiala

T25-1B Effects of social experience on neural function in *Drosophila*
Frederic Alexander Römschied

T25-2B Differential effects of starvation on different forms of short-term memory in Drosophila melanogaster

Juliane Thoener, Svea Königsmann, Thomas Niewalda, Sevval Demirci, Latafat Guliyeva, Fatima Amin, Isabel Walther, Mozhdeh Besharatifar, Bertram Gerber, Christian König

T25-3B Contribution of the neural network to the consolidation of generalized motor content during sleep
Nesa Ahmadi, Farzin Kamari, Olga Garaschuk, Lisa Marshall

T25-4B Cell-type specific actions of Nogo-A in controlling spatial memory formation by modulating neuronal excitability
Jan Flechtner, Martin Korte, Marta Zagrebelsky

T25-5B Exploring structural and functional properties of the lizards' cortical regions.
Niels Röhrdanz, Ceylan-Scarlett Steinecke, Anil Menon, Kira Balueva, Elke Edelmann, Peer Wulff

T25-6B Integration of Information in the Absence of Action in Drosophila
Johanna Aurelia Schweizer, Johannes Felsenberg

T25-7B Neuronal Modulation by Latent Inhibition in Antennal Lobe and Mushroom Body Output
Cansu Arican, Martin Strube-Bloss, Brian H Smith, Martin Paul Nawrot

T25-8B Modulation of contextual fear memory circuits by acute phase delay
Lara Mariel Chirich Barreira, Hannah Gapp, Julia Henschke, Janelle M. P. Pakan, Anne Albrecht

T25-1C The role of epigenetic mechanisms for body size memory in *Drosophila melanogaster*
Natalie von Hattingberg, Burkhard Poeck, Roland Strauss

T25-2C Unraveling the role of sleep in vocal learning
Artemis Gkinakou, Hamed Yeganegi, Janie M. Ondracek

T25-3C Brain-wide networks for category learning in the mouse
Selina Majaj, Sandra Reinert, José-Maria Martinez de Paz, Mark Hübener, Pieter M. Goltstein, Emilie Macé, Tobias Bonhoeffer

T25-4C Pattern completion of contextual fear memory: Modulation by hippocampal somatostatin-positive interneurons
Gina Marie Krause, Oliver Stork, Anne Albrecht

T25-5C Behavioral algorithms underlying flexible decision-making
Ashrit Mangalwedhekar, Sydney Hunt, Armin Bahl

T25-6C Unlocking visual pathways: Enhanced visual learning through olfactory deprivation in *drosophila*
Büsra Çoban, Johannes Felsenberg

T25-7C Olfactory-visual integration in input and output regions of the mushroom bodies in the honeybee, *Apis mellifera*
Andrea Rafaela Nicolaidou, Claudia Groh, Martin Strube-Bloss, Keram Pfeiffer, Wolfgang Rössler

T25-8C Action, valence, dopamine- Drosophila as a study case
Fatima Amin, Oliver Barnstedt, Salil Bidaye, Ilona C. Grunwald Kadow, Marcel Heim, Christian König, Ashok Litwin-Kumar, Utsab Majumder, Nino Mancini, Kazuma Murakami, David Owald, Anna Pierzchlinska, Jasmine T. Stone, Bertram Gerber

T25-9C Oppositional and competitive instigation of hippocampal synaptic plasticity by the VTA and locus coeruleus
Hardy Hagena, Denise Manahan-Vaughan

T25-1D Optogenetic Control of Mitochondria in PV+ Interneurons Alters CA1 Function
Rina Patel, Matthias Haberl, Silvia Viana Da Silva

T25-2D Nogo-A regulates fear memory processes and memory engram formation by modulating neuronal excitability in a sex-specific manner
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T25-7D Acute circadian rhythm disturbance impairs contextual-memory engrams in the dentate gyrus
Harini Srinivasan, Anne Albrecht, Oliver Stork

T25-8D Changes in neural representation of social conspecifics in response to reward learning
Lars-Lennart Oettl, Cristina Mazuski, Chenyue Ren, John O`Keefe

Dopamine modulates the excitabilty of dopaminergic neurons involved in feeding in *Drosophila*.

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Activity of dopaminergic neurons in higher animals, invertebrates, and vertebrates alike, is critical for associative learning and motivation. In *Drosophila*, a large subset of dopaminergic neurons project into the mushroom bodies. This higher order brain center is essential for olfactory learning and memory. Modulation of the mushroom bodies' circuitry has been intensely studied in the context of learning. However, it is still unclear whether dopaminergic modulation of dopaminergic neurons affects mushroom body function. To address this, we performed focal dopamine injections onto dopaminergic neurons of the mushroom bodies. Using the genetically encoded voltage indicator ArcLight, we demonstrate that applying dopamine dendritically hyperpolarizes dopaminergic neurons. Moreover, we provide evidence that this signaling cascade is mediated by the receptor Dop2R and that its absence elevates food seeking. Our results are reminiscent of mammalian Dop2R auto-receptor signaling, indicating an evolutionarily conserved role for Dop2R signaling in learning and reward driven behavior.

The role of recurrent long- and short-range connections in experience-dependent modulation in *Drosophila*

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Odor-driven innate behaviors dictate an animal's survival and reproduction. Life decisions in *Drosophila melanogaster*, such as mating, oviposition choice and parasitoid avoidance are also triggered by specific olfactory cues that activate hard-wired neuronal circuits leading to stereotyped behaviors. The hedonic valence and intensity of such odors are known to be encoded in the lateral horn of the fly brain. However, depending on new experiences, physiological state and context, the representation of stimuli can be overwritten to assign new values, allowing the fly to adapt to changing environments. In a novel ecologically relevant oviposition assay, we show that mated female flies innately prefer parsnip food for oviposition, but change their preference to apple food after oviposition training, e.g. after having oviposited on the apple food (Otarola-Jimenez et al. 2024). Also, when these flies are tested in a Y-maze assay, apple-trained flies choose the apple odor over the parsnip odor. Results from these behavioral experiments demonstrate that even innate olfactory behaviors are modulated by prior experience. We propose that this modulation occurs in primary and secondary brain centers mediated by recurrent connections between these olfactory neuropils (i.e. antennal lobe - lateral horn - mushroom body). Using the recently established oviposition learning as a behaviorally relevant learning paradigm, we are currently monitoring the modulation of second- and higher-order neurons via two-photon functional imaging. Furthermore, based on the published Drosophila brain connectome data, we are analyzing the role of candidate centrifugal and modulatory neurons for mediating this modulation through recurrent connections within and between olfactory neuropils.

Memory induction in drosophila using a virtual olfactory arena

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Here we introduce a virtual olfactory arena set up, wherein a fly walking on an air supported ball setup is inducted with memory by activating different dopaminergic neurons (DANs). We use different DANs while pairing it with different odours thereby producing attractive and aversive associative learning using a closed loop feedback system. The performance index of individual flies are quantified by velocity of walking, turning direction and location preference of the fly. We further show immediate/longer-term memory performance across
multiple genotypes while activating different DANs.

Maturation of decision making across adolescence in mice

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Cognitive flexibility is the ability to rapidly adjust behavior in response to environmental changes and results from the convergence of multiple processes, such as goal-directed behavior, working memory, and decision making. It is obvious that such processes involve a neural circuitry that extends over much of the brain, yet it is commonly held that the prefrontal cortex (PFC) is a critical hub for cognitive flexibility. Flexible adaptation to new situational task emerges towards to the end of the development and have been linked to the functional maturation of the PFC. However, the developmental trajectories of distinct behaviors leading to cognitive flexibility is still poorly understood.

Here, we assess the maturational dynamics of decision-making in mice that perform a four-choice odor discrimination and reversal task from postnatal day (P) 22 to 60.

We show that pre-juvenile mice (P22-23) show a tendency of requiring more trials. Similarly, trial latency in pre-juvenile mice showed a tendency towards an increased trial duration compared to the older groups (Juvenile P30-31; Adolescent P38-39). Whereas in contrast, juvenile mice (P30-31) trend towards requiring fewer trials and reduced trial duration during the reversal phase compared to younger (Pre-juvenile P22-23) and older (Adolescent P38-39) mice.

The results reveal the developmental dynamics of decision-making in mice and identifies time windows of particular relevance for distinct task phases.

Memory Patterns across Synaptic Boutons: Compartmentalized Dopamine Effects along the Mushroom Body Gamma Lobe

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The insect mushroom body (MB) is a widely studied model system for memory computation. High-resolution 2-photon calcium imaging experiments in the MB gamma lobe have revealed a distributed synaptic memory encoding at the cholinergic Kenyon cell (KC) – mushroom body output neuron (MBON) synapse (Bilz et al., 2020, Stahl et al., 2022). During olfactory conditioning, calcium response in KC boutons changes in a compartment-specific manner, suggesting the presence of independent modulatory mechanisms.

In a computational neural network model, we investigate fundamental synaptic, cellular and circuit mechanisms that we hypothesize to underlie the observed phenomenon. To this end, we model KC adaptive firing rate response dynamics (Rapp & Nawrot, 2020) to odor stimuli and the related calcium responses at KC axon boutons across multiple compartments. In addition, we consider lateral muscarinic G protein-coupled rceptor (GPCR) mediated inhibition among KC axons (Manoim et al., 2022). Memory formation is facilitated by dopamine-dependent heterosynaptic plasticity at the KC-MBON synapse that leads to a differential bouton-specific reduction or increase in calcium influx reflecting presynaptic strength at the KC-MBON synapse.

Our results resemble key experimental findings (Bilz et al., 2020) such as the learning-induced decorrelation of calcium responses across the KC bouton matrix in the gamma lobe and allow for novel experimental predictions.

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Determinants of trace- delay- and relief conditioning in fruit flies

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Receiving punishment feels bad, but relief upon its termination feels good. These experiences result in aversive and appetitive learning of cues respectively associated with the occurrence versus the termination of punishment. Our work has established such timing-dependent valence-reversal as an across-species principle (Gerber et al. 2019). From a clinical perspective, considering distortions of timing-dependent valence reversal may offer new views of pathological behaviors. For example, distortions in favor of relief processing may promote self-cutting to bring about relief, or may establish maladaptively strong ties to places where fear or panic subside.

We report on our ongoing experiments, in Drosophila melanogaster as a study case, to uncover determinants of timing-dependent valence reversal. Our focus is on the contribution of biogenic amine systems and on the role of the cAMP and PKC cascades in this respect. Also, we probe for determinants of memories from procedures with versus procedures without a time gap between the presentation of predictive cues and punishment (‘trace’ versus ‘delay’ conditioning, respectively).

Open plasticity window during memory consolidation in *Drosophila melanogaster*

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Memory allows the storage and retrieval of acquired information. Memories are encoded as specific cellular changes, product of the stabilisation of synaptic plasticity after learning.
The location of olfactory memories in Drosophila melanogaster is well known, and the availability of genetic tools permits the dissection of the circuit underpinnings associated with different stages of memory, e.g. during consolidation, storage and retrieval.
In Drosophila, the mushroom bodies (MBs) are involved in storing different elements of reward and punishment associative memories. Our work now shows that blocking synaptic output of M4/6 MB output neurons interferes with memory consolidation, reverting aversive to appetitive memories. We show that this memory is distinct from appetitive sugar memories as it is not gated by the animal's hunger state. Moreover, in vivo imaging reveals that aversive training triggers ongoing activity in M4/6 MB output neurons, indicating post training network activity. Thus, post training interventions of single neuron output can reprogram circuit activity and change memory outcome

Cortex and hippocampus differentially contribute to spatial coding in subiculum

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To find food or shelter, animals need to spatially navigate through their environment. One of the main brain structures responsible for this is the hippocampal formation. Neuronal firing patterns in this brain region are strongly correlated to various aspects of the animal's location and its navigation behaviour. The subiculum is the primary output structure of hippocampal information processing and provides output to various cortical and subcortical areas. It is hypothesised that its main role lies in the integration, compression, and distribution of hippocampally processed information to the wider brain. Two major inputs to the subiculum arise from the hippocampus' CA1 region and parahippocampal entorhinal cortex. How these two input streams are processed within the subiculum and give rise to subicular spatial coding properties remains largely unknown.

Here, we have used in vivo whole-cell patch clamp recordings in mice running freely on a circular track to show that dorsal subicular neurons receive spatially tuned input. Channelrhodopsin-assisted circuit mapping demonstrates that the two major input streams target specific regions in the dendritic tree of dorsal subicular neurons. Specifically, CA1 input is located more proximally, while EC input forms synapses in the distal part of the dendritic tree of dorsal subicular neurons. Finally, individual contributions of both input streams on the spatial tuning of dorsal subicular neurons were investigated using two-photon calcium imaging in mice running on a linear treadmill. Simultaneous chemogenetic inactivation of either CA1 or entorhinal cortex inputs via viral transduction of the inhibitory DREADD and local application of CNO by micro-infusion through the imaging window reveals district contributions of both input paths: CA1 inputs are necessary for place and velocity tuning, while EC inputs are only necessary for place tuning of dorsal subicular neurons.

Taken together, our data reveal that (1) subicular neurons receive spatial and velocity tuned input (2), that subicular neurons maintain a functional input segregation between CA1 and entorhinal cortex synapses and (3) that both input streams play differential roles in shaping the spatial tuning of subicular neurons with respect to place and movement speed. We thus demonstrate the input-output transformation of a key hippocampal output node.

Dissection of neuronal circuits underlying olfactory sensitization – The role of a neuronal circuit in the mushroom body calyx

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Olfactory sensitization is a form of non-associated learning characterized by a nonspecific enhancement of a response towards an odor stimulus. In previous studies in honeybees, the depolarization of a particular octopaminergic neuron (VUMmx1) has been shown to mimic the effects of sugar reinforcement during associative olfactory learning, highlighting this neuron's role in food-induced arousal and reward. In *Drosophila melanogaster*, an analogous neuron - known as OA-VUMa2 - has a similar pattern of innervation as the VUMmx1 neuron in honeybees, innervating the calyces of the mushroom bodies as well as the antennal lobes and the lateral horns. Despite these anatomical similarities, the functional role of the OA-VUMa2 neuron in *Drosophila* remains unknown. To investigate whether OA-VUMa2 and potentially other calyx-innervating octopaminergic neurons, as well as calyx-innervating mushroom body output neurons, contribute to food-induced arousal in *Drosophila*, we established a sensitization paradigm modeled after previous studies in honeybees. Using the light-activated channel CsChrimson, we selectively activated the neurons of interest. Fifteen seconds after optogenetically induced neuronal activation, an odor stimulus was presented and the proboscis extension response (PER) towards the odor was measured as an indicator of arousal. The results revealed that activating the OA-VUMa2 neuron or the mushroom body output neuron MB-CP1 significantly increased the PER in response to the odor. The findings suggest an involvement of OA-VUMa2 and MB-CP1 neurons in the neural circuits underlying food-induced olfactory arousal in *Drosophila*.

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Effects of social experience on neural function in *Drosophila*

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Learning from social feedback is crucial for success in a social world. Conversely, lacking such social flexibility can lead to social isolation and strong impairments to an individual's quality of life. Therefore, understanding the neural basis for social flexibility is key to understanding how circuit malfunctions lead to social disorders. Gaining such understanding is challenging, since it requires high-resolution measurements of social interactions and characterization of neural function, the combination of which is not available in most systems.

We therefore focus on the social flexibility of the vinegar fly, *Drosophila melanogaster*, which exhibits highly quantifiable social behavior and allows for optogenetic neural interrogation in unrestrained and freely behaving animals [1]. Prior work has established that past social experience influences the behavioral strategies of male flies. Specifically, experiencing ongoing sexual rejection from female flies leads to subsequent suppression of male courtship even towards receptive females. How this and other types of social experience influence the function of individual neurons to facilitate social flexibility remains elusive.

To test how different types of past social experience shape the function of individual neurons along the neural circuitry controlling social behavior in male *Drosophila*, we combine real-time pose estimation, closed-loop optogenetic behavioral manipulation and neural interrogation, and unbiased behavioral quantification. We first let individual males experience one of several possible 'alternate social realities', in which females provide different types of social feedback to the male. We then quantify the function of targeted neurons in the male, using a combination of optogenetic neural characterization with stochastic stimuli and unbiased behavioral classification [2]. We then investigate how this behavioral readout of neural function differs between males that experienced different social realities in the past, with a female that was either a) receptive, b) rejecting, or c) walking backwards every time the male extends a wing to produce courtship song. To facilitate c), we use optogenetic activation of Moonwalker Descending Neurons [3] in the female, triggered on male wing extension in closed loop. To ensure specific stimulation of the female and to prevent unintended optogenetic stimulation of the male during this experience phase, we track location and identity of the male and female in real time [4] and use this information to guide a laser onto the female, generalizing a previous approach for rapid neural manipulation in freely behaving *Drosophila*[5] to socially interacting animals.

Together, this novel approach can reveal how social experience shapes the function of individual neurons to enable learning from social feedback.

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Differential effects of starvation on different forms of short-term memory in Drosophila melanogaster

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Humans and animals must constantly adapt their behaviour to the stimuli of a complex and changing environment. However, behaviour is not only shaped by external circumstances, but also by internal states. Indeed, internal states such as fear, arousal or hunger can profoundly influence perception, cognition and action and thus shape a variety of animal behaviours. Using Drosophila melanogaster as a study case, we investigate how ‘being unwell’ in terms of hunger - induced by starvation - affects associative short-term memory. Testing two different developmental stages of Drosophila, different appetitive and aversive USs (‘real world’ or artificial), single or multiple trials and mapping out multiple time intervals we found no effects on aversive memories. In the appetitive domain, however, our results suggest a remarkably complex picture on increased, decreased or unchanged levels of short-term memory across the employed tasks. These results suggest that the effects of ‘being unwell’ in terms of hunger on associative short-term memory involve complex underlying mechanisms in Drosophila.

