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Cellular neuroscience

MouseFlow: a Python toolbox for quantifying facial and body movements in head-fixed mice

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There is increasing recognition that understanding neuronal function requires understanding complex behaviours at high granularity. To allow stable optical or electrophysiological recordings in rodents, animals are often head-fixed. While high-resolution camera tracking is becoming more widely adopted under such conditions, the quantification and interpretation of these data remains challenging. Here, we present MouseFlow: an open-source Python toolbox that allows the quantification of fine-grained facial and body movements with minimal user input, using a combination of established machine learning tools with kinematic analyses and computer vision algorithms. A pretrained and highly generalisable DeepLabCut (DLC) network allows us to faithfully track pupil diameter, eye movements, and eye blinks, regardless of camera angles or lighting conditions. Furthermore, this network allows us to automatically segment facial regions such as whisker pad, nose, or mouth regions. To overcome known constraints when applying single markers to infer global movements such as whisking and sniffing, we instead use dense optical flow algorithms to infer movement magnitude and directionality in facial regions of interest. This allows us to infer whisking and sniffing activity with high fidelity, including frequency and phase information. DLC markers also allowed us to resolve differences in mouse gait that varied with different treadmill running speeds. Importantly, we find that whisking and sniffing oscillations modulate a variety of neural activity across various brain regions recorded either electrophysiologically or optically. Overall, we present an open-source Python toolbox that allows easy-to-use fine-scale behaviour quantification of head-fixed rodent face and body video data.

AMPK/mTOR pathway regulates the neuroprotective effects of caffeine following neonatal hypoxic-ischemic brain injury

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Introduction: Neonatal hypoxic-ischemic encephalopathy (HIE) is the most common cause of death and long-term disabilities among term neonates. Caffeine is a drug that exerts anti-inflammatory effects and has been used in neonatal intensive care units to treat neonatal apnea. The AMPK/mTOR/autophagy pathway plays an important role in sensing stress response following brain injury. The potential role of caffeine in the prevention of developmental brain injury after neonatal HIE and the underlying molecular mechanisms involved are not well understood. **Materials and Methods:** We used a neonatal rat model of HI brain injury, following treatment with a single dose of vehicle or caffeine at different dosages. For the analysis of AMPK/mTOR/autophagy, samples were analyzed at 4 and 24h after HI. Tissue loss and microgliosis were analyzed by immunohistochemistry 7-days after HI. **Results:** At 7-days post HI, a dose of 40 mg/kg caffeine showed a significant reduction in brain tissue loss compared to the vehicle group (11.34% vs. 35.04% area loss). The brains treated with caffeine showed reduced microgliosis compared whit the vehicle group. Analysis of the AMPK/mTOR pathway after caffeine treatment; showed that, at 4h and 24h after HI, caffeine had a significant inhibitory effect on AMPK/mTOR activity. **Conclusion:** Caffeine treatment (40 mg/kg) reduced hypoxic-ischemic brain damage and microgliosis in neonatal rats following hypoxic-ischemic brain injury. Caffeine treatment directly regulates the AMPK/mTOR/autophagy pathways, being one potential mechanism of neuroprotection

Neuroprotective effect of a Sonic hedgehog agonist in a rat model of neonatal hypoxic-ischemic brain injury by decreasing brain inflammation and enhancing myelination

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Introduction: Neonatal hypoxic-ischemic encephalopathy (HIE) is the most common cause of death and long-term disabilities among neonates. Following HIE, gliosis plays an important role in the resolution of inflammation and defines the efficiency of white matter injury regeneration. The Sonic hedgehog (Shh) signaling pathway is critical for central nervous system (CNS) development, neuroprotection, and regeneration. Here, we demonstrate the neuroprotective effect of Sonic hedgehog agonist (SAG) in the injured neonatal brain after HIE and its effect on neuroinflammation and white matter regeneration. **Materials and Methods:** We used a P7 rat model of HI brain injury, following treatment with a single dose of vehicle or SAG at different dosages. Tissue loss, microgliosis, astrogliosis, oligodendrocyte proliferation, and re-myelination were analyzed after seven days of survival. **Results:** We observed a dose-dependent neuroprotective effect of SAG, with the most effective dose of 50mg/kg. A significant decrease in microgliosis and astrogliosis was observed in the animals treated with SAG. In addition, we observed a significant increase in proliferating oligodendrocyte Olig-2 and a significant decrease in mature oligodendrocyte CC1 levels in animals treated with SAG. We also found that SAG significantly increased myelination after HI. **Conclusion:** Our results demonstrate that SAG is a potential neuroprotective agent for the treatment of neonatal HIE by decreasing brain inflammation and enhancing myelination.