Contribution of the neural network to the consolidation of generalized motor content during sleep

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The brain is not in an idle state during sleep but rather continues to process information encoded during prior wakefulness. Memory formation consists of encoding, consolidation, and recall. It is assumed that sleep-associated memory consolidation benefits particularly the abstraction of “gist” information for its storage in long-term memory [1]. Few studies have investigated the gist abstraction of motor memory in animal models [2], and the time course of brain inter-regional communication across successive days of learning [3]. A hippocampus-dependent Complex Wheel task, which requires mice to learn a general strategy is employed [4].
Our aim is to verify the sleep-dependence of a complex motor learning task and disclose shifts in activity between brain regions such as motor cortex and hippocampus across days of motor learning on the Complex Wheel, in which the distances between rungs of the wheel are irregularly spaced.
For this purpose, performance on the Complex Wheel of head-fixed mice (C57BL/6J; 3-6 months, male) and subsequent sleep are recorded on Days 1-4 and 10-11 of an 11-day experimental period. During the task and the subsequent 6-h in the sleep box activity in the different brain regions (such as motor cortex and dorsal hippocampus) is recorded. To assess behavioral performance, both limb trajectory length and speed are tracked by cameras, and movements are subsequently quantified using DeepLabCut (version 2.3.9) and DLC2Kinematics (version 0.0.7) to provide motor learning metrics. Local field potentials and electromyography are recorded with a Neuralynx system.
Preliminary results demonstrate that learning was associated with a gradual minimization of fore and hind limb speeds from Day 1 to Day 3 (here and below Wilcoxon signed-rank test with Bonferroni correction, $n = 5$ mice, 10 trials per day; $p = 0.021$, $p = 0.011$, respectively). Already, from the last trial on Day 1 to the first trial on Day 2, with an intermittent sleep period, limb speed had decreased significantly ($p = 0.018$), indicating offline learning. During the primary 4-day learning period the limb speed saturated at a minimum, reflecting an optimized trajectory length (mean ± SEM = 72324 ± 11343 traveled pixels in the 40th trial). Recent memory tested on Day 10, after a 5-day interval of no training, did not differ significantly from prior performance using the same irregular rung pattern ($p > 0.98$). Similarly, using a novel rung pattern on Day 11, performance did not differ as compared to Day 4 ($n = 4$ mice; $p > 0.99$).
Results on the influence of sleep vs. sleep deprivation on task performance and associated electrophysiological activity in a larger sample will be presented.

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Cell-type specific actions of Nogo-A in controlling spatial memory formation by modulating neuronal excitability

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Learning and memory processes activate specific ensembles of neurons distributed across different brain regions, the so-called engram, and induce lasting physical and chemical changes in them. The allocation of neurons to an engram depends on their relative excitability. Therefore, mechanisms regulating the balance between excitation (E) and inhibition (I) in the brain influence memory formation. Nogo-A negatively regulates neuronal plasticity and influences the E/I balance by promoting inhibitory while suppressing excitatory synaptic transmission. Moreover, the loss of function of Nogo-A leads to improvements in spatial learning in the Morris water maze. This suggests that Nogo-A may influence learning and memory acquisition by modulating neuronal excitability and thus, the allocation of neurons to the engram. Nogo-A is expressed by excitatory and Parvalbumin-positive (PV+) inhibitory neurons throughout the hippocampus, but especially in the CA3 region, which is crucial for the acquisition of episodic spatial memory. This work aims to elucidate the cell type-specific effects of Nogo-A signalling on E/I balance in the hippocampus and spatial learning. Conditional knockout mice lacking Nogo-A in either excitatory or inhibitory PV+ neurons were used to generate organotypic hippocampal cultures (OHCs) or trained in the Morris water maze. Patch-clamp recordings, simultaneously measuring mEPSCs and mIPSCs from CA3 pyramidal neurons, revealed no significant differences in the E/I balance between the groups. Furthermore, escape latency and the use of hippocampus-dependent search strategies were not altered. Consistent with these results, there was no increase in c-Fos expression in CA3 and CA1 neurons. Current experiments are investigating whether the lack of cell type-specific effects on learning might be due to compensatory effects during brain maturation. Nogo-A expression will be deleted in adult mice locally in CA3 and CA1 by stereotactic AAV injection, and after recovery these mice will perform the Morris water maze to assess their spatial learning.

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Exploring structural and functional properties of the lizards' cortical regions.

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The lizard *Anolis carolinensis* is an interesting model organism in cognitive neuroscience, offering a unique perspective with its evolutionary early three-layered hippocampal homologue. Behavioral studies show the lizards' ability to perform hippocampus-dependent cognitive tasks similar to mammals. However, it is unknown whether the functional organization in reptiles is the same as that of mammals.
We combined different approaches, including behavioral experiments, gene expression analysis and patch clamp recordings to investigate the hippocampal homologue in *Anolis carolinensis*.
To reveal hippocampal substructures in Anolis carolinensis, we analyzed the expression of genes known to be specifically expressed in the murine hippocampal formation and its subregions. We find a homologous region to the dentate gyrus in the lizard's medial cortex and homologous regions to the cornu ammonis region in the lizard's dorsomedial to the dorsal cortex.
We investigated the participation of hippocampal substructures in processing spatial input using immediate early gene imaging in *Anolis carolinensis*. This method allows us to visualize the activation of genes that are rapidly and transiently induced in response to a stimulus. After exposure to a novel context, we find an increase in active cells, especially in the medial cortex. Preliminary patch clamp data in acutely isolated brain slices of Anolis carolinensis reveal several differences in action potential properties (e.g., amplitude or frequency), passive cell properties and synaptic signaling compared to murine hippocampal slices, which will be currently investigated for the different regions of the lizard's hippocampal homologue and compared to existing data of the murine model.
Our data support the presence of an early hippocampal structure in the cortex of *Anolis carolinensis*. This structure shares functional principles with the mammalian hippocampus. Further research combining different behavioral paradigms with immediate early imaging, as well as electrophysiology will help us to further probe for functional homology and identify hippocampal sub-circuits in the lizard.
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Integration of Information in the Absence of Action in Drosophila

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In dynamic environments learned information must be continuously revised to predict the most feasible outcome of a situation and initiate appropriate behavioral responses. Associations that turn out to be unreliable must be updated by new learning. Thus, learning from repeated non-reinforced re-exposure to cues that elicit inadequate fear or favor drug-related relapse provides an opportunity to alleviate the consequences of maladaptive memories. Extinction based therapy has the potential to weaken associations between cues and reinforcement thereby helping to prevent relapse and promoting new learning. However, whether such extinction learning requires the expression of learned responses is not known. Here we provide evidence that extinction of reward memories does not require conditioned food seeking behavior. Satiated flies (*Drosophila melanogaster*) do not express learned approach behavior to a sugar predicting odor. However, despite the absence of food seeking behavior, satiated flies learn about the omission of the predicted sugar and adapt their behavior accordingly. This learning in the absence of seeking behavior depends on peripheral sensing of the sweet taste of the food. However, interference of novel stimuli can perturb extinction learning in satiated but not hungry flies. Thus, although extinction learning can take place in the absence of behavioral expression of the memory, it seems to be more sensitive to contextual information. Understanding how extinction learning does or does not depend on behavioral responses will be essential to improve its application in therapeutic approaches. Furthermore, the decoupling of computation of information from action might allow to understand cognitive operations.

Neuronal Modulation by Latent Inhibition in Antennal Lobe and Mushroom Body Output

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The familiarity of sensory stimuli strongly influences animal behavior and cognitive processing. Observations in honeybees demonstrate that the differentiation between familiar and novel olfactory stimuli significantly affects learning performance. On a neurophysiological level, it is established that olfactory projection neurons undergo modulation during the process of familiarizing with an odor. However, an unexplored area for investigation involves the potential modulatory effects on higher-order brain areas, particularly at the mushroom body output, known as the center for learning and memory.
In this study, we investigate potential neuronal modulation in the antennal lobe and mushroom body output pathways following both associative and non-associative learning paradigms. Utilizing extracellular single-unit recordings, we examine how these distinct forms of learning influence the activity patterns within the output regions of the antennal lobe and the mushroom body in honeybees.

Modulation of contextual fear memory circuits by acute phase delay

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Circadian disturbances may lead to memory deficits and behavioural changes that are associated with a disrupted prefrontal cortex (PFC) to hippocampus (HIP) information flow in humans and in rodent models. In the present study, we demonstrate that an acute six-hour phase delay as a model of 'jet-lag' in mice resulted in a reduced contextual fear memory when applied prior retrieval. This impairment was accompanied by an increased c-Fos activation of the hilus in the dentate gyrus (DG) and in the supramammillary nucleus (SuM), relay station connecting the PFC and the DG. The overactivation of SuM and DG by acute phase delay was the mimicked by a chemogenetic activation of the SuM or the DG before contextual fear memory retrieval. Overactivation of these regions reduced freezing levels as well, indicating a direct role for these regions in modulating context fear memory under phase delay conditions.

Interestingly, phase delay also over-activated orexinergic neurons in the lateral hypothalamus. Since the wake-promoting neuropeptide orexin can regulate memory and fear behavior, a potential orexinergic modulation of the PFC-SuM-DG pathway was determined. To this end, the expression of orexin receptor 1 (OXR1) was characterized for subpopulations of SuM and DG neurons. Applying a dual tracing technique by anterograde tracing in the PFC together with retrograde tracing in the DG, we identified SuM relay neurons for information transfer from mPFC to DG. Immunohistochemical staining for OXR1 in slices from dual traced mice confirmed that 1/3 of the relay neurons in the SuM express OXR1. Within the DG, expression of OXR1 mRNA was characterized in different populations of hilar interneurons by RNAscope. Here, a major portion OXR1 mRNA-positive hilar interneurons also co-express Calb2 and Drd2 mRNA, both markers for mossy cells. In order to identify key areas for this significant memory lost we simulated the activation previously observed under phase delay.

To begin to investigate the role of an overactivation of orexinergic neurons during acute phase delay, orexin brain levels were externally increased by intranasal orexin administration before retrieval. This resulted in a variable freezing response, including mice with reduced freezing levels but also individuals with higher freezing than control mice treated with saline. Immunostainings with cFos revealed a lower activation of orexinergic neurons in high freezing mice, suggesting that the impact of external intranasal orexin application may dependent on internal states of the orexinergic neurons and interconnected areas. The freezing levels were further negatively correlated with the activation of Drd2-Calb2 neurons in the hilus and neurons in the SuM, as indicated by cFos co-labelling.

Altogether, our results reveal that an acute jet-lag applied during late consolidation stages elicits fear memory deficits, which are closely associated with a disrupted information flow between the PFC-SuM-DG pathway. This pathway is potentially sensitive to an orexinergic modulation via OXR1 targeting different subpopulations of neurons. Our study offers new insights for future therapeutic approaches for cognitive deficits induced by acute deregulations of the light-dark rhythm and potentially other stressors.

The role of epigenetic mechanisms for body size memory in *Drosophila melanogaster*

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Knowledge of its own body size is crucial for precise distance estimation to reach out for objects and also essential for navigating complex environments. While humans continuously update this knowledge about their body size and body reach throughout their lives, the body size of *Drosophila melanogaster* imagines is once learned after metamorphosis and stored as a remote memory. To investigate this potentially for a lifetime lasting memory, flies were raised in constant darkness and tested later in a gap-crossing paradigm, in which they faced a clearly insurmountable gap. The dark-reared, thus naïve flies tried to cross this gap that exceeded their body reach by far. In contrast, flies raised in light/dark cycles in a structured environment, thus experiencing parallax motion, made significantly fewer crossing attempts. These results indicated that integrating information about number of steps taken while receiving discrete amounts of parallax motion is used to learn body size and reach. Rescue experiments showed that the activity of the adenylyl cyclase encoded by the *rutabaga* gene is essential for body-size memory formation. Additionally, the transcriptional regulator cAMP response element-binding protein (dCREB2) is required for both the formation and maintenance of this memory. Unlike the well-studied olfactory long-term memory in *Drosophila*, body-size memory requires continuous activity the of dCREB2 transcriptional activator. Moreover, the requirement for cAMP/dCREB2 signaling was localized in the so-called Δ7-neurons, which provide intrinsic connectivity within the protocerebral bridge, one of four neuropiles comprising the central complex in the flies brain. Unlike the well-studied olfactory long-term memory encoded in the mushroom body of *Drosophila*, body-size memory requires continuous dCREB2 activity in the Δ7-neurons (Krause et al., 2019). Furthermore, it is hypothesized that the maintenance of body-size memory is sustained through epigenetic mechanisms. An RNA-interference (RNAi) mediated gene-specific knock-down screen in the Δ7-neurons identified e.g. a histone acetyltransferase, a histone methyltransferase and chromatin remodeling complexes that may be involved body size learning, memory consolidation and/or memory maintenance. Future studies will focus on the identification of further epigenetic factors and their continuous requirement in long-term memory mechanisms.

Krause, Tammo; Spindler, Laura; Poeck, Burkhard and Strauss, Roland (2019): *Drosophila* Acquires a Long-Lasting Body-Size Memory from visual Feedback. In: Current Biology 29 (11), 1833-1841.e3. DOI: 10.1016/j.cub.2019.04.037.

Unraveling the role of sleep in vocal learning

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As a juvenile bird learns to sing, it must undergo a complex memory task that involves the formation of auditory memories, sequences of motor output, and associative higher-order representations of learned vocalizations (Margoliash & Schmidt, 2010). As soon as a juvenile bird is exposed to the song of another male bird (usually its father, the “tutor”), the young bird begins to imitate aspects of those songs in squeaky and noisy subsongs, which are often compared to the babbling of human babies (Aronov et al., 2008; Brainard & Doupe, 2000). Through a process of auditory feedback and motor learning, juvenile subsongs transition from acoustically simple songs to complex and stereotypical adult songs in a process known as crystallization.

How do neural circuits change to incorporate these newly learned events? Numerous studies in mammals have shown that offline periods like sleep might provide a ideal state to facilitate the reactivation and consolidation of recent events in the absence of new sensory input (Maquet, 2001).
Could sleep serve a similar function during vocal learning in songbirds? Indeed, compelling behavioral (Brawn et al., 2013; Derégnaucourt et al., 2005), electrophysiological (Dave & Margoliash, 2000; Elmaleh et al., 2021; Rauske et al., 2010; Shank & Margoliash, 2009), and molecular (Phan et al., 2006) evidence indicates that sleep is crucially involved in vocal learning.

In this work, we investigated brain activity during natural sleep and singing behavior as juvenile birds transitioned from variable subsong to crystallized songs. We used advanced clustering algorithms to track the spectral features of song syllables over the course of learning. We found that song syllables that changed substantially over the course of learning deteriorated overnight during sleep and were subsequently practiced and improved over the next days of singing. In contrast, syllables that did not change substantially over the course of learning were improved and consolidated overnight. We found that this vocal behavior was correlated with changes in the durations of electrophysiological sleep states during the night. Overall, our results provide the first mechanistic insight into the interplay between sleep and learning in songbirds.

Brain-wide networks for category learning in the mouse

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Learning to group objects and experiences into categories is an important cognitive ability that helps us to efficiently respond in complex and unfamiliar situations. Multiple regions in the neocortex, hippocampus and subcortex have been identified to underlie category learning in many species. However, most of these studies have focused on one brain region at a time or the interaction between two regions. Thus, there is a need to investigate the spatial and temporal interactions of brain regions involved in category learning in an unbiased manner.

Here we present an approach utilizing functional ultrasound imaging (fUSI) to record brain-wide dynamics while mice are engaged in a category learning task. The principle of fUSI is to detect cerebral blood volume changes induced by neuronal activity. We used this method over a period of several months during which mice learned to discriminate visual categories in a head-fixed 2- alternative forced choice (2-AFC) paradigm.

Our initial data show that we achieved sufficient spatiotemporal resolution to examine brain-wide activity during task trials across different stages of category learning. We identify distinct sensory and motor neural correlates representing instructed task variables (visual categories, licks, reward and running). Additionally, our data show brain -wide dynamics with different temporal and functional profiles in mice that learned or failed to learn the task. Using this approach, we aim to characterize the learning related recruitment of specific brain regions that constitute functional networks that process and generalize visual categories.

Pattern completion of contextual fear memory: Modulation by hippocampal somatostatin-positive interneurons

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Pattern completion is an important function of the hippocampal formation allowing for the generalization of memories in response to incomplete sensory inputs. Pattern completion of contextual information during fear conditioning involves the mossy fiber system that connects the dentate gyrus (DG)-and the cornu ammonis (CA) 3 regions. Within the DG the salience of hippocampus-dependent contextual fear memories is controlled by local somatostatin (SST)-positive interneurons that regulate the excitability of DG granule cells. Recent evidence suggests that neuronal excitability and plasticity is also dependent on autophagy, a cellular process shaping proteostasis and contributing to the uptake and recycling of proteins and receptors in response to neuronal activity.
In the current study, we investigate the contribution of SST-positive interneurons to contextual fear memory specificity via pattern completion and describe the associated changes in autophagy levels. SST-positive interneurons were chemogenetically inactivated in the dorsal DG during re-exposure to either the original training context, a novel, or an altered context. The inactivation of SST-positive interneurons in the DG-CA3 system impaired the differentiation between familiar and novel contexts observed in control mice. Immunohistochemistry for the neuronal activation marker cFos indicated a heightened activation of DG granule cells specifically in the infrapyramidal (lower) blade of the DG. This was accompanied by a reduction in expression levels of the autophagy marker p62 specifically in the proximal CA3 pyramidal cell layer, a target region of infrapyramidal DG granule cells. To test whether lowered p62 levels may suggest elevated autophagy in CA3 pyramidal cells after inhibition of SST-positive interneurons during pattern completion, a high-resolution analysis of CA3 sublayers for LC3-postive autophagosomes is conducted.
Together, the inactivation of SST interneurons during contextual fear memory retrieval decreases memory recall specificity via disinhibition of the DG-CA3 mossy fiber system. This is of relevance for stress-induced neuropsychopathologies such as posttraumatic stress disorder, where an over-generalization of aversive memories is observed.

Behavioral algorithms underlying flexible decision-making

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Animals live in a complex, heterogenous, world where environmental features change dynamically, quickly within seconds, and slowly over minutes and hours. For survival, animals need to integrate sensory information and generate behavioral responses. Across diverse species, including worms, flies, fish, and primates, decision-making appears to follow surprisingly similar algorithmic principles, characterized by evidence accumulation over multiple timescales. In fruit flies and larval zebrafish sensorimotor transformations and decision-making have been studied using the innate motion-following behavior, the optomotor response. Larval zebrafish integrate sensory information over several seconds to make decisions in noisy environments, a process that can be effectively modeled as a low-pass filter. While we are beginning to understand neural circuitry and computation underlying such evidence accumulation, we still do not know how memories of past trials modulate behavior. In this study, we show a strong history-dependent response across timescales. On a shorter timescale, at the onset of optic flow, the optomotor response amplitude rapidly rises and then gradually declines over tens of seconds. On stopping optic flow, fish swim opposite to the direction of the previously perceived motion. Over the course of several hours, repeated exposure to optic flow significantly reduces the strength of optomotor response without affecting swim speed or swim rate. In many fish, this effect can even lead to a persistent reversal of the optomotor response. Mathematical modeling suggests that larvae use a high-pass filter in addition to the previously discovered low-pass filter for sensory integration and decision-making. The model further shows that short timescale dynamics arise as a weighted output of these high-pass and low-pass filters. Over the timescale of hours, the individual weights are updated. Through brain-wide calcium imaging, we find neural correlates of the high-pass and low-pass filter in the zebrafish hindbrain. We will now search for the neuromodulatory mechanisms that regulate the weights within the hindbrain. Our findings demonstrate that the optomotor response, previously thought to be purely innate and reflexive, is in fact a strongly adaptable, goal-oriented and experience-dependent behavior, which cannot be explained only by basic retinal stabilization mechanisms.

Unlocking visual pathways: Enhanced visual learning through olfactory deprivation in *drosophila*

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The brain is flexible and can adjust to lasting perturbations, such as severe injuries. Thus, the loss of one sensory modality can lead to compensatory improvements in some of the remaining senses. However, the extent to which such sensory deprivation can affect the cognitive capacities of the remaining modalities remains less clear. Here, we show that depriving flies of olfactory input by surgically removing the main olfactory organ, the antenna, improves visual learning. This perturbation leads to a shift of neuronal activity in the memory center from olfactory to visual pathways. We find that the improvement in visual learning capacity depends on the recruitment of a small set of putative visual Kenyon cells, the α/βp neurons. In intact flies, these neurons show relatively little responses to visual stimulation and are dispensable for visual learning. However, in olfactory-deprived flies, α/βp neurons gain sensitivity to visual input and become crucial for color learning. Together, these findings suggest that the loss of olfaction leads to an increase in visual learning capacity, not by recruiting former olfactory pathways but by utilizing an otherwise restricted parallel visual pathway. These insights into the plasticity of the hierarchical memory circuits of the fly might help explain compensatory adjustments in more complex memory systems.

Olfactory-visual integration in input and output regions of the mushroom bodies in the honeybee, *Apis mellifera*

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The European honeybee, *Apis mellifera*, is an excellent experimental model for investigating olfactory-visual integration at both the behavioral and neuronal levels. Such integration takes place in high-order processing centers like the mushroom bodies (MBs). The MBs receive information from primary olfactory and visual sensory input regions, the antennal and optic lobes. Projection neurons (PNs) of the two input regions form complexes of synaptic contact with ~184.000 MB intrinsic neurons, the Kenyon cells (KCs), in each brain hemisphere. KCs are classified into two distinct classes (class I and II KCs) based on the morphology of their dendritic specializations. Their dendrites innervate distinct divisions of the MB calyx: the lip, collar, and basal ring subcompartments, receiving segregated olfactory and visual input, and input from both modalities, respectively. How this multisensory information within the MB calyx is organized at the KC level, particularly whether multimodality already arises at the KC level in the honeybee MB, is still unknown. The information is transferred further via the KC axons through the peduncle to the MB output regions, the medial and vertical lobes. Here, KC axons synaptically converge on ~400 MB output neurons (MBONs), some of which were shown to provide centrifugal long-range feedback to the antennal lobe (AL), and input to the lateral horn (LH), and other regions of the protocerebrum, as well as short-range recurrent connections to the MB calyx (Menzel & Rybak 1993, *J. Comp. Neurol.*; Kirschner et al 2006, *J. Comp. Neurol.*). Recent multiunit electrophysiological recordings from MBONs have revealed that individual MBONs are sensitive to either light or odor stimuli, while a substantial proportion of MBONs responds to both light and odor stimuli (Strube-Bloss & Rössler 2018, *R. Soc. Open Sci.*). This demonstrates that a large portion of MBONs is multimodal and that multisensory convergence takes place at least at the MB output level. Is multimodal convergence possibly already implemented at the MB calyx input, more specifically, at the level of KCs? Are differences in responses to the two sensory modalities potentially expressed by morphologically distinct MBON subpopulations? How is crossmodal information distributed to upstream and/or downstream processing centers? We performed micropipette dye injections in the MB VL of adult honeybees to morphologically characterize synaptic connections of individual KCs. Furthermore, we physiologically and anatomically characterize individual MBONs using intracellular recording techniques. To elucidate the origin of multimodal integration in the honeybee MB, we anatomically map potential sites of multimodal integration of KCs within the MB calyx and of the target innervation patterns of uni- and multimodal MBONs in upstream and downstream sensory neuropils. We combine this with the immunohistochemical characterization of pre- and postsynaptic structures in the proto- and deutocerebrum using a recently published new atlas of the honeybee central brain as a reference (Habenstein et al. 2023, *J. Comp. Neurol.*). So far, we found no indications of multimodal connections across the MB calyx lip and collar in both types of KCs. Ultimately, our studies are aimed at understanding crossmodal interactions underlying context and experience-related modulation of olfactory processing and olfactory-visual perception.

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Action, valence, dopamine- Drosophila as a study case

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Darwin´s 1872 book on the relationship between emotions and behaviour (Darwin 1872) sparked a controversy continuing to this day. He suggested that there can be mutual causation between these two: not only can a particular emotional state engage a corresponding expressive behaviour, but, conversely, we can adopt the emotional state corresponding to the behaviour we engage in.
To see whether a neurobiologically tractable study case to investigate these processes can be established in the fruit fly Drosophila melanogaster we focused on basic behaviours and emotions, namely moving backward and feeling 'bad'. We induce backward locomotion by activating the 'moonwalker neurons' (Bidaye et al. 2014) and find that odors presented during such backward locomotion can establish negative valence.
Through a combination of behavioral analyses, optogenetics, pharmacology, connectomics, neurophysiology and modelling, we investigate the punishing effect of activating the moonwalker neurons in detail, with a focus on the pathways from the mushroom body towards the motor periphery, and the role of movement and of the dopaminergic reinforcement system in this paradigm. Through a normative model as well as behavioral experiment, inspired by the uncovered processes, suggest the plausible role to maintain successful learned avoidance, shedding new light on what is known in experimental psychology as the 'avoidance paradox'.

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Oppositional and competitive instigation of hippocampal synaptic plasticity by the VTA and locus coeruleus

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Introduction: Persistent hippocampal synaptic plasticity in the form of long-term potentiation (LTP) and long-term depression (LTD) encode different kinds of spatial information. Whereas LTP has been shown to be associated with the acquisition of novel spatial representations (Kemp and Manahan-Vaughan, 2004; Whitlock et al., 2006), LTD is associated with the acquisition and updating of information about spatial content (Kemp and Manahan-Vaughan, 2004; Hagena and Manahan-Vaughan, 2024). This kind of synaptic plasticity is modulated by noradrenaline that is released from the locus coeruleus (LC) and dopamine that is released from the ventral tegmental area (VTA) and the LC. It is unclear as to how the VTA and LC affect the direction of change of hippocampal synaptic plasticity and thus the content of stored information.

Methods: Eight to 10 week old TH::Cre rats were anesthetized and underwent chronic implantation of a monopolar recording electrode in the dorsal CA1 region of the hippocampus, a bipolar stimulation electrode in the Schaffer-collateral pathway and a cannula into the ipsilateral cerebral ventricle. For optogenetic activation or inhibition, channelrhodopsin or halorhodopsin were injected into the VTA or LC and an optical fiber was implanted above both structures.

Results: Optogenetic activation of tyrosine hydroxylase positive (TH+) neurons of the VTA at 25 Hz in conjunction with test-pulse stimulation of Schaffer collateral – CA1 synapses resulted in dopamine D1/D5-receptor dependent LTP, whereas activation of TH+ neurons of the LC at 5 Hz resulted in D1/D5- and β-adrenergic receptor dependent LTD. Inactivation of dopaminergic projections from the VTA to CA1 prevents facilitation of LTP that usually results during weak input-specific induction of short-term potentiation in association with novel spatial exploration and inactivation of TH+-neurons of the LC prevents the facilitation of short-term depression (STD) into LTD that usually occurs during acquisition of spatial content.

Conclusion: The VTA and LC decisively shape hippocampal synaptic plasticity in an oppositional fashion and thereby promote the integration of state-dependent experience into hippocampal representations.

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Optogenetic Control of Mitochondria in PV+ Interneurons Alters CA1 Function

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Parvalbumin-positive (PV+) interneurons are crucial for maintaining spatial memory functions within the hippocampus that are otherwise impaired in neurodegenerative diseases. To maintain such an extensive control, PV+ interneurons require a high energy demand, which requires highly functional mitochondria to produce ATP quickly and efficiently. Previous studies have observed mitochondrial dysfunction within the hippocampus (HPC) in early cases of Alzheimer's Disease (AD), even preceding the major pathological hallmarks: tau aggregation, amyloid-beta plaques, and impairments in memory. Optogenetic tools have been instrumental in discovering and understanding the function of specific neuronal subtypes within various different brain networks. Recently developed optogenetics tools have allowed for light-control of intracellular organelles, such as mitochondria. To understand how the function of mitochondria within PV+ interneurons contributes to the learning and memory processes regulated by the hippocampal CA1 circuit, we packaged a previously developed optogenetic construct, mitoChR2, into an adeno-associated vector (AAV) and performed viral vector injections in PV-Cre mice targeting the CA1 region of the hippocampus. The mitoChR2 construct targets the inner membrane of the mitochondria (IMM), and in the presence of light, the channel opens and causes a disruption of the proton motive force that drives ATP production, which in turn decreases the amount of ATP produced. Through the use of this technique, in conjunction with performing electrophysiological recordings while the mice are freely moving, we discovered stimulation of mitoChR2 impaired the firing activity of both interneurons and pyramidal cells and consequentially altered spatial properties of place cells within the HPC during exploration of a familiar environment. Our findings emphasize the importance of mitochondria in learning and memory mechanisms, and suggest mitochondria be considered for potential therapeutic targets for the treatment of AD.

Nogo-A regulates fear memory processes and memory engram formation by modulating neuronal excitability in a sex-specific manner

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The neuronal engram coding for fear memory relies on the plasticity, for its formation and the stability of the neuronal network for its persistence. The allocation of neurons to a specific engram depends on their excitability. Molecules regulating the excitation / inhibition balance (E/I balance) in the brain are therefore considered key players in engram formation. Nogo-A has been shown to influence the E/I balance, restrict activity-dependent functional and structural plasticity and modulate learning and memory formation. However, whether Nogo-A controls the memory engram formation and the mechanisms of this action are still unknown. Behavioural experiments were combined with histochemical analysis to address this hypothesis. Moreover, the cell type-specific role of Nogo-A was assessed comparing the full Nogo-A knockout (KO) to conditional KOs missing Nogo-A either in Parvalbumin (PV) expressing interneurons or in excitatory neurons in a contextual fear conditioning paradigm. Nogo-A KO in excitatory neurons shows an increased freezing time accompanied by a higher number of cFOS expressing neurons in the basolateral amygdala. The effect of a Nogo-A deletion on fear memory could only be observed in female mice, suggesting a sex- and cell type-specific effect of Nogo-A in this context. Nogo-A loss-of-function in acute hippocampal slices shows an increased Ca^{2+}-influx upon chemically induced long term potentiation indicating a higher neuronal excitability, supporting the results above. Current 2-Photon Ca^{2+}-imaging experiments address whether the activity of Nogo-A on engram formation may be related to its ability to modulate neuronal network synchronization. In addition, electrophysiological LTP experiments will be performed to further characterize the cell type-specific Nogo-A KOs.

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The role of gamma oscillations in stimulus encoding and memory maintenance during a sequential memory task in the human Medial Temporal Lobe

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The encoding and maintenance of sequential information is a fundamental component of episodic memory, though the underlying mechanisms are still open to investigation. A prominent theory proposes that maintaining sequential information in memory is reflected in ordered firing of neurons at different phases of theta oscillations (1). One study has recently challenged this view using single-unit and LFP recordings from epilepsy patients in the human medial temporal lobe (MTL, 2). In addition to predicting a specific temporal relationship between spiking and theta oscillations related to temporal order, the theory also assumes that 1. gamma-frequency activity encodes stimulus-relevant information that is maintained within memory and temporally aligned to spiking of individual neurons and 2. proposes a temporal relationship between gamma activity and theta oscillations during memory. Both aspects were not directly explored in the previous study. To address these questions, we utilized the same dataset as in (2) consisting of local field potentials (917 channels) and SUA (1411) from the MTL of epilepsy patients performing a working memory task for temporal order. When assessing whether gamma power (60 - 100 Hz) encoded stimulus information, 31% of the channels exhibited increased activity during visual presentation of stimuli as compared to baseline (test $p<0.001$, Binomial test). Stimulus identity could both be successfully decoded from firing rates of single units and gamma power ($p < 0.05$, Mann Whitney U test). Interestingly, stimulus-encoding channels also showed increased gamma power and higher theta-gamma phase amplitude coupling than non-responsive channels during memory maintenance ($p < 0.001$, Mann Whitney U test). Further, we identified a subset of LFP-neuron pairs (N = 70) with similar stimulus preferences as the corresponding LFP-gamma power. These pairs exhibited increased gamma-spike-field coherence during the encoding and maintenance period as compared to pairs with non-overlapping stimulus preferences ($p < 0.05$, Mann Whitney U test). Taken together, our preliminary analyses show that stimulus information is encoded in 1. gamma power, 2. increased temporal alignment between spiking and gamma during encoding and delay and 3. Increased phase-amplitude coupling between gamma and theta during the delay, as predicted by the theory. We plan to further explore how these findings relate to the temporal order of stimuli, and whether the model holds true in its major prediction about the relationship between stimulus- and theta phase order.

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Neural circuits that regulate exploratory odor-driven behavior

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To adapt to environmental changes, animals must adjust their behavior, overriding innate drives and adopting new strategies when expectations are not met. The neural circuits responsible for this behavioral flexibility remain largely unclear. For instance, flies innately pursue odor cues signaling a food source, but abandon the odor when it is not associated with a reward. This behavioral shift is not a generalized odor habituation; it is odor valence-dependent. While flies reevaluate appetitive odors in the absence of rewards, innate odor avoidance remains unchanged even if no punishment is present.
In insects, odor stimuli are processed by the mushroom body (MB) and lateral horn (LH), which respectively encode learned and innate odor preferences. The MB is innervated by two clusters of dopaminergic neurons (DANs) critical for associative learning. During learning, sensory information is integrated with rewards or punishments via DANs, altering the predictive odor value represented in MB output neurons (MBONs). We hypothesize that the switch from pursuing an odor to exploring the surroundings arises from constant updates in the MB's odor value, driven by DANs, allowing for re-evaluation of the innate odor value encoded in the LH.
To test this hypothesis, we are investigating the role of the MB and DANs in promoting flexibility in exploratory odor-driven behavior. Using a free walking assay, where flies can move freely within an arena while they are exposed to an odor, we aim to simulate a naturalistic environment in which they behave as they would in nature—freely exploring, deciding how long to follow the odor, and when to give up on it. Our experiments demonstrate that blocking synaptic output from the dopaminergic system prevents flies from abandoning an unrewarded odor source, leading them to persist and accumulate at the odor's location over time. We conducted a targeted screen to identify specific subsets of DANs involved in this behavioral re-evaluation.
To further understand the computations carried out by DANs in this unconditioned context, we used 2-photon imaging to quantify DANs' responses to odor stimuli. Our data show that several DANs respond to both innate appetitive and aversive odors, with dynamics that are neuron-specific. Notably, different DANs exhibit diverse decay times in response to sustained stimulation, providing a possible mechanism for updating odor value over time.
Overall, our findings demonstrate the role of the dopaminergic system in modulating innate odor expectations, even in the absence of external reinforcement.

Towards establishing a cocaine preference model in *Drosophila melanogaster*

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With five percent of all Europeans and 4.8 million people in the US, age 12 or older, encountering cocaine abuse, cocaine is a major cause of drug-related health issues. Despite its detrimental health effects, cocaine appears to drive similar rewarding properties as natural rewards. How the positive value of cocaine is provided to the brain and if it engages the same neural and molecular mechanisms as natural rewards, however, remains largely unclear and difficult to address in complex mammalian brains. Here we aim to establish a *Drosophila* model employing classical conditioning and using cocaine as a reinforcer to investigate preference and avoidance behavior to cocaine-associated cues. We build on preceding insights as to how rewarding and punishing reinforcement signals underlying Pavlovian conditioning are transduced to the *Drosophila* Mushroom Body, the fly's olfactory learning center, via previously identified sets of dopaminergic neurons.

In vivo imaging and optogenetics reveals a role of the mammillary body in spatial reward memory

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The ability to form and recall episodic memories is vital for leading a meaningful and self-determined life. The hippocampus is the core brain region for memory processing. The subiculum, the main output node of the hippocampal formation, routes mnemonic information to downstream centers. The main subicular output is a small hypothalamic nucleus, the medial mammillary body (MMB). Lesion studies in humans and animal models revealed that damage to the MMB leads to antero- and retrograde amnesia. However, the contribution of the MMB in memory processing is poorly understood as the MMB is extremely difficult to access due to its small size and deep location.

In this study, we combined single-cell calcium imaging and pan-neuronal and projection-specific neural activity manipulations in freely moving animals to investigate the contribution of the MMB to different stages of memory processing. Single-cell calcium imaging in the MMB enabled us to characterize the stimulus-response profiles and spatial tuning of individual neurons in freely exploring animals. To test the contribution of MMB neurons and their subicular inputs to recognition memory, we optogenetically activated MMB neurons, or their subicular inputs, respectively, during the acquisition or the recall phase of the spontaneous object recognition task. To evaluate the relevance of MMB neurons and their subicular inputs to spatial reward memories, we optogenetically activated MMB neurons, or their subicular inputs, respectively, in the holeboard task. The activation of neuronal MMB populations in the test phase of the holeboard task impaired memory performance, while inhibition of subicular input to MMB during training impaired the acquisition of reward locations. To test the contribution of MMB neurons to social recognition memory, we activated MMB neurons in the three chamber test. Here, activation of MMB populations did not affect social recognition memory.