Neutrophil extracellular traps released following hypoxic-Ischemic brain injury and hypothermia treatment in newborn rats

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Introduction: Neonatal hypoxic-ischemic encephalopathy (HIE) is the most common cause of death and long-term disabilities among neonates. Therapeutic hypothermia (TH) is the current standard treatment. Peripheral neutrophils provide major immunomodulatory effects during sterile inflammation such as HI. Neutrophil extracellular traps (NETs) have been identified as contributor to ischemic neuropathology. The nucleotide-binding domain, leucine-rich repeat protein (NLRP-3) inflammasome has been involved in the assembly of NETs. **Materials and Methods:** We used a P7 rat model of HI brain injury following normothermia (NT) or TH treatment. The brains were analyzed at different time points (0, 6, 24, and 48 h after HI). **Results:** Flow cytometry revealed a significant increase in the percentage of infiltrating RP-1^+/$CD11_{b/c}{}^+$ neutrophils in injured brains. Six hours after HI/TH, a significant increase compared to the HI/NT and sham groups was observed, whereas 24 h after HI/NT, a significant increase compared to the HI/TH and sham groups was observed. Neutrophils in the blood samples showed a significant increase at all time points for both treatments compared to the sham group. A significant increase in Cit-H3 (NET marker) in brain samples at 6-48 h after the HI/TH group was observed compared to the HI/NT and sham groups. We also demonstrated that the NLRP-3 inflammasome was highly expressed at the same time points, as observed with high expression levels of NETosis after HI/TH. **Conclusion:** Our results confirmed the contribution of NETosis formation in the neonatal HI brain and a possible mechanism of TH by promoting NET formation in the injured brain.

Suppression of optomotor processing during escape saccades in flying Drosophila

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During voluntary behaviors, animals need to disable any reflexes that could interfere with the intended movements. With the optomotor response, flies stabilize a straight flight path by correcting for unintended deviations sensed as the panoramic motion of the surround. HS cells of the fly are thought to mediate optomotor responses to horizontal motion. During spontaneous flight turns, a putative efference copy acts on HS cells with the right sign to counteract the visual input elicited by the fly's own behavior. Here, we investigated, whether looming-elicited turns in flying Drosophila have a similar effect on HS cells, and how this may affect the optomotor response via descending neurons. We show that looming-elicited escape turns have a similar effect on HS cells. Perception of excitatory panoramic motion is suppressed during turns in both directions in a subtype of HS cells only. Concurrently the visual experience of a looming stimulus itself contributes to reduced responses to panoramic motion in all HS cells. We are now studying how optomotor responses are controlled by neurons downstream of HS cells, focusing on a pair of descending neurons that receives preferential input from the HS subtype that receives an efference copy signal.

Theta-phase locking of human single neurons during memory encoding and retrieval

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The electrophysiological mechanisms of human memory encoding and recall are incompletely understood. Precise interactions between single-neuron spiking and brain oscillations presumably play a critical role in this process. Here, we used human single-neuron recordings from an object-location memory task with separate periods for encoding and retrieval (Kunz et al., Neuron, 2021) to investigate the timing of single-neuron activity in the medial temporal lobe relative to the local theta rhythm (1–10 Hz) while epilepsy patients encoded and retrieved object–location memories in a virtual environment. 18 epilepsy patients participated in this task and contributed a total of 27 experimental sessions. We extracted single-neuron action potentials from hybrid depth electrode recordings (1025 neurons in total) using previously established spike-sorting algorithms (Chaure et al., Journal of Neurophysiology, 2018) and computed the Hilbert transform of the local field potential to estimate the instantaneous theta phase of each spike. For each neuron, we then examined its phase locking to the local theta rhythm, tested how this theta-phase locking varied as a function of theta power and task variables, and compared the phase distributions of action potentials between encoding and retrieval. We found that a significant portion of the recorded neurons phase locked to the theta rhythm, which was similarly strong during the encoding and retrieval of object–location memories. Neurons generally locked to the trough of theta oscillations, replicating previous observations (Jacobs et al., Journal of Neuroscience, 2007). Theta-phase locking was most prevalent during periods with high theta power. In some of the phase-locked neurons, we furthermore observed small but significant shifts in the preferred phases between encoding and retrieval, which is in line with theoretical models suggesting separate phases for encoding and retrieval (Hasselmo et al., Neural Computation, 2002). Overall, our results are consistent with the idea that human memory involves specific timing relationships between single-neuron action potentials of individual neurons and the theta rhythm during both memory encoding and retrieval.