In this study, we developed a protocol for single-cell calcium imaging of MMB neurons in freely moving animals. Our initial optogenetic experiments suggest that MMB populations, and their subicular inputs, play an important role in the recall of spatial reward memories.

Acute circadian rhythm disturbance impairs contextual-memory engrams in the dentate gyrus

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Endogenous circadian rhythms synchronize organisms' physiological processes and behavior with environmental cues. Modern disruptions, such as artificial lighting, digital device use, irregular work hours, and poor sleep, are associated with negative health outcomes. Rodent studies suggest these disruptions impair hippocampal plasticity and memory, yet research on the effects of acute circadian shifts, like sudden changes in light (e.g., jet lag), on hippocampus-dependent memory and engram formation remains limited.In this study, we examined whether a 6-hour experimental phase shift in the light-dark cycle in mice affects fear memory retrieval and engram formation in the dentate gyrus (DG). Following a 6-hour delay in light onset, mice tested in an open-field arena displayed increased mobility with no significant change in anxiety-like behavior. Engram cells were labeled using intrahippocampal adeno-associated virus injections expressing the Robust Activity Marker (RAM) in adult C57Bl6 male mice during contextual and cued fear-conditioning. After applying the 6-hour phase delay post-training, fear memory retrieval was assessed at two time points. Immunohistochemical analysis of cFos expression revealed reduced reactivation of DG engram neurons and decreased conditioned freezing behavior during context memory retrieval, suggesting impaired memory recall after the acute phase delay. Notably, while retrieval time-point influenced fear response form (e.g., freezing vs. risk assessment), it did not impact engram activation in the DG, indicating that the DG does not encode state-specific memory information. In contrast, female mice displayed no impairment in memory retrieval 24 hours post-conditioning despite the phase delay. The underlying cellular mechanisms, sex differences, and stability of memory content retrieved are currently under further investigation.

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Changes in neural representation of social conspecifics in response to reward learning

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Recognizing and remembering social conspecifics is crucial for the survival of an organism, but it remains unclear how this information is represented in the brain. In previous research, we identified populations of basolateral amygdala (BLA) neurons in the freely moving rat that were highly tuned to distinct naturalistic stimuli such as male or female conspecifics. To understand how discrimination learning modulates this social representation, we recorded from large populations of neurons in the rat amygdala and surrounding areas using 4-shank Neuropixels probes during a novel, fully automated go/no-go task using social conspecifics as stimuli.
In the BLA, we observed strong modulation of neural activity during social discrimination learning, leading to enhanced decoding of identity. In contrast, identity decoding in neighboring regions, such as the posterior piriform cortex, remained relatively stable throughout learning.
To understand how discrimination learning affected social representation during naturalistic behavior, we allowed implanted rats to freely interact with conspecifics after completing task learning. The similarities between neural responses during the task and in free interaction highlight distinct learning dynamics observed throughout the task, suggesting that the amygdala and piriform cortex may play unique roles in social reward learning and identity encoding.

Poster Topic

T26: Computational Neuroscience

T26-3C Spatiotemporal Deep Learning Pipeline for Decoding Stimulus-Driven Whole-Brain Calcium Imaging
Amina Abdelbaki, Paul Bandow, Karen Cheng, Ilona C. Grunwald Kadow, Martin Paul Nawrot, Vahid Rostami

T26-4C Dissociation of multisensory processing in superior colliculus and primary sensory cortex
Daniel Gerber, Peter Severin Graff, Björn Kampa, Simon Musall

T26-5C How pronounced refractoriness prevents resurgent excitation in bidirectional motor nerve nets of the jellyfish
Peixuan Wu, Jan-Hendrik Schleimer, Susanne Schreiber

T26-1D Excitatory-Inhibitory Interaction Shapes Activity-Dependent Self-Organization of Neuronal Networks
Richmond L. Crisostomo, Shreya Agarwal, Ulrich Egert, Samora Okujeni

T26-2D A Novel AI-based Tool for Real-Time USV Detection as Unbiased Markers of Distinct Social Interactions
Ali Mohammadi, Jens F. Tillmann, Martin K. Schwarz

T26-3D Estimating Latent Variables of Decision-Making Behavior in Zebrafish Larvae
Roberto Garza, Ahmed El Hady, Armin Bahl

T26-4D Interaction with third parties shapes courtship behavior in groups of fruit flies
Amirmohammad Naderi, Adrián Palacios Muñoz, Sarath Ravindran, Jan Clemens

T26-5D Beyond firing rate homeostasis: How neurons can tune their excitability class using higher-order statistics of the Calcium signal
Hannah Schultheiss, Nelson Niemeyer, Jan-Hendrik Schleimer, Susanne Schreiber

Adaptive mechanism in clustered spiking attractor model explains Experimental Firing Rates and Variability

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Recent studies, such as Rostami et al.[1], have demonstrated that spiking attractor networks with excitatory and inhibitory clustering can robustly exhibit metastable dynamics, providing a explanation for observed cortical activity. Rostami et al. applied this model to motor cortical activity during a delayed-center-reach-out task[2], which we also use here. These dynamics are consistent with experimental findings on firing rates and trial-to-trial variability (Fano Factor). Building on this network architecture, we explore whether cellular adaptive mechanisms or adaptation of the input can further enhance the model's ability to replicate these experimental findings.
We compare the effects of spike-frequency adaptation on network dynamics with those of variable external inputs. Using a Gaussian Process-based optimization framework, we fitted the model's output to match experimental firing rates and Fano Factors. Preliminary results show that optimizing the structure of external inputs alone achieves a close match to experimental observations. This finding underscores the critical role of external input in shaping neural dynamics. Additionally, we demonstrate that cellular adaptation serves as a mechanism to modulate the stability of metastable states.

This work highlights the potential of input-driven strategies for refining neural network models and provides insights into how external drive underpins neural computations in complex networks. While input optimization proves sufficient to shape the dynamics, cellular adaptation may be influenced by the animal's global behavioral states. Moreover, our findings suggest that input optimization reflects an adaptive mechanism that likely operates at upstream processing levels.

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Distributed reinforcement signals among Drosophila larva dopaminergic neurons guide learning in individual mushroom body compartments

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Dopaminergic neurons play a central role in mediating synaptic plasticity within the insect mushroom body, a higher-order brain region involved in learning and memory. In this network, individual dopaminergic neurons target distinct compartments, each influencing behavior by biasing mushroom body output towards approach or avoidance. Specifically, the activity of dopaminergic neurons that predominantly encode punishment drives avoidance behaviors, as the mushroom body output is relayed onto pre-motor areas.

Here, we utilize a computational model of the Drosophila larva mushroom body, in which individual compartments are innervated by either punishment-encoding dopaminergic neurons of the DL1 cluster or reward-encoding neurons of the pPAM cluster. By fitting the neuron models of the dopaminergic neurons using calcium imaging data that shows their relative responses to salt or fructose, we predict their dynamic responses over the duration of learning experiments. This model enables us to investigate the time-resolved contributions of individual dopaminergic neurons to learning during classical conditioning with salt or optogenetically induced punishment. We separately quantify the contributions of individual dopaminergic neurons to a distributed reinforcement signal and manipulate them in simulated knockout experiments.

Bridging Tuning and Invariance with Equivariant Neuronal Representations

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Our current understanding of neuronal computations in visual brain areas is heavily influenced by how individual neurons respond to rotated bars. For example, the primary visual cortex is traditionally characterized by edge-detecting neurons, even though many neurons do not fit into this category. Furthermore, extending this understanding to naturalistic stimuli has proven challenging.

We propose a complementary approach to characterizing brain areas by describing populations of neurons rather than individual ones and using rotations of many naturalistic images rather than rotations of a single bar.[1] We introduce a mathematical framework that explains how populations of neurons can achieve both tuning and invariance to image rotations, regardless of the underlying image. The framework is based on the principle of equivariance,[2] which asserts that the response of a neuronal population transforms in a systematic way when the input image undergoes a geometric transformation, such as a rotation. Large-scale calcium imaging data in mouse visual cortex show that neuronal responses become progressively more invariant in higher-order areas, similar to what has been observed in non-human primates,[3] and are more tuned in primary and secondary visual cortex. This progression from tuned to invariant responses supports robust object recognition.

A key finding is that while both biological and artificial visual systems (such as deep convolutional neural networks) display trends of increasing invariance across layers, artificial networks lack the structured, rotation-equivariant representations seen in mouse visual cortex. This difference suggests that incorporating equivariance into artificial models could enhance their robustness to geometric transformations, inspiring improvements in computer vision algorithms.

Our work bridges the fields of neuroscience and machine learning, providing a critical step toward understanding how biological visual systems achieve complex computations, such as object recognition, through a combination of tuning and invariance to image transformations. The framework also opens up new avenues for designing more biologically inspired artificial neural networks.

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Computing in neuronal networks with plasticity via all-optical bidirectional interfacing

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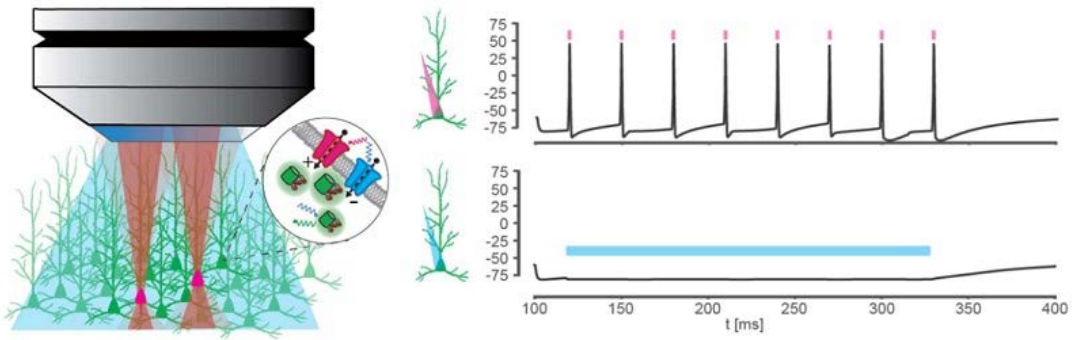
All-optical techniques play a pivotal role in the direct interrogation of biological neural networks and the investigation of their computational properties. Such techniques combine spatiotemporally precise optogenetic stimulation at cellular resolution to manipulate neuronal states in the network with optical imaging of fluorescent genetically-encoded indicators for simultaneous readout of neuronal activity. The biophysical models of various optogenetic tools have been studied computationally in single neurons, accurately fitting experimental data. However, the effects of optogenetic manipulations with complex excitation and inhibition patterns have been explored in less detail, especially in the context of the plasticity induction and the computation in neuronal networks. Thus, we can ask the following questions: (i) how can different stimulation protocols modify functional connectivity in isolated neuronal systems, and (ii) what computations can be practically realized with such an approach?

In this work, we introduce optogenetic manipulation and fluorescence readout in spiking neuronal networks with recurrent connectivity and spike-timing dependent plasticity. We propose a protocol for "programming" the functional connectivity of the network and use this protocol to implement a simple computational task, memory storage and recall. Notably, during the "programming" phase, the protocol separates interdependence between the activity of the network and the induction of the associated changes in functional connectivity. It also permits a simplification of the description of the neuronal network as a dynamical system and guarantees reproducible initial conditions for its temporal evolution. This work represents a significant advance by providing a realistic simulation of a neuronal system with a bidirectional optogenetic interface that takes into account both biophysical and computational aspects, and suggests a strategy for further investigation of computation in neuronal networks.



DENOISING: Dynamic Enhancement and Noise Overcoming in Multimodal Neural Observations via High-density CMOS-based Biosensors

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Large-scale multimodal neural recordings on high-density biosensing microelectrode arrays (HD-MEAs) offer unprecedented insights into the dynamic interactions and connectivity across various brain networks. However, the fidelity of these recordings is frequently compromised by pervasive noise, which obscures meaningful neural information and complicates data analysis. To address this challenge, we introduce DENOISING, a versatile data-derived computational engine engineered to adjust thresholds adaptively based on large-scale extracellular signal characteristics and noise levels. This facilitates the separation of signal and noise components without reliance on specific data transformations.

DENOISING is uniquely capable of handling a diverse array of noise types (electrical, mechanical, and environmental) and multidimensional neural signals, including stationary and non-stationary oscillatory local field potential (LFP) and spiking activity. This adaptability makes it applicable across different recording modalities and brain networks. Applying DENOISING to large-scale neural recordings from mice hippocampal and olfactory bulb networks yielded enhanced signal-to-noise ratio (SNR) of LFP and spike firing patterns compared to those computed from raw data.

The hippocampus and olfactory bulb are critical regions for spatial contextual learning, episodic memory, and olfactory processing, demonstrating remarkable neuroplasticity. By improving the clarity of neural signals from these regions, DENOISING facilitates a deeper understanding of the complex interplay of neural circuits involved in cognitive functions. Comparative analysis with existing state-of-the-art denoising methods, employing SNR and root mean square noise (RMS), underscores DENOISING's superior performance in improving data quality and reliability.

Through experimental and computational approaches, we validate that DENOISING improves signal clarity and data interpretation by effectively mitigating independent noise in spatiotemporally structured multimodal datasets. This advancement unlocks new dimensions in understanding neural connectivity and functional dynamics, paving the way for more accurate and comprehensive brain research.

The non-human primate connectome

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Generating a connectome of non-human primates (NHP) is critical because their brains closely resemble human brains in terms of structure and function, making them an ideal model for understanding human neural networks. A comprehensive connectome based on tract-tracing studies offers high-resolution mapping of neural pathways, revealing precise connectivity patterns that are not fully captured by non-invasive imaging techniques like DTI. Furthermore, it facilitates translational neuroscience, providing crucial insights into brain disorders and potential therapeutic targets that can be applied to human health. Lastly, by integrating data from a wide range of tract-tracing studies in a metastudy, the resulting connectome provides a more reliable and generalized map, overcoming limitations of individual studies and ensuring robust conclusions about primate brain organization.

To construct a weighted and directed connectome of NHP, we collated connectivity data from over 1000 tract-tracing studies published in peer-reviewed journals. These studies provided detailed descriptions of neural connections across both the bilateral CNS and PNS. The data were extracted systematically, capturing the presence, directionality, and strength of connections reported in each study. Both contralateral and reciprocal connections were carefully considered, ensuring the inclusion of bilateral and bidirectional interactions between brain regions. Each connection was weighted based on the density of the reported connectivity, where available, and all connections were categorized as directed, reflecting the source and target of each neural projection. The dataset was further refined by cross-referencing studies to account for inconsistencies and redundancy, ensuring the robustness of the final connectome. The resulting connectome captures the complex, multi-level architecture of neural pathways in NHP.

Graph theoretical features of of the NHP connectome reveal fundamental organizational principles of primate brain networks, but also species-specific differences. All NHP connectomes exhibit small-world characteristics, which means they balance efficient local clustering of connections with short global path lengths. This allows for efficient information processing. The NHP connectome has a modular organization which refers to the presence of tightly interconnected modules that perform specialized functions while maintaining intermodule communication. It has a highly developed modularity, reflecting distinct functional systems such as sensory, motor, cognitive, and associative networks, which are more complex and differentiated than those in non-primate species like rodents. Rich-club organization is a feature where high-degree nodes (hubs) are more densely interconnected than would be expected by chance, supporting robust, efficient communication. NHP have a strong rich-club organization, with high-degree hubs in association areas like the prefrontal cortex, involved in higher cognitive functions. Hierarchical organization refers to the presence of high-level nodes that integrate information across different network levels. In NHP, there is a more pronounced hierarchical structure, with higher-level integrative areas (e.g., prefrontal cortex) that coordinate activity across the brain, allowing for complex behaviors and cognitive processes. Assortativity refers to the tendency of nodes to connect to other nodes with similar degree (i.e., hubs connecting to other hubs). The NHP connectome exhibit higher assortativity when compared with rodent connectomes, meaning that high-degree hubs tend to form more interconnected, robust networks that are essential for global integration. The non-human primate connectome, meticulously collated and analyzed by integrating data from all available published studies, provides a reliable and comprehensive map of the brain's connectivity, capturing the intricate hierarchical organization of multilevel neuroanatomical regions with unprecedented precision.

Establishing functional ultrasound imaging in crows

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Functional ultrasound imaging (fUSi) is an emerging method to examine functionality of brain regions by detecting subtle changes in the cerebral blood volume (CBV) which correlates with neuronal activity through neurovascular coupling. Compared to more established imaging methods, such as functional magnetic resonance imaging (fMRI), this technique offers higher spatiotemporal resolution and greater flexibility for the subject. Here, we imaged the telencephalon of two carrion crows (*Corvus corone*) in both anesthetized and awake states, marking the first use of this method in a songbird species. From within a larger brain volume, we successfully localized the primary auditory region of the crows' telencephalon, known as the Field L complex, capitalizing on this method's ability to cover a broad range of brain activity. fUS imaging revealed a clear dorso-ventral tonotopy in the Field L complex, with low frequencies activating dorsal regions and higher frequencies activating more ventral regions. This pattern is consistent with electrophysiological mappings in other songbirds, highlighting the high spatial specificity of fUSi. Thanks to the high spatiotemporal resolution and the broad anatomical coverage, just a few seconds of scanning are sufficient to obtain clear images of responses to various auditory stimuli. With univariate analysis methods and multi voxel pattern analysis (MVPA), we were able to robustly reproduce tonotopic mapping for various acoustic stimuli, including pure tones and bandpass filtered noise, across anesthetic versus wake states, as well as among individual subjects. As a proof of principle, these results pave the way for large-scale measurements of CBV in awake, behaving crows, aiming to identify the functional networks involved in cognitive processing.

Data-Driven Pipeline for Characterizing and Simulating Sensory Neurons Using Electrophysiology Recordings

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Sensory systems extract relevant attributes from incoming stimuli and encode them into neural activity patterns. In rate coding, neural populations modulate their firing rates in response to specific stimulus features. While electrophysiology allows direct measurement of sensory neuron responses, translating these complex datasets into meaningful insights remains challenging. In this project, we propose a data-driven analysis pipeline using a tailored integration of statistical modeling, machine learning, and computational neuroscience algorithms. Our pipeline detects relevant stimulus events and extracts spike trains from voltage traces. It also fits stimulus-response transfer functions and quantifies the variability in neural responses at the single-neuron level, providing a fine-grained analysis of neuronal behavior. Additionally, our approach classifies neurons based on their response patterns and estimates parameter distributions for transfer functions. By integrating these results, the pipeline generates spike train simulations that mimic experimental data, enabling decoders with various network architectures to assess neuronal populations' stimulus sensitivity across diverse settings. Tested on example datasets, our user-friendly and flexible pipeline is applicable to numerous sensory systems, providing single-neuron models that enable deeper investigation of sensory neural coding through model-driven experiments that extend beyond the limits of traditional electrophysiology.

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An automated behavioral setup for multisensory perception and cortical activity in awake mice

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Multisensory perception is a fundamental aspect of sensory processing, yet investigating its neural mechanisms in awake, behaving animals poses challenges. Traditional paradigms demand labor-intensive training and are often affected by human-animal interactions and environmental variability. To overcome these limitations, we developed automated behavioral setups, aimed at probing multisensory perception and cortical activity in awake mice. The systems offer streamlined solution for perceptual decision-making tasks, supporting high-throughput experimentation with minimal human intervention.
We created setups for both freely moving and head-fixed configurations, providing flexibility for different experimental designs. RFID-based tracking enables precise monitoring of individual animals, reducing variability and ensuring consistency in data collection. By automating the training process, the platforms minimize external factors to produce more reliable results. In general, mice are trained to discriminate visual stimuli, with the added capacity to investigate multisensory integration by combining visual and tactile inputs. The setups therefore allow detailed study of unimodal and multimodal sensory processing and capture real-time behavioral and high-speed video data from multiple cameras via the Bpod platform.
To improve scalability and throughput, we also implemented a server-based architecture for centralized control and data management. This infrastructure supports simultaneous operation of multiple experimental setups from a centralized server. Data management is then handled through a user datagram protocol (UDP), enabling low-latency communication and reliable synchronization. This allows researchers to conduct parallel experiments, facilitating large-scale studies and improving reproducibility.

To demonstrate the utility of the approach, we then used the head-fixed setup to record cortex-wide widefield imaging data as mice were learning a multisensory decision-making task. We developed an analysis pipeline to align the data to the Allen Common Coordinate Framework and developed an algorithm to project data from all imaging sessions across learning in a common low dimensional space where cortical areas are functionally-identified as spatially-restricted activity patterns in cortical space. Using this approach we found that training enhances the representation of visual evidence over time, while the representation of tactile evidence was enhanced in more frontal areas. Lastly, changes in decision-making accuracy where reflected in earlier and more robust activation of frontal cortical areas. These results demonstrate the evolution of specific cortical dynamics in parietal and frontal cortex during task learning and the general utility of our behavioral approach for future studies of multisensory perception and decision-making.

A bio-physically inspired model for synaptic tagging and capture

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Dendritic spines are small protrusions of dendrites that form the morphological basis of most excitatory synapses. During plasticity, these structures undergo reorganisation of their receptor content (functional plasticity) as well as shape remodelling (structural plasticity), which are thought to be fundamental for memory storage and retention.
The longevity of these alterations has been shown to depend on synaptic tagging and capture of newly synthesized proteins (STC). This mechanism has so far been mostly modelled phenomenologically, such that models could not be readily mapped to biology and their capability to extapolate to unknown stimulation protocols remains questionable.

Here, we present a simple model capturing the complex biophysical processes that give rise to long-term plasticity. Particularly, we consider the dynamics of cytoskeletal structures such as actin filaments, which -- in the spine -- occur in two distinct pools: a dynamic one with a fast molecule turnover rate, and a more static one, in which filaments are stabilized by cross-linking proteins. During plasticity, there are various phases in which the dynamics of actin filaments is modulated -- e.g., through crosslinker unbinding -- and as a result the spine can undergo dramatic changes in volume. Following this, also the postsynaptic density hosting the receptors can undergo size changes, which ultimately lead to changes in the receptor content and thus functional LTP or LTD.

Using a coarse grained approach we reduced these complex interactions to three fundamental biological variables of spine dynamics: the two actin pools and the PSD size, which serves as a proxy for the synaptic transmission efficacy. Their dynamics is captured by simple differential equations, which are temporally modulated during plasticity according to experimental observations.

When analyzing this model, we find that it can reproduce not only standard plasticity events like LTP and LTD, but also a number of experimental findings associated with the STC mechanism -- from conversion of early-LTP into late-LTP, to the transient property of the synaptic tag, and also more complex mechanisms such as tag resetting through the use of low frequency stimuli after strong tetanization.

Hence, we present a biophysically interpretable model for synaptic tagging and capture that can reproduce various experiments on functional plasticity and is simple enough network simulation.

Primary Neuronal Cell Culture in Ambient CO_2

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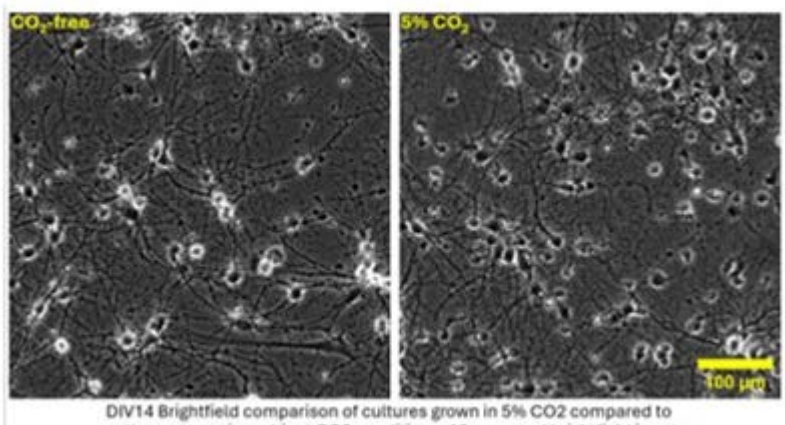
Dissociated neuronal cell culture is a well-established method that is universally performed in warmed, humidified incubators with 5% CO_2. Cultures in these conditions require a suitable culture medium that is buffered with a bicarbonate-, HEPES-based buffer system. Removing cells from the incubator and placing them in atmospheric conditions leads to significant cell death, which is a major limitation of this culture system.

We have developed and tested a method for growing primary, dissociated cells outside of CO_2 environments. Using a modified culture medium, we can grow primary neuronal cultures for up to two weeks in ambient CO_2 conditions. These neurons develop axons and dendrites, allowing them to form synaptic connections and develop active networks. By using a combination of calcium imaging, electrophysiology, and immunocytochemistry, we are characterizing these cells functionally and morphologically.

A CO_2-free system presents several advantages. Our culture system enriches for neurons rather than glia which could be beneficial for certain applications. Not requiring a continuous supply of CO_2 lowers the barrier to establishing culture systems in labs around the world and removes the need for CO_2 supply, which is a toxic, potentially lethal gas. Furthermore, the role of bicarbonate in CNS cell survival and development could be selectively studied in a controlled system, and CO_2-free cultures could potentially be used for biological studies in space, where CO_2 supply is expensive and problematic. Beyond culturing, this modified culture medium could be used in the future to transport living cultures without CO_2 either immediately after plating or once they reach maturity without significant changes to neuronal structure and function.



DIV14 Brightfield comparison of cultures grown in 5% CO2 compared to cultures grown in ambient CO2 conditions. 10x cropped brightfield images

Spatiotemporal Deep Learning Pipeline for Decoding Stimulus-Driven Whole-Brain Calcium Imaging

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Whole-brain calcium imaging has emerged as a powerful technique for capturing neuronal dynamics with high spatial and temporal resolution, offering valuable insights into brain-wide activity patterns. However, the complexity of these recordings poses substantial challenges for analysis. We present a deep learning-based pipeline that enables end-to-end analysis of 3D whole-brain continuous recordings, directly decoding the spatiotemporal dynamics of raw imaging data to reveal responses to various stimuli. This approach eliminates the need for extensive manual preprocessing and feature engineering by allowing the model to learn relevant features directly from the raw data. We validate this approach using Light Field Microscopy (LFM) data of calcium activity in *Drosophila melanogaster* - comparing well-fed and food-deprived animal groups in response to different odor, taste, or combined stimuli. This pipeline effectively identifies brain-wide neuronal patterns in response to chemosensory stimuli as influenced by the animal's metabolic state, advancing our understanding of how global brain activity encodes stimulus processing and providing new insights into the neural mechanisms of chemosensory processing.

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Dissociation of multisensory processing in superior colliculus and primary sensory cortex

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The superior colliculus (SC) plays a crucial role in integrating multisensory stimuli and is associated with various cognitive functions, such as decision-making. It receives inputs from different sensory modalities, either directly from sensory organs or from primary sensory regions in the cortex. However, the distinctions between multisensory integration in the SC and the cortex remain unclear. In particular, it is unclear to what extend multisensory responses in cortex and the SC are due to the integration of unisensory inputs or driven by other factors, such as widespread movement-related activity. To study the physiological underpinning of multisensory integration in these areas, awake mice were exposed to visual, tactile, and multisensory stimuli, while neural activity was recorded in primary visual cortex (V1), primary somatosensory cortex (S1) and the SC simultaneously using high-density Neuropixels electrodes.

Analysis of the spiking activity in cortex and SC revealed significant disparities in multisensory responses compared to the sum of tactile and visual responses. Moreover, these responses included additional activity that was not directly related to sensory processing, but movement-related activity. To dissociate the sensory and movement components of the neural activity and isolate multisensory processing, we used generalized linear models and investigated the variance and different response components that were uniquely explained by different model predictors. Interestingly, the activity of SC neurons was largely explained by the sensory-related components. In contrast, the activity in V1 is mostly decoded by movement components.

Because trial-by-trial fluctuations of neural activity are influenced by varied movements of the mouse, further analysis considered only the stimulus-related activity extracted by the generalized linear model. In this case, the difference between multisensory responses and the sum of unisensory responses is reduced compared to the analysis results of the unfiltered spiking activity. This indicates that the model's dissociation provides a more detailed view of multisensory integration in the SC.

How pronounced refractoriness prevents resurgent excitation in bidirectional motor nerve nets of the jellyfish

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Scyphozoa jellyfish are among the earliest branching organisms that evolved nervous systems. Their motor nerve net is composed of bidirectional neurons with excitatory, chemical synapses. Previous modeling studies have simulated the dynamics of single neurons, network architecture, and swimming behavior, primarily focusing on the effects of motor nerve net geometry on swimming. However, the computational properties of single motor nerve neurons and their roles in jellyfish behavior remain largely unexplored. One interesting property is their particularly large refractory period. Here, we tried to understand how this feature of the neurons' electrical dynamics relates to the bidirectionality of their synaptic transmission, their forest-fire-like network dynamics and how it eventually affects jellyfish behavior. Through exploring the computational model of jellyfish motor neurons, we found two unique properties in these neurons. Firstly, neurons exhibit class-III excitability for most constant inputs except for a narrow range of input amplitudes leading to oscillations. Secondly, the duration of the refractory period is not only considerably long but also depends on the ion channel dynamics and internal state during firing onset. Our further investigation uncovered that the refractoriness of the neuron is gated by voltage thresholds: the neuron exits the refractory state after firing as soon as the voltage decreases below a threshold. In order to understand the underlying mechanism analytically, we reduced the conductance-based model to two dimensions. Through analysis of the bifurcations and the phase space, we discover that a ghost of the saddle-node fixed point is responsible for the slow dynamics these neurons exhibit after the upstroke. The consistent, slow decay of the membrane potential, plus the juxtaposed arrangement of the relative refractory zone and upstroke zone in the phase space eventually manifest as a voltage threshold of refractoriness. Compared with neuronal models with a rapid downstroke following the upstroke of a spike (where a fixed refractory period is sufficient to describe the refractoriness of most models), the phase space geometry enables jellyfish motor neurons to achieve a long, extendable refractory period. Effectively, this mechanism prevents pathological firing of the network and undesirable repetitive contractions during swimming. The study bridges from the level of single neurons to the level of functionally relevant networks, allowing us to gain mechanistic insights into the influence of cellular biophysics on animal behavior.

Excitatory-Inhibitory Interaction Shapes Activity-Dependent Self-Organization of Neuronal Networks

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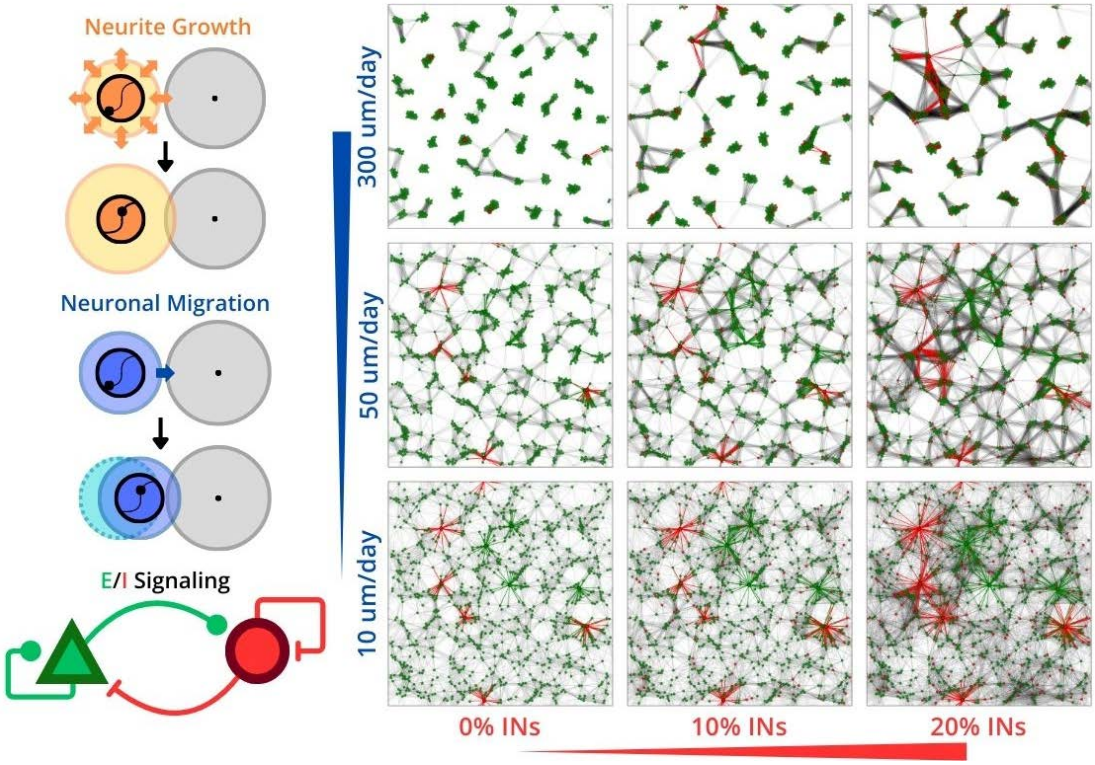
Activity-dependent structural plasticity (ADSP) plays an essential role in the development of neuronal networks. The regulation of neuronal growth and migration by activity introduces a homeostatic control loop involving the developing network structure, connectivity, and activity.
In the course of development, GABAergic signaling transitions from depolarizing to hyperpolarizing, suggesting that excitatory and inhibitory (E/I) interactions interfere with ADSP in network formation. Such interplay becomes particularly relevant for understanding the biological mechanisms underlying neuronal network development, with computational models of cell cultures providing a controlled dynamic environment for exploration. In growth models of spatially constrained networks of excitatory neurons (ENs), activity-dependent neuronal outgrowth and migration interact in shaping network architectures with varying degrees of clustering and modularity. Here, the role of E/I signaling in ADSP-driven regulation of connectivity and activity remains unclear.
This work explores how inhibition contributes to the self-organization of network structure. Extending the computational growth model, we examine the influence of inhibitory neurons (INs) on network development under different migration conditions. The extent of inhibition is determined by the proportion of INs, their synaptic strength, and how they integrate into the mesoscale network architecture. We assess the effects of varying IN fractions on network stabilization and spatial distribution, and we explore how changes in synaptic strength or delayed inhibitory signaling reorient local circuit embedding.
Our simulations demonstrate that asymmetric E/I interactions play a critical role in network development. Due to the homeostatic regulation of activity, inhibition requires compensatory growth and connectivity which delays the developmental stabilization of the network. This can lead to instability in networks that fail to achieve E/I balance.
With the model imposing repellent inhibitory signaling and attractive excitatory signaling, neuron clusters become dominated by ENs and are surrounded by INs, particularly with high migration rates promoting clustering of neurons. In addition, varying E/I synaptic strength affects the manner of spatial embedding of INs. Finally, introducing a delayed maturation of inhibition results in a more random embedding of GABAergic neurons. This underscores the importance of a precisely timed maturation of inhibition for the proper integration of neurons into local circuits.
Our computational framework not only provides insights into the mechanisms underlying network development but also offers a platform for testing hypotheses with experiments in vitro using dissociated cortical cultures.



Interactions among neuronal growth, migration, and E/I signaling shape network architecture. Neurons establish connections to reach homeostatic target activity by expanding neurite fields or migrating towards the direction of excitation. Fast-migrating neurons form well-delineated clusters with enhanced connectivity in networks having larger IN-fraction.

A Novel AI-based Tool for Real-Time USV Detection as Unbiased Markers of Distinct Social Interactions

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Ultrasonic Vocalizations (USVs) are integral in the complex world of rodent communication especially during social interactions. Unfortunately, these interesting communicative cues have remained elusive for a long time, hidden beyond the human range of hearing. Only in the mid-1950s USVs were discovered and became the subject of extensive research.

Notably, researchers identified USVs as reliable biomarkers for neurological conditions, including Autism Spectrum Disorder and Parkinson's Disease. Yet, the process of manually identifying USVs proved challenging and demanded significant human effort. Recent advances in Digital Signal Processing and Machine Learning allowed the development of novel tools offering automatic and semi-automatic identification and analysis of USVs.

Within this context, we will introduce DeepFisFis, a novel end-to-end solution for real-time detection of USVs utilizing a 1-Dimensional Convolutional Neural Network (1D-CNN). Here, we demonstrate the functionality of DeepFisFis by analyzing the USV emission pattern of mice during a social familiarization task monitored in a purpose-built multi-modal experimental arena.