Neuronal and astrocytic sodium-calcium exchanger-1 contribute to ionic changes in both neurons and astrocytes during periinfarct depolarization

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Disruption of cerebral blood flow (CBF) in stroke leads to an imbalance of ionic and neurotransmitter gradients in neuron and glial cells, which triggers the occurrence of peri-infarct depolarizations (PIDs) in the ischemic penumbra, which may negatively affect infarct size and clinical outcome. The cellular pathways which govern intracellular calcium and sodium changes in neurons and astrocytes during PIDs, and how they affect extracellular glutamate dynamics, remain incompletely understood. We here investigated the role of sodium-calcium exchanger-1 (NCX-1) for sodium and calcium homeostasis during PIDs in a rodent stroke model, using neuron-specific NCX1 KO mice. Calcium and sodium changes in neurons and astrocytes as well as extracellular glutamate kinetics were monitored using in vivo multiphoton microscopy. Lesion volume was determined using intravital MRI 1 and 3 days after stroke. We found that neuron-specific KO of NCX-1 resulted in significantly lower cellular calcium transients but higher sodium transients during PIDs, indicating that neuronal NCX1 may operate in the reverse mode during PIDs. Interestingly, extracellular glutamate levels were lower in neuron-specific KO mice. In line with this, the threshold for the occurrence of PIDs after stroke was higher in KO mice, and MRI-measured stroke volume appeared to be lower 3 d after stroke. Hence, NCX1 may represent a prominent translational target for stroke therapy.

Short- and long-term consequences of chronic stress on neuronal properties in the mouse motor cortex

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Chronic stress plays a key role in understanding the development of affective disorders such as depression and anxiety. In particular, social stress plays an important pathogenetic role in short- and long-term changes in brain and body function in humans and animals. Chronic social stress models in mice have been validated to induce depression-like symptoms. We have recently discovered that the structure and function of the motor cortex is chronically altered by social stress. Specifically, we have demonstrated damage to structural neuroplasticity as a function of individual stress vulnerability and have seen initial evidence for substantial changes in neuronal function and network activation. We now aim to further characterize these changes in excitation and inhibition in relation to the post-stress interval. Adult male wildtype mice were behaviorally classified as either stress-susceptible or -resilient after 10 days of chronic social defeat stress. Intrinsic neuronal properties of layer V principal neurons in the motor cortex were measured electrophysiologically in acute slices with and without the influence of the surrounding inhibitory networks. These recordings were made either immediately after the end of the stress period or 4-6 weeks later, after a re-evaluation of the stress behavior had taken place. First analyses corroborate the hypothesis of a disinhibition of excitatory neuronal activity in the mouse motor cortex after chronic social stress. Whether the cause is a primary change in the properties of the excitatory neurons, the inhibitory neurons, or a change in the interaction of both networks, is part of the ongoing analyses.

Illuminating the hippocampal-thalamic-retrosplenial network via prism: a miniature two-photon microscopy study

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The Retrosplenial cortex (RSC) is a complex and heterogeneous brain region with diverse connectivity, yet its precise function remains elusive. Our study aims to explore the role of RSC in spatial behavior during free movement, specifically focusing on the behavioral implications of hippocampal gating of thalamic input and its modulation of RSC activity. Previous clinical studies and animal lesion research have highlighted the critical involvement of RSC in the interactive processing of egocentric and allocentric information, which are crucial for navigational behavior. RSC exhibits prominent connections with brain regions involved in cognitive mapping, such as the hippocampal formation, and its granular subdivision receives long-range inhibitory input from hippocampal CA1. Each RSC subdivision incorporates distinct intracortical and thalamic inputs that interact with hippocampal afferents, facilitating the integration of spatial and sensory information necessary for supporting behavior. However, the lack of a method to access individual RSC subdivisions hampers our understanding of the specific signals transmitted to RSC and the interaction between hippocampal and thalamic input in signal integration. To overcome the challenge posed by the deep anatomical location of granular RSC cells, we employ prism implantation with miniature two-photon microscopy (MINI2P). This innovative approach enables targeted access to RSC, allowing for chronic monitoring of each RSC subdivision and their long-range axonal projections during freely moving behavior. By investigating the underlying mechanism of the hippocampal-thalamic-retrosplenial network, this study aims to shed light on the functional organization of RSC and its role in spatial behavior.

Lipid uptake at the neurovascular unit

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The brain maintains a lipid pool largely separated from that of the body. However, some key lipids must be obtained from the circulation as these essential lipids cannot be synthesized *de novo* in the brain. This projects studies uptake and metabolic routing of lipids across the neurovascular unit. For that new technologies based on alkyne lipid tracers and mass spectrometry are developed. The complex biological process is investigated using co-cultures of primary endothelium and astroglia in an established transwell-setup. The transport and metabolism of different saturated and (poly)unsaturated lipids are studied in competition experiments. We find profound differences between these lipids pointing to their differential metabolic processing at the neurovascular unit.