We show that DeepFisFis was able to reliably detect ultrasonic vocalizations of mice. Interestingly, the vast majority of USVs were emitted during periods of intense social contact, classifying USVs as a facile indicator of close social interactions. Notably, the number and total duration of USVs decreased as a function of repeated social interactions, identifying them as ethologically relevant and unbiased biomarkers for social habituation. These findings are corroborated by A-SoiD- guided automated postural analysis, underlining the strong correlation between USV emission during close social contact and suggesting the association between USVs with different types of social behavior in mice.

Estimating Latent Variables of Decision-Making Behavior in Zebrafish Larvae

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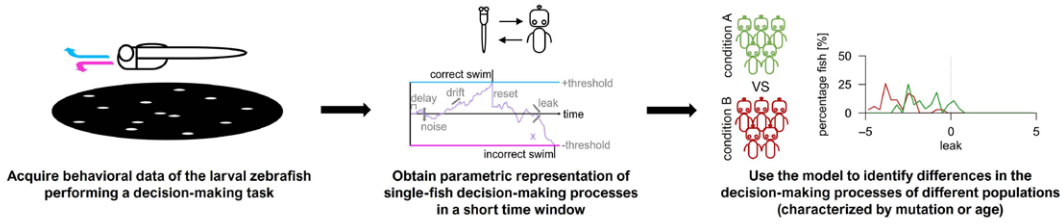
Animals rely on integration strategies to make reliable decisions in natural environments, where they frequently encounter sparse and noisy visual cues. In their 2021 study, Harpaz et al. proposed a behavioral algorithm describing how animals integrate sensory information over time to make decisions. However, how this algorithm varies between individuals and how it is influenced by changes in visual stimuli and internal states of the animal remains unexplored.

To address these questions, we study the decision-making processes underlying the optomotor response of the larval zebrafish (Danio rerio) presented with noisy visual stimuli. We model the behavioral recordings from this well-established experimental paradigm using generalized drift-diffusion models (DDMs). These models assume information is gradually integrated until a threshold is reached, prompting a decision. This strategy has already been successful in capturing behavioral outputs — specifically, swim events— under noisy conditions.

What makes these models especially valuable is their interpretability. With a small set of parameters, each representing a distinct aspect of the decision-making process, the models offer a clear view of the underlying cognitive mechanisms. Once a model is fitted to experimental data, we can thus analyze these parameters as cognitive latent variables that drive different facets of behavior, providing deeper insight into the integration process.

To validate the robustness of our parameter estimation, we benchmarked the fitting procedure using synthetic data generated from various model instances. Our analysis demonstrated that the model parameters can be accurately estimated from small datasets, such as those generated by a single fish in approximately 30 minutes.

We then applied this model at the level of individual fish to examine temporal and inter-individual differences in the integration process. By fitting the model to data from fish with variations in age and genotype, we uncovered significant differences in the distributions of the latent variables across groups, especially in the timescale of integration. This approach provided valuable insights into the variability of decision-making processes across individuals, conditions, and time.



Interaction with third parties shapes courtship behavior in groups of fruit flies

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Courtship behavior is commonly considered a pairwise interaction between a single courter male and a single target female. While courter preferences and target receptivity are key factors in shaping social structure, interactions with third parties, such as rival courters and alternative mating targets, within the group can significantly influence target selection. Compared to the well-studied courtship rituals in pairs, little is known about how flies regulate mate selection in groups. To address this, we introduced a novel assay and an information-theoretic framework to understand the structure of social interactions during courtship behavior in groups of the fruit fly *Drosophila*. We recorded interactions in groups of 8 flies and defined a dynamic social interaction network based on the proximity and alignment of flies.

To quantify males' dependence on third parties in courtship target selection, we compared the group structure in the data to a model where males made independent decisions from other males.

We find that social interactions depend on group composition, genotype, and the availability of sensory information: Group structure in single-sex groups of only males or females or mixed-sex groups in the dark can be explained using an independent model. In contrast, in mixed-sex groups of 4 males and 4 females, the pair of a single male and a single female emerges as the predominant type of interaction occurring more frequently than expected from a model of independent pairwise interactions, with larger group sizes, such as triplets or quadruplets, occurring less frequently. Interestingly, two different wild-type strains of *Drosophila melanogaster*, exhibiting varying levels of aggressive and courtship behavior, show distinct social strategies and higher-order interactions.

A maximum entropy model of the social network that included interaction with third parties, shows that the overexpression of pairs is due to the instability of male-male-female triplets. This instability is because males either defend the female target by repelling rivals or avoid courting a female already being courted by another male. Although repulsive male-male interactions are enough to explain group size distribution, even higher-order interactions are necessary to explain the full structure of the social network.

To investigate the neural circuits that regulate higher-order social interactions, we currently use this information-theoretic approach to quantify how manipulating aggression and courtship circuits modulate the structure of the social network.

Overall, this study enhances our understanding of courtship strategies and social interactions within a complex group scenario. Quantifying interactions with third parties sheds light on the integration of sensory cues and their contribution to courtship choice and enables us to study the underlying neural circuits.

Beyond firing rate homeostasis: How neurons can tune their excitability class using higher-order statistics of the Calcium signal

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Biophysical models of intrinsic neural homeostasis usually focus on stabilising general features such as average firing rates or bursting behaviour. However, recently published analyses of the Drosophila wing motor system shows that beyond periodic spiking, the actual onset bifurcation, i.e. the excitability class, and its associated phase response curve (PRC) are crucial to the functioning of the central pattern generator that controls wing movement. This begs the question of how neurons can tune themselves into the parameter region that shows the correct PRC and supports proper network function. In particular, this is a difficult question if a neuron does not have immediate access to its own onset bifurcation and if it cannot read out the network state.
Here, using a biophysical neuron model we show that in the presence of noise, the intracellular calcium signals contain enough information to identify the appropriate parameter region. Simple rules are sufficient to tune the neuron into this appropriate region following diverse perturbations. A biophysically realistic model supports that tuning the onset bifurcation based on calcium first and second moments is biologically plausible. These results highlight the need for at least two sensors to tune switches in excitability and showcase that noise can play a constructive role in tuning intrinsic neural properties.

Poster Topic

T27: Techniques and Demonstrations

T27-1A MouseFlow: a Python toolbox for high-resolution behavioral tracking in head-fixed mice using optical flow and kinematic analyses
Lam Q. Bui, Felix Kuhn, Janelle M. P. Pakan, Simon Musall, Anne Petzold, Oliver Barnstedt

T27-2A A genetic tool for tracing electrical synaptic connectivity
Rachita Taneja, Stefanie Ryglewski, Carsten Duch, Marion Silies, Christopher Schnaitmann

T27-3A Multiscale light sheet fluorescence expansion microscopy reveals region-specific synaptic innervation of HDB projections within the olfactory bulb
Juan Eduardo Rodriguez Gatica, Ulrich Kubitscheck, Martin K. Schwarz

T27-4A A stimulus and analysis framework for self-consistent estimations of ionic current dynamics from voltage clamp experiments
Lukas Sonnenberg, Stephan Lauxmann, Jan Benda

T27-5A Eliminating the Edge Effect in Neuronal Cell Culture
David Daniel Murphy, Piotr Aleksander Niziolek, Paul Turko

T27-6A Cardiac Dysfunction and Immune Infiltration of Cardiac Tissue in Murine Model of Recurrent Stroke
Laura Kate Ismajli, Polina Bugaeva, Sylwia Piatek, Eduart Temaj, Amido Daugardt, Marco Foddis, Ronja Marion Dörk, Tingting Wang, Amelie Weber, Susanne Mueller, Philipp Boehm-Sturm, Nikolaus Wenger, Christian Hoffmann, Linda Hammerich, Christian Oeing, Christoph Harms

T27-7A Minimally invasive holographic microendoscope for subcellular deep brain imaging
Hana Cizmarova, Sergey Turtaev

T27-1B Limitations of Click Chemistry in Neuroimaging: Non-specific Alkyne Binding in Neurons
Piotr Aleksander Niziolek

T27-2B The quantification of cellular structures in the intact cochlea by advanced sub-micron light sheet imaging
Lennart Roos, Aleyna M. Diniz, Mostafa Aakhte, Anupriya Thirumalai, Elisabeth Koert, Jakob Neef, Bettina Wolf, Constantin Pape, Jan Huisken, Tobias Moser

T27-3B Implementation of the CRISPR/Cas9 toolbox enables targeted genome editing in the parthenogenetic stick insect *Medauroidea extradentata*
Elina Dirksen, Benjamin Altenhein, Sigrun Korsching, Ansgar Büschges, Giulia Di Cristina

T27-4B Analysis of Glial Cell Morphology in EAE Mice and Olfactory Stimulation Experiments Using Advanced Imaging and Tissue-Clearing Technologies
Insa Gudrun Kreimer, Hanna Hartwig, Greta Hartmann, Hannah Gäb, Anne-Wienke Nissen, Charlotte Schubert, Manuel Friese, Daniela Hirnet, Christian Lohr

T27-5B An innovative approach for conducting 3D electrophysiological recordings within intact organoids
Kerri Kukovetz, Tom Stumpp, Sara Mirsadeghi, Michael Mierzejewski, Angelika Stumpf, Haein Chang, Udo Kraushaar, Ali Hosseini, Michele Giugliano, Jenny Hsieh, Peter Jones

T27-6B Oxygen imaging of hypoxic pockets in the mouse cerebral cortex
Felix Ralf Michael Beinlich, Antonios Asiminas, Verena Untiet, Zuzanna Bojarowska, Virginia Plá, Björn Sigurdsson, Vincenco Timmel, Lukas Gehrig, Michael H. Graber, Hajime Hirase, Maiken Nedergaard

T27-1C Inverse BiPOLES: Expanding the Toolkit for
Bidirectional Optogenetic Control of Neuronal Activity
Yilmaz Arda Ates, Niklas Meyer, Johannes Vierock, J. Simon Wiegert

T27-2C Fused Fiber Photometry 2.0:
intensiometric and ratiometric monitoring of neuronal activity
Alexander Dieter, Andrey Formozov, Andrei Kalinichenko, Marton Molnar, Lena Susann Eschholz, J. Simon Wiegert

T27-3C ChReef – An improved ChR for Future Optogenetic Therapies
Alexey Alekseev, Victoria Hunniford, Maria Zerche, Aida Garrido-Charles, Isabel Witzke, Kathrin Kusch, Tobias Moser, Thomas Mager

T27-4C Investigating fluorescent neurotransmitter sensor dynamics using fast patch-clamp fluorometry
Latife Sönmez, Tim Ziebarth, Laura Moreno Wasielewski, Stefan Pollok, Andreas Reiner

T27-5C RNA splicing revisited: New molecular tools for analysis of cryptic splice donors at single cell resolution
Magnus Harnau, Barbara Schweisstahl, Leonie Emde, Steffen Fricke, Jochen Meier

T27-1D Advancements towards high-throughput array tomography for hippocampal circuit analysis
Pelin Ayyildiz, Laura-Jane Neßler, Rina Patel, Silvia Viana Da Silva, Matthias Haberl

T27-2D Optimizing Viral Transduction of the Locus Coeruleus: A Comparison of Model Systems and Strategies
Lena Susann Eschholz, Chantal Wissing, Maxime Maheu, Kathrin Sauter, Fabio Morellini, J. Simon Wiegert, Alexander Dieter

T27-3D Epidural focused ultrasound stimulation in a rodent model of depression
Lisa Ratz, Lidia Miguel Telega, Tiago Costa, Volker Arnd Coenen, Máté Daniel Döbrössy

T27-5D Primary Neuronal Cell Culture in Ambient CO_2
John Carl Begley, Yi Lien, Camin Dean, Paul Turko

MouseFlow: a Python toolbox for high-resolution behavioral tracking in head-fixed mice using optical flow and kinematic analyses

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There is a growing appreciation that a deep understanding of brain function requires analyzing detailed and complex behaviors. In rodent studies, animals are typically head-fixed to allow for stable optical or electrophysiological recordings. High-resolution cameras are increasingly used to capture these behaviors, but analyzing the resulting data poses significant challenges. To address this, we introduce MouseFlow, an open-source Python tool designed to quantify subtle facial and body movements with minimal user effort. By leveraging machine learning techniques alongside kinematic analysis and computer vision algorithms, MouseFlow simplifies the process of extracting fine-grained behavioral insights.

MouseFlow employs a pre-trained, highly generalizable DeepLabCut network to faithfully track features such as pupil diameter, eye movements, and blinks, regardless of camera angle or lighting conditions. Additionally, this network allows for automatic segmentation of key facial regions, including the whisker pad, nose, and mouth. To address limitations posed by single-marker approaches for inferring global movements, we use dense optical flow algorithms to quantify both the magnitude and direction of movements such as whisking and sniffing. This approach allows for high-fidelity measurement of whisking and sniffing activity, providing detailed information on their frequency and phase. MouseFlow also tracks changes in mouse gait at different treadmill speeds, revealing distinct movement patterns that are related to neural activity across various brain regions.

By providing a simple yet powerful tool for fine-scale behavior quantification, MouseFlow enables researchers to study detailed behaviors in head-fixed rodents and better understand the relationship between specific behaviors and neural activity.

A genetic tool for tracing electrical synaptic connectivity

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In the nervous system, neurons establish synaptic connections to transmit information to one another. These connections can be either chemical synapses, which rely on neurotransmitter signaling, or electrical synapses, which enable direct communication between cells via ion and metabolite exchange. Electrical synapses are formed by gap junctions, which consist of homo- or heteromeric hemichannels made of innexin or connexin proteins that dock between adjacent cells. Recent advances in technology have enabled neuronal reconstructions and connectivity analysis at the whole-brain level in various organisms using ssSEM (serial-section scanning electron microscopy). While these datasets provide detailed annotation of chemical synapse networks, electrical connectivity between neurons remains largely elusive. Although several methods have been developed to identify and characterize gap junctions, none of these approaches enable non-invasive, high-throughput and cell type specific tracing of electrically coupled neurons. Here, we present a genetic method for tracing electrical coupling based on the diffusion of small molecules (<1kDa) in *Drosophila melanogaster*. Our approach involves the targeted expression of a non-endogenous enzyme in specific cell types. This enzyme catalyzes the conversion of a naturally abundant substrate into a small, non-endogenous molecule capable of diffusing through gap junctions into electrically coupled neurons. The spread of this small molecule is detected post-hoc through immunohistochemistry using a highly specific antibody. We are currently testing this method within the giant fiber escape circuit, a well-characterized electrically coupled circuit responsible for mediating escape behavior in the fruit fly.

Multiscale light sheet fluorescence expansion microscopy reveals region-specific synaptic innervation of HDB projections within the olfactory bulb

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Sensory perception is modulated in a top-down manner by higher brain regions and consequently shapes behavioral responses. Notably, the horizontal limb of the diagonal band (HDB) in the basal forebrain modulates olfactory bulb (OB) output computations in a top-down fashion. To better characterize in 3D the synaptic target sites of different HDB (Parv-, Chat-, and Vglut1-positive) from meso- to nanoscale-resolution, we differentially labeled them with fluorescent proteins. For subsequent circuit analysis we then employed an optimized light sheet fluorescence expansion microscopy (LSFEM) protocol (1,2).

Using this optimized LSFEM method (3) allowed us to bridge mesoscopic to nanoscale (super-resolution) resolutions and an accurate quantification of differential HDB fiber distribution plus their synaptic innervation within OB subregions.

To overcome challenges in imaging expanded samples exceeding the high-resolution objectives working distance, we combined LSFEM with automated serial expanded-block sectioning using a purpose-built microtome. This novel setup allowed us to image expanded samples with extended axial extensions (~1cm^3 after expansion).

Here we present a novel pipeline for expanding, clearing, and analyzing large neuronal tissue samples with expansion factors of 1.5 to 4-fold. Our technique allow us to reveal the differential innervation of OB subregions by distinct HDB projection subtypes, highlighting the complexity in top-down modulation of olfactory sensation by HDB.

These results are in line with two-photon *in vivo* Ca^{2+}-imaging in awake head-fixed mice which revealed sensory-driven activity in a majority of HDB axons in the glomerular layer.

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A stimulus and analysis framework for self-consistent estimations of ionic current dynamics from voltage clamp experiments

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Voltage clamp experiments are gold standard for studying voltage-dependent ion channel dynamics, but their interpretation is complicated by intrinsic limitations and artefacts. A major issue is the trade-off between voltage error and intrinsic noise: large ionic currents increase voltage error, distorting the data, while small currents suffer from poor signal-to-noise ratios. Additionally, the presence of series resistance between the amplifier and the cell, as well as the capacitance of the patch pipette, hinders direct measurement of the membrane voltage, making it difficult to accurately extract ion channel dynamics. Traditional voltage clamp step protocols can only sample discrete voltages for these dynamics and often cannot be used to provide information for the relevant voltage range around the spiking threshold. For example, sodium inactivation can only be measured at voltages around -30 mV or higher, while its subthreshold counterpart, sodium channel recovery, requires long and tedious stimulation protocols, especially when applied across multiple voltage steps. Often times, the results of these protocols are also not self-consistent. For example an activation curve, estimated from step stimuli, cannot reproduce the same activation curve when experimental protocols are tested in simulations. Overall, step protocols can fail to capture ion channel dynamics in a self-consistent manner, or require excessively long stimulation protocols that are impractical for standard experimental conditions.

To adress these challenges, we developed a comprehensive stimulation and analysis framework, aimed at improving the precision, efficiency and stimulation time of voltage clamp experiments. First, we parameterized the pipette and cell properties, allowing us to mitigate artefacts and achieve a more reliable estimation of membrane voltage. Second, we designed short, randomized stimulation protocols that efficiently sample the full range of ionic current dynamics while minimizing experimental time. Finally, we implemented a model-fitting framework that uses the acquired data to predict ionic current behavior, providing a more robust and accurate representation of the underlying dynamics.

This framework reduces the stimulation time for sodium activation and inactivation dynamics from minutes to about 10 seconds. It also provides ionic current dynamics in the threshold region, which was previously difficult to access. Properties of slow inactivation can be estimated for the inactivation curve, but also their corresponding time constant over a wide voltage range, which was previously not possible with traditional step stimuli.

Overall, this integrated approach significantly enhances the reproducibility and precision of ion channel measurements in voltage clamp experiments and their subsequent analysis. It offers a powerful tool for predicting ion channel behavior, with broad applications in understanding disease mechanisms and in the prediction of pharmacological effects on channel and neuronal level.

Eliminating the Edge Effect in Neuronal Cell Culture

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The edge effect is a persistent problem in cell culture, characterized by cultures exhibiting different properties at the edges of multiwell plates compared to the center such as differential growth rates, metabolic activity, responses to treatment and reduced neuronal cell density. Variations in these qualities across the plate can either result in unfair comparisons between groups or, if groups are formed to ensure fair comparison, lead to high intragroup variability. Low inter-well variation facilitates efficient assays by reducing the need for excessive sample sizes to achieve the required statistical power, thereby facilitating high-throughput methods. We have identified reduced neuronal density due to the edge effect in rat mixed neuron-glia cultures, with 50% greater density in the central wells compared to the corners. Methodologically reducing this variability within neuronal cell cultures would have a substantial impact on research quality, increasing efficiency, reducing sample sizes and thereby saving animals, money, and critically, time.

A clearly identifiable factor was greater evaporation in the edges and corners of the well plate, the pattern of which was found to inversely correlate to the decrease in neuron density. We developed a robust, simple and cheap intervention preventing evaporation across the multiwell plate. Using standard laboratory parafilm, sterilized with hydrogen peroxide, without stretching the parafilm, we sealed the plate. This method, while preventing evaporation, failed to ameliorate the edge effect, suggesting that factors other than evaporation were responsible.

Neurons are particularly sensitive to pH shift, at ambient CO2 neurobasal A culture medium will become more alkaline, causing toxicity to neurons. We hypothesised that exposure to ambient CO2 during feeding and lowered CO2 levels within the incubator due to door openings could result in an alkaline shift of the culture medium that could be greater in the edge and corner wells of the plate, leading to the observed edge effect. We then implemented two interventions to prevent this. We stopped all feeding of cultures and began keeping culture plates within sealable airtight containers in the incubator. Prior to these interventions we had found the 4 central wells of the plate to have 50% greater neuronal density compared to the 4 corner wells, this difference was found to be reduced to 20% greater neuronal density in the central wells by implementing these methodological adjustments.

Following this, we observed that in corner wells of the plate there was an uneven distribution of neurons where cells had accumulated against the well edge nearest to the edge of the well plate, hypothesised as being caused by exposure to thermal gradients following seeding (Lundholt et al., 2003). To counteract this, we let the newly seeded plates incubate at room temperature to allow the cells to settle before being exposed to the thermal gradients within the incubator. This was done in conjunction with glass-bottomed plates to maximize the even distribution of cells. Preliminary findings from the additional use of these methods, further reduced the edge effect to only 10% greater cell density in the central wells compared to the corners.

Cardiac Dysfunction and Immune Infiltration of Cardiac Tissue in Murine Model of Recurrent Stroke

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Introduction: Recurrent stroke occurs in approximately 19% of patients following incident stroke, and as high as 54% in patients with pre-existing cardiac conditions. In addition to the known mechanism of cardiovascular disease leading to stroke, many cardiac complications, such as Takotsubo syndrome, sudden cardiac death, or cardiac muscle damage have also been reported following both ischemic and hemorrhagic stroke. Following damage to the Heart-Brain axis, activation of systemic inflammation and autonomic dysregulation may lead to further cardiac injury, rather than repair. The combination of cardiovascular damage and immune infiltration into the heart tissue has not been previously studied in recurrent ischemic stroke and may have severe implications on further cerebrovascular events and health.

Methods: Twelve-week-old male and female mice underwent a 30-minute cerebral artery occlusion (MCAo) with reperfusion, followed by a distal middle cerebral artery occlusion (dMCAo) two weeks later, and perfusion 35 days following the recurrent stroke. Stroke lesion size was accessed 24 hours after each stroke on T2-weighted MR images. Retroorbital blood withdrawal was performed after MRI to examine the blood for presence of troponin. One day prior to dMCAo and perfusion, cardiac function was accessed using Echocardiography (EchoCG) to determine if changes in heart structure or systolic and diastolic function had occurred. Following perfusion, immune cells from heart tissue was extracted and examined using spectral flow cytometry.

Results: Four groups of mice with different stroke conditions were obtained but showed no statistically significant differences in macrophage infiltration. Additionally, no group had elevated troponin levels, nor did troponin levels correlate with stroke lesion size.

In summation, more risk factors including age, metabolic diseases, or hematological conditions are needed in order to model Stroke-heart syndrome in mice. Future directions aim to investigate stroke-heart syndrome in aged animals and animals harboring mutations in immune cells.

Minimally invasive holographic microendoscope for subcellular deep brain imaging

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In-vivo observations of neuronal cells and their interactions are essential for understanding brain functions and advancements in diagnostics and treatment of neuronal disorders. Over the last two decades, optical microscopy fueled neuroscience research by enabling routine visualization of biological structures and functions at the sub-cellular level.
The ongoing challenge is to extend the reach of high-resolution imaging methods towards the deeper brain regions, where the complexity of light transport in living tissue lead to severe optical aberrations. Modern multiphoton microscopes with active aberration correction techniques are already capable of accessing the whole cortical region in small animal models. The alternative approach allows for bypassing of optical distortions by implanting thin endoscopes, usually based on graded-index lenses, directly into the deeper brain regions, where removing the overlaying tissue could be tolerable for the functions under study.
Recent studies propose the use of holographic control of light transport through multimode optical fibres, turning a single optical fibre into an imaging element for much less traumatic application and superior imaging performance to reach deep and sensitive tissues.

Here we present a compact design of a holographic endoscope that enables laser-scanning fluorescent imaging at the tip of a hair-thin fibre probe. Relying on active wavefront shaping, the device is capable of random-access observations across a field of 100um in diameter with submicrometric lateral resolution and on-the-fly adjustment of the focal distance.
We demonstrate in-vivo imaging of fluorescently labelled neurons, in anaesthetized as well as awake animal models acquired through an acute insertion up to the depth of 5mm. We present high-resolution imaging of subcellular structures like dendritic spines and recording of neuronal activity with calcium and voltage indicators in deep structures like amygdala, ventral tegmental area and ventral hippocampus.
Postmortem analysis of insertion-induced inflammation indicates lower tissue damage compared to state-of-the-art endoscopic solutions.

The compact holographic microendoscope is developed by a biophotonics company DeepEn GmbH, that is a spin-off of Leibniz Institute of Photonic Technology in Jena, Germany. The technology has been in advanced through research groups at Leibniz Institute of Photonic Tehcnology Jena, Germany and Institute of Scientific Instruments of the CAS, Brno, Czechia. DeepEn aims to bring it into practical use in neuroscience laboratories and thus promote advances in neuroscience research.

Limitations of Click Chemistry in Neuroimaging: Non-specific Alkyne Binding in Neurons

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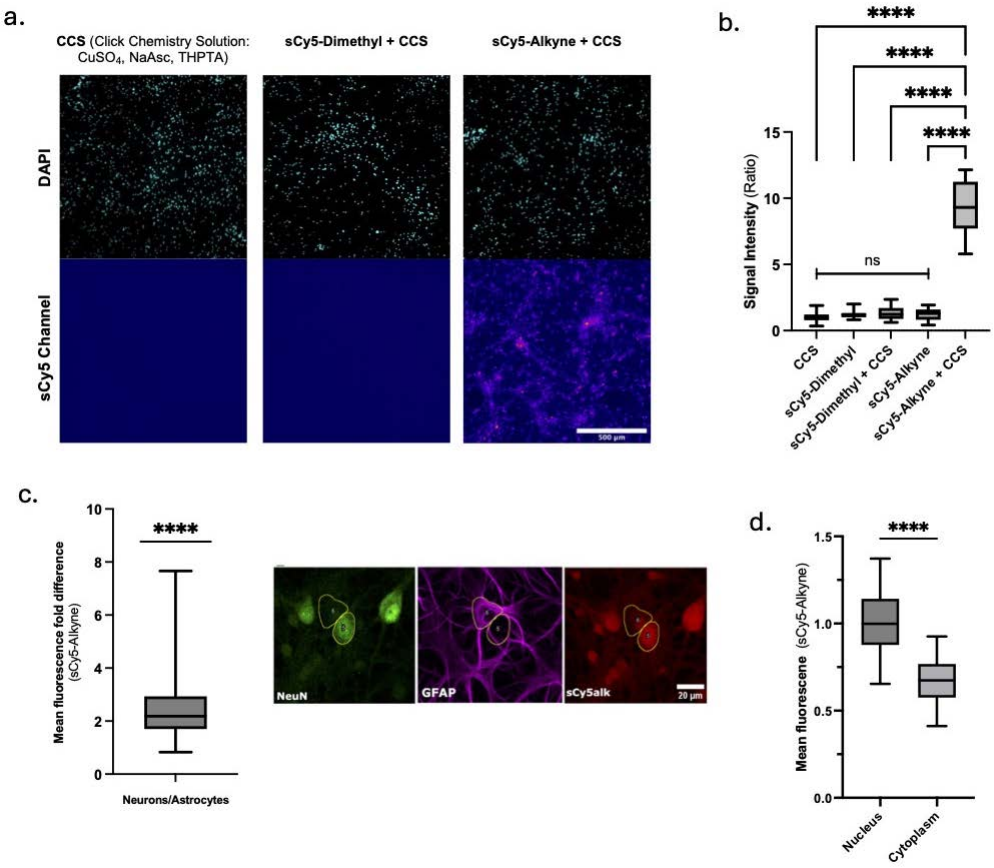
Click chemistry, awarded the Chemistry Nobel Prize in 2022, is a widely used method for highly selective labelling of biomolecules under mild conditions and using simple reagents.
The most famous example of a click reaction is the copper-catalysed azide-alkyne cycloaddition (CuAAC), in which an azide group and a terminal alkyne group bind together to form a 1,2,3-triazole ring. In order to perform the staining, one of the functional groups is introduced into the cells while the other is conjugated to the fluorophore.
The main advantage of click chemistry reactions is their bio-orthogonality of the reagents, which means that they can be carried out in living organisms without interfering with them. Unfortunately, we have found that contrary to popular belief, this method is not specific when used in brain tissue or neuronal cell cultures (a,b). Using immunocytochemistry and confocal microscopy we were able to establish that fluorophores containing terminal alkyne bind non-specifically and with more than twice the affinity to neurons than to other cells, producing a high background signal (c). The distribution of the signal in the cell is also not uniform - higher in the nucleus than in cytoplasm (d). This suggests that neurons contain a specific molecule that is a binding partner for the alkyne.
We determined that among the different functional groups used in click chemistry reactions, only the terminal alkyne leads to non-specific binding. To prevent this problem we searched for the binding partner in the cells and ways to block it.
Based on chemical properties, cysteine was tested as the reaction partner. We found that the terminal alkyne reacts with the free thiol group of cysteine (alkyne cannot bind to the methylthiogroup of methionine). The use of thiol blockers such as n-ethylmalemide significantly reduces but does not prevent non-specific binding. It suggest that cysteine is a binding partner but not the dominant one.
Further experiments showed that iodide-containing molecules, such as thyroid hormones, increase the unspecific signal in the tissue. The higher the iodide content in the molecule, the greater the increase in binding (triiodothronine 3-fold and thyroxine 5-fold), suggesting a direct interaction between the iodide and the alkyne.
Together these findings show that the CuAAC reaction interferes with the neuronal cell environment by binding to functional groups such as thiols and idodide present in thyroid hormones, leading to unspecific binding and false results. In our project, we present the potential mechanism of non-specific binding and solutions to optimise labelling.


a.
CCS (Click Chemistry Solution: CuSO4, NaAsc, THPTA)
sCy5-Dimethyl + CCS
sCy5-Alkyne + CCS
DAPI
sCy5 Channel
500 µm
b.
****
****
****
****
Signal Intensity (Ratio)
15
10
5
0
ns
CCS
sCy5-Dimethyl
sCy5-Dimethyl + CCS
sCy5-Alkyne
sCy5-Alkyne + CCS
c.
****
Mean fluorescence fold difference (sCy5-Alkyne)
10
8
6
4
2
0
Neurons/Astrocytes
NeuN
GFAP
sCy5alk
20 µm
d.
****
Mean fluorescene (sCy5-Alkyne)
1.5
1.0
0.5
0.0
Nucleus
Cytoplasm

The quantification of cellular structures in the intact cochlea by advanced sub-micron light sheet imaging

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[8]University of Göttingen, Multiscale Biology, Department of Biology and Psychology, Göttingen, Germany
[9]University Medical Center Göttingen, Auditory Circuit Lab, Göttingen, Germany
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According to the World Health Organization (WHO), a growing number of people worldwide, about 0.5 billion, currently suffer from disabling hearing loss (HL). While the causes of HL are diverse, many originate in dysfunctions of the inner ear. Recent improvements in light sheet fluorescence microscopy allow nanometric examination of the whole intact cochlea in immunohistochemistry. These findings may help to identify and quantify disease mechanisms at a new scale and aid in the design of novel treatments and their efficacy in the cochlea of patients with severe HL. In this study, we evaluate the nanoarchitecture of the cochlea utilizing a cutting-edge, custom-built cleared tissue light sheet microscope (CTLSM), which provides an isotropic resolution of 0.8µm with yet unprecedented clarity and detail. Native and genetically modified mouse cochleae were decalcified, immunolabelled, and cleared using an adapted iDisco$^+$ protocol. The generation of robust immunolabelling protocols, together with the innovative CTLSM enables the study of distinct fluorescence patterns at a cellular to subcellular level. To quantify cochlear architecture, such as spiral ganglion neurons (SGNs) and hair cells, we established a U-Net-based machine learning algorithm to detect and segment cellular structures. The high, isotropic resolution of the CTLSM allows for precise analysis of SGN density, their subtypes and targeted therapies along the Rosenthal's canal. Here, machine learning based automated segmentation allows us to quantify the number and spatial distribution of SGNs using models uniquely adapted and trained on data from the new CTLSM. Thus, we can report first automated SGN counts of 7,394 cells/ cochlea, in agreement with previously reported SGN counts of the whole mouse cochlea of 9,106 ± 724 (Keppeler et al., PNAS, 2021: doi: 10.1073/pnas.2014472118.) and 7,920 ± 4,230 SGNs (Duque Afonso, Doctoral Dissertation, 2020: doi.org/10.53846/goediss-8106). For the first time, we can also map inner hair cells and their synapse distributions throughout the whole intact cochlea, with the aim of adapting our AI-based data analysis to these and other cell types, and subcellular structures. Nanoscale analysis of cochlear architecture will contribute to the understanding of hearing and its disease mechanisms, as well as to improving hearing restoration e.g., by future auditory prostheses.

Implementation of the CRISPR/Cas9 toolbox enables targeted genome editing in the parthenogenetic stick insect *Medauroidea extradentata*

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The understanding of neural circuit composition and action can markedly profit from species-specific availability of genetic tools enabling researchers to manipulate an animal's genome. Ideal are combinations of electrophysiological recordings with fluorescent tagging or optogenetic control of specific neuronal populations. Compared to other insects, the stick insect presents an ideal model-organism for studies of motor control due to its easily accessible nervous system. However, no genetic tools were available for stick insects so far.

Here, we optimize and apply CRISPR/Cas9 (Jinek et al. 2012) as a technique to modify the genome of the stick insect *Medauroidea extradentata*. Their parthenogenetic life cycle with its initial haploid developmental stage quickly leads to the efficient generation of a genetically modified and self-reproducing isogenic line. For proof of principle, we targeted genes involved in the ommochrome pathway of eye pigment transport and synthesis (*cinnabar* and *white*; Summers et al. 1982), performing microinjection of eggs within 24h after oviposition to generate homozygous knockout mutants. We observe highly efficient gene targeting for both genes.

Cinnabar (*Mexcn*) and *white* (*Mexw*) knockouts resulted in distinct eye and cuticle colour phenotypes. Homozygous *Mexcn* knockouts showed white eyes and cuticle, but died during the process of hatching. However, one mosaic animal reached adulthood and laid eggs, consequently producing viable homozygous knockout mutants. This shows that induced CRISPR/Cas9 events are stable and can be transmitted to the next generation by parthenogenetic mechanisms. Homozygous *Mexw* knockout resulted in a completely unpigmented and transparent embryonic phenotype, but was lethal in later embryonic stages shortly before hatching.

Follow-up experiments focused on the efficient integration of exogenous DNA by testing for the different pathways of double-strand-break repair, finally revealing that microhomology-mediated end joining (MMEJ) efficiently integrates short co-injected DNA templates (6bp) at the cutting site.
This insight now builds the foundation for the potential creation of fusion proteins that are needed for the experimental approaches mentioned above.

In conclusion, we showed that CRISPR/Cas9 can be successfully applied on the genome of *M. extradentata* by creating mutants with distinct phenotypes and first targeted knock-in events. This genetic toolbox can now be employed and further developed to create genetically modified lines to enable further unravelling of motor control (for recent review see Bidaye et al. 2018) using state-of-the-art methods in an upcoming parthenogenetic model organism.

Analysis of Glial Cell Morphology in EAE Mice and Olfactory Stimulation Experiments Using Advanced Imaging and Tissue-Clearing Technologies

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Glial cells, including astrocytes and microglia, are essential regulators of central nervous system (CNS) function, maintaining homeostasis and modulating neuronal activity. Their morphology and activation states are highly responsive to both inflammatory processes and environmental stimuli, such as enriched environments and learning tasks. These changes offer crucial insights into disease mechanisms and glial plasticity in both healthy and pathological conditions.

This project investigates the effects of inflammatory processes on microglia in an experimental autoimmune encephalomyelitis (EAE) model as well as potential morphological adaptations of astrocytes in response to olfactory stimulation in the olfactory bulb of mice. For the EAE model, immunohistochemical labeling is performed using anti-GFAP (Glial Fibrillary Acidic Protein) antibodies to reveal astrocytic filamentous structures and anti-Aquaporin-4 antibodies to assess astrocytic polarization and water regulation. Microglial activity is visualized using anti-Iba1 (Ionized Calcium-Binding Adapter Molecule 1) antibodies, a marker that reflects various microglial activation states. anti-Collagen IV antibodies are used to evaluate the integrity of blood vessels by labeling the basal lamina of the blood-brain barrier, helping to identify potential disruptions under disease conditions.

The application of X-Clarity tissue-clearing technology allows deep-tissue visualization by removing lipids and rendering the tissue transparent, thereby enabling high-resolution imaging through several hundred micrometers of intact brain tissue. The cleared samples are then analyzed with the advanced 3D image analysis software IMARIS, which provides a comprehensive set of tools for quantifying and visualizing glial morphology. IMARIS's 3D Surface Rendering, Filament Tracer, and Volume Rendering modules facilitate precise morphometric analyses, such as measurements of filament structure, branching complexity, and cell volume. The Sholl-Analysis module is used to evaluate the complexity and density of cellular processes, while the Colocalization tool assesses spatial relationships between glial cells and surrounding structures. Automated cell detection and segmentation minimize user bias and ensure reproducibility across experiments.

The combination of tissue-clearing technologies and advanced image analysis will provide a more refined understanding of glial cell adaptations to various environmental and pathological stimuli, offering new insights into their roles in CNS health and disease. Ultimately, this project aims to lay the foundation for insights that target glial function in neuroinflammatory and neurodegenerative diseases.

An innovative approach for conducting 3D electrophysiological recordings within intact organoids

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Organoid technology is a pivotal tool for exploring human physiology and diseases. Despite its potential, current readout capabilities constrain organoid electrophysiological research. Classical microelectrode arrays (MEA) fall short in capturing data from intact organoids, which may flatten in the 2D-MEA surface, jeopardizing physiological responses and data validity. To overcome this, we pioneered a mesh MEA, reducing morphological deformations, fostering 3D growth, and facilitating electrical activity recording within intact organoids over an extended period. Electrophysiological recordings of human brain organoids were performed in an MEA-2100 head stage from MultiChannel Systems, accommodating classical MEA and mesh MEA chips. Extracellular neural activity, sampled at 25 kHz and filtered at 400 Hz for spike detection, accurately reflected action potential events on the membrane. Neuronal migration around the mesh was monitored using light microscopy. The mesh MEA integrates 60 titanium nitride electrodes (30 µm diameter) at the nodes of a 2D polymer mesh with a pitch of 200 µm and filament width of ~20 µm and thickness of ~10 µm. The mesh scaffold is suspended 2 mm from the bottom of the well. From preliminary measurements, spike time analysis revealed heightened activity after seven days on the mesh MEA (mean firing rate 34 Hz) compared to acute recordings on a classical MEA (5 Hz). Microscopy images illustrated neuronal migration, dendritic growth, and axon development around the mesh structure and electrodes. These findings suggest that the mesh MEA holds great promise for comprehensive, long-term organoid electrophysiological studies, providing deeper insights into human functions and disorders.

Oxygen imaging of hypoxic pockets in the mouse cerebral cortex

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Loss of consciousness occurs within seconds when cerebral blood flow stops, as the brain cannot store oxygen, leading to fatal interruption of oxidative phosphorylation within minutes. However, our understanding of cortical partial oxygen tension (Po_2) dynamics under physiological conditions remains limited. In this study, we present the Green enhanced Nano-lantern (GeNL), a genetically encoded bioluminescent oxygen indicator for Po_2 imaging. Using this tool in awake, behaving mice, we discovered spontaneous, spatially defined “hypoxic pockets” and demonstrated their connection to local capillary flow disruption. Exercise was found to reduce the occurrence of hypoxic pockets by 52% compared to rest. This research offers insights into cortical oxygen dynamics in awake animals and introduces a tool to explore the role of oxygen tension in physiological processes and neurological disorders.

Inverse BiPOLES: Expanding the Toolkit for Bidirectional Optogenetic Control of Neuronal Activity

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In neuroscience, the ability to precisely manipulate neuronal activity is critical to understanding complex neural networks. The development of optogenetic tools has revolutionized the field. Modern tools have been optimized to allow multimodal manipulations, such as bidirectional two-color control of neuronal excitation and inhibition with BiPOLES, which consists of a red-light-sensitive cation channel and a blue-light-sensitive anion channel. Building on this foundation, we introduce inverse BiPOLES (iBiPOLES), an optogenetic construct designed for bidirectional control of neurons using spectrally opposite action spectra compared to the original BiPOLES. iBiPOLES consists of the blue light sensitive cation channel Chrome2S for neuronal excitation and the red shifted anion channel raACR for neuronal inhibition.
When used in conjunction with the original BiPOLES, iBiPOLES extends the possibilities for neuronal manipulation. It allows for mutually exclusive excitation and inhibition of two neuronal populations, providing a versatile tool for complex experimental designs that test the function of one neuronal population of interest independently of a second, distinct population of neurons. In the future, these tools may be useful for disentangling the contribution of small, molecularly defined nuclei in close proximity to each other, such as brainstem neuromodulatory centers, to various physiological functions of the brain. Future development of bidirectional optogenetic actuators with action spectra compatible with optical voltage or calcium indicators and other optogenetic tools opens new avenues for multidimensional control of neural circuits.

ChReef – An improved ChR for Future Optogenetic Therapies

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Optogenetic approaches carry huge potential for future therapies, because they enable remote control of excitable cell activity with unique spatiotemporal resolution. Due to the low single-channel conductance of ChRs (1), which can be employed for excitable cell photostimulation, the optogenetic control of cellular activity relies on a combination of strong ChR expression and high light intensity stimulation. This is particularly relevant for clinical applications as it bears a risk for proteostatic stress and phototoxic effects. In this regard, ChRmine, a recently identified so-called bacteriorhodopsin-like-cation channelrhodopsin (BCCR; (2)) is of great interest. ChRmine is optimally activated with green light and shows large photocurrents. Using a high performance automated patch-clamp system (Syncropatch 384, Nanion), which we operated in synchrony with LED-based illumination, we recently showed by noise analyses that the single channel conductance of ChRmine is considerably bigger than the single channel conductance of the state-of-the-art ChR CatCh. However, ChRmine utility is impaired by a strong, light dependent desensitization, which can be mainly attributed to a light-dependent inactivation process, which resembles a substrate inhibition of the partial type. The noise analyses moreover revealed that photocurrent inhibition by light is likely associated with the presence of a parallel photocycle open state of lower conductance and/or low open probability. We recently engineered the ChRmine mutant T218L/S220A, which we named ChReef (“ChR that excites efficiently”). In ChReef the light-dependent inactivation process that resembles a substrate inhibition of the partial type, was abrogated by the mutations, which led to a pronounced reduction of photocurrent desensitization. Moreover, comparative experiments revealed that the stationary photocurrent of the ChRmine mutant ChReef was considerably larger than the photocurrents of other widely-used ChR variants. We therefore anticipated an increase in the efficiency of sustained excitable cell photostimulation and accordingly assessed the suitability of ChReef for future optogenetic therapies, thereby focusing on cardiac defibrillation, vision restoration and hearing restoration.

1 Feldbauer, K. et al. Channelrhodopsin-2 is a leaky proton pump. Proc Natl Acad Sci U S A 106, 12317-12322, doi:10.1073/pnas.0905852106 (2009).
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Investigating fluorescent neurotransmitter sensor dynamics using fast patch-clamp fluorometry

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Neurotransmission is a fast process with many receptors responding in the millisecond to subsecond range. The kinetics of ionotropic receptors have been well studied over the last decades using electrophysiological methods, whereas the kinetics of GPCRs are less easy to measure. Moreover, little is known about the actual concentrations and dynamics of the various neurotransmitters. Current progress in the field is driven by the development of genetically encoded fluorescent sensors, which change their fluorescence in response to ligand binding. This can be used to detect when and where in the nervous system neurotransmitters are released. Over the last years, many sensors have been designed for different neurotransmitters, such as *sDarken* for serotonin (5-HT) or nLightG for norepinephrine (NE). To measure the kinetics of these GPCR-based sensors we combined fast perfusion with patch-clamp fluorometry (fast-PCF). Fast, piezo-driven perfusion has originally been used to measure the kinetics of ligand-gated ion channels in outside-out patches. For this, the pipette containing the patch is directly positioned in front of a double-barreled Θ-glass pipette, with one channel containing the ligand and the other containing extracellular solution. Through a piezo-driven movement of the pipette, ligands can be applied and removed within milliseconds. We combined these fast electrical recordings with EMCCD camera-based imaging (100-200 Hz).
Using fast-PCF, ligands can be rapidly applied and removed, yielding direct information on the resulting ON and OFF kinetics. For instance, the 5-HT sensor *sDarken* responded to 100 µM 5-HT with single-exponential kinetics of τ_{ON} = 43.5 ± 9.7 ms and τ_{OFF} = 323 ± 61.5 ms [1]. Additional experiments, in which we varied the ligand concentration, showed that the ON kinetics became faster with increasing concentration until reaching a plateau, whereas the OFF kinetics were concentration-independent. This behavior can be explained by a simple kinetic model that describes ligand binding and subsequent conformational changes in the sensor as two distinct steps. Next to the *sDarken*, we used fast-PCF to compare the kinetics of two recent NE sensors, nLightG and $GRAB_{NE1m}$ [2]. nLightG, which is based on α1a adrenergic receptor, showed faster kinetics (τ_{ON} = 23 ± 5 ms, τ_{OFF} = 194 ± 42 ms at 5 µM NE) compared to $GRAB_{NE1m}$, which is based on α2a adrenergic receptor (τ_{ON} = 192 ± 23 ms, τ_{OFF} = 593 ± 118 ms at 5 µM NE). In summary, fast-PCF is a useful method to monitor rapid changes in fluorescence upon ligand binding and unbinding and thus provides valuable information for the kinetic characterization of new fluorescent sensors. This information may also be useful for guiding their application in various experimental settings.

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2. Kagiampaki, Z., Rohner, V., Kiss, C., Curreli, S., Dieter, A., Wilhelm, M., Harada, M., Dus, S. N., Dernic, J., Bhat, M. A., Zhou, X., Ravotto, L., Ziebarth, T., Wasielewski, L. M., Sönmez, L., Benke, D., Weber, B., Bohacek, J., Reiner, A., Wiegert, J. S., Fellin, T. & Patriarchi, T. (2023). *Nature Methods*, 20: 1426.

RNA splicing revisited: New molecular tools for analysis of cryptic splice donors at single cell resolution

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RNA splicing and editing contribute to functional diversification of proteins encoded by single genes. Previous and current research primarily focus on sequencing of bulk material and bioinformatics to identify editing and splice sites, but these technologies ignore by nature the role of individual cell types in the regulation of RNA splicing and editing. We present new molecular tools for analysis of RNA splicing at single cell resolution using synthetic and natural introns. We show that cryptic splice site usage is not that rare than assumed previously, because the results identify consensus sequence motifs involved in effective cryptic RNA splicing. Furthermore, cellular heterogeneity of cryptic splice site usage demonstrates cellular regulation of this type of RNA processing and may reveal a novel dimension of cellular regulation by RNA processing and vice versa.

Advancements towards high-throughput array tomography for hippocampal circuit analysis

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Fluorescence array tomography is a high-resolution imaging technique used to visualize and reconstruct 3D structures of biological samples by slicing tissues into ultrathin sections and imaging each slice using fluorescence microscopy. This method allows for the detailed mapping of molecular markers across large tissue volumes with high precision, preserving spatial relationships between molecules in their native tissue environments. With new developments in the processing of fluorescent array tomography sections we achieve enhanced visualization of neural circuits in the hippocampal region, offering new insights into its structural and functional organization. In this study, we employed an ATUMtome (Automated Tape Collecting Ultramicrotomy) to cut thin and ultrathin (50 nm) sections of stained and embedded brain tissues of mice, focusing on the hippocampal region. Our work emphasizes technological advancements that have significantly improved the quality of sectioning and imaging.

We introduced a new resin formulation, optimized for reduced autofluorescence and faster curation times, which improves the process of embedding and allows for clearer imaging of fluorescence-labeled structures. In addition, we refined our staining protocols to improve the contrast and specificity of cellular components, further minimizing background noise and maximizing signal intensity. These innovations are crucial for detailed and accurate circuit mapping, which was previously hindered by technical limitations.

Additionally, we have characterized a range of materials and identified a new substrate for sectioning using the Automated Tape-Collecting Ultramicrotome (ATUM). This substrate supports high-quality sectioning and imaging workflows. We will present data on new circuit analysis enabled by these technological developments, demonstrating their impact on the precision and depth of hippocampal connectivity studies.

This poster presentation will highlight the application of these advancements to neural circuit analysis, showcasing how they can improve our insight into the hippocampal structure through 3D segmentation and reconstruction. The integration of improved materials and methodologies represents a significant leap forward, providing new tools for exploring complex brain regions at the nanoscale.

Optimizing Viral Transduction of the Locus Coeruleus: A Comparison of Model Systems and Strategies

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The locus coeruleus (LC) noradrenergic (NE) system is involved in a plethora of physiological and pathophysiological processes. Refining our understanding of LC function largely relies on selective transgene expression in molecularly defined cells, allowing targeted manipulation and readout of noradrenergic neurons. Here, we performed a side-by-side comparison of the most commonly used strategies to genetically access the LC including different cre driver lines and promoter-mediated transgene expression. Additionally, different injection volumes, promoters and serotypes were analyzed to optimize viral transduction of LC noradrenergic cells. We report differences between these strategies in terms of transgene expression efficacy and molecular specificity. Notably, we found no behavioral alterations performing anxiety tests and memory tasks in cre-expressing mice of any mouse line as compared to wild-type littermates. Finally, to further ease the investigation of LC-NE function, we created a suite of constructs, including reporter proteins, calcium indicators, and optogenetic actuators whose expression is mediated by the previously described PRS×8 promoter. These constructs allow for monitoring and manipulation of LC-NE activity either in wild-type mice, or in combination with tissue-specific manipulations of different cre driver lines. The results of our study are crucial for the interpretation of results from previous experiments using the respective targeting strategies, as well as for the design of future studies.

Epidural focused ultrasound stimulation in a rodent model of depression

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Depression is one of the most prevalent psychiatric disorders, with approximately one-third of patients classified as treatment-resistant. For these individuals, various neuromodulation approaches have been explored, including deep brain stimulation (DBS) of the medial forebrain bundle (mfb), which has shown promising results in clinical studies. Transcranial focused ultrasound (FUS) at low intensities has been emerging as another form of stimulation. However, the clinical utility of FUS is currently limited by the short-lived effects of acute treatments and challenges due to skull-induced ultrasound attenuation, necessitating lower frequencies and therefore resolutions.

We report on the initial phase of the UPSIDE project, a collaborative effort to develop an implantable epidural FUS chip. This novel chip allows for less invasiveness but high network coverage and resolution compared to common neuromodulation techniques, using a steerable, focused beam of ultrasound waves. Combined with an innovative epidural recording electrode, we aim for the development of a closed-loop system to be tested in the Flinders Sensitive Line (FSL) rodent depression model.

The current phase investigated targeting accuracy as well as possible auditory side effects of the stimulation and optimized mfb stimulation parameters for epidural FUS (eFUS). The analysis of c-Fos expression and neurotransmitter release after mfb eFUS in narcotized animals gives insight into the comparability of effects to mfb DBS, where FSLs and controls showed a robust dopamine and noradrenaline release in the reward related network after electrical stimulation. To inform future closed-loop applications using eFUS, we also recorded prefrontal ECoG signals in both the experimental animals under resting and stress conditions to identify potential electrophysiological biomarkers relevant to the depression model.

This study provides a proof-of-concept for an innovative epidural focused ultrasound neuromodulation device and highlights its potential as a precise, implantable tool for treating depression. The evaluation of parameters in a rodent model contributes to a better understanding of the effects of FUS in general, as well as neuromodulation in depression.

Primary Neuronal Cell Culture in Ambient CO_2

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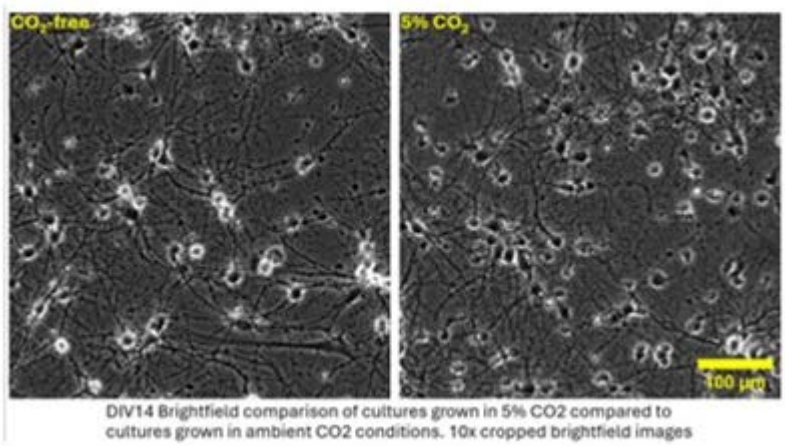
Dissociated neuronal cell culture is a well-established method that is universally performed in warmed, humidified incubators with 5% CO_2. Cultures in these conditions require a suitable culture medium that is buffered with a bicarbonate-, HEPES-based buffer system. Removing cells from the incubator and placing them in atmospheric conditions leads to significant cell death, which is a major limitation of this culture system.

We have developed and tested a method for growing primary, dissociated cells outside of CO_2 environments. Using a modified culture medium, we can grow primary neuronal cultures for up to two weeks in ambient CO_2 conditions. These neurons develop axons and dendrites, allowing them to form synaptic connections and develop active networks. By using a combination of calcium imaging, electrophysiology, and immunocytochemistry, we are characterizing these cells functionally and morphologically.

A CO_2-free system presents several advantages. Our culture system enriches for neurons rather than glia which could be beneficial for certain applications. Not requiring a continuous supply of CO_2 lowers the barrier to establishing culture systems in labs around the world and removes the need for CO_2 supply, which is a toxic, potentially lethal gas. Furthermore, the role of bicarbonate in CNS cell survival and development could be selectively studied in a controlled system, and CO_2-free cultures could potentially be used for biological studies in space, where CO_2 supply is expensive and problematic. Beyond culturing, this modified culture medium could be used in the future to transport living cultures without CO_2 either immediately after plating or once they reach maturity without significant changes to neuronal structure and function.



DIV 14 10x brightfield comparison of cultures grown in ambient CO2 (left) and in 5% CO2 (right)

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