Ripple-locked coactivity of stimulus-specific neurons and human associative memory*

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Associative memory enables the encoding and retrieval of relations between different stimuli. To better understand its neural basis, we investigated whether associative memory involves temporally correlated spiking of medial temporal lobe (MTL) neurons that exhibit stimulus-specific tuning. Using single-neuron recordings from epilepsy patients performing an associative object–location memory task, we identified the object- and place-specific neurons that represented the separate elements of each memory. When patients encoded and retrieved particular memories, the relevant object- and place-specific neurons activated together during hippocampal ripples. This ripple-locked coactivity of stimulus-specific neurons emerged over time as the patients' associative learning progressed. Between encoding and retrieval, the ripple-locked timing of coactivity shifted, suggesting flexibility in the interaction between MTL neurons and hippocampal ripples according to behavioral demands. Our results are consistent with a cellular account of associative memory, in which hippocampal ripples coordinate the activity of specialized cellular populations to facilitate links between stimuli.

*Also a short talk

Neuroprotective effect of melatonin in a neonatal hypoxia-ischemia model is regulated by AMPK/mTOR pathway

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Introduction: Hypoxic Ischemic Encephalopthy (HIE) is one of the common cause of mortality and life-long morbidities among neonates. Melatonin has been shown to be a neuroprotective agent due to its anti-inflammatory, anti-apoptotic and anti-oxidative features, and also can penetrate the blood brain barrier. The AMPK/mTOR/autophagy pathway plays an important role sensing stress response after injury in the central nervous system. The purpose of this study is to investigate the neuroprotective effect of melatonin and analyze one potential pathway. ***Methods:*** We used a P7 rat model of hypoxic-ischmic (HI) brain injury, following treatment with vehicle or melatonin. For the analysis of AMPK/mTOR/autophagy, samples were analyzed 5h and 24h after HI. Tissue loss and microgliosis were analyzed by immunohistochemistry 7-days after HI. ***Results:*** At 7-days post HI, we observed a significant decrease of tissue loss in the melatonin treated animals compared to the vehicle group. We did not observe a significant decrease in mircogliosis at the analyzed time-point. At the AMPK/mTOR pathway analysis we observed a decrease in the mTOR pathway, with a significant decrase at the autophagy level and NF-$\tau\beta$ at both time points (5h and 24h) in the melatonin group compared to the vehicle group. ***Conclusion:*** Melatonin treatment at a dose of 25 mg/kg; reduces hypoxic-ischemic brain injury in newborn rats by inhibiting autophagy and reducing the NF-$\tau\beta$ signaling pathway regulated by via the AMPK/mTOR pathway.

Cell-type-specific rescue of hippocampal CB1 receptor leads to age-related changes in adult and middle-aged mice

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Cannabinoid receptors type 1 (CB1) are highly expressed in the hippocampus, where they are involved in the regulation of learning and memory and they exert anti-inflammatory activity. Furthermore, they modulate the release of excitatory and inhibitory neurotransmitters, maintaining a proper balance between excitation/inhibition. Previously we have shown that constitutive deletion of CB1 receptors in mice leads to premature signs of ageing, namely increased neuroinflammation, reduced cell proliferation and memory impairments. This study aims to investigate whether rescue of the CB1 receptor signaling in the hippocampus can prevent these effects. We performed stereotaxic injections of rAAV1/2 expressing Cre in 2-month-old CB1-deficient (CB1-STOP) animals. After 4 and 10 months from viral injections, we tested the social memory in the partner recognition paradigm and their learning abilities and spatial memory in the Morris Water Maze test. Next, we performed pro-inflammatory cytokine expression analysis by RT-PCR and we investigated the glial cells and neuronal densities as well as lipofuscin autofluorescence in the hippocampus by immunohistochemistry. Unexpectedly, instead of improved cognitive abilities, rescued animals showed a lower social memory and impaired flexibility of learning and spatial memory compared to the constitutive knockouts. The phenotype observed in the CB1 conditional rescued animals was associated with enhanced glial cell activity, increased neuronal loss and lipofuscin accumulation, most prominently in the CA1 region, compared to their age-matched constitutive knockouts. We hypothesize that the hippocampal CB1 receptor was rescued in a cell-type-specific manner, thus leading to an imbalance excitation/inhibition and to the age-related changes observed in conditional rescued animals.

Brain endothelial cell-derived angiocrine factors and their role in neurodevelopment

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The central nervous system (CNS) of mice starts forming during embryonic development and continues postnatal with synaptic refinement, myelination, and gliogenesis. These processes require precise and accurate communication between the vascular and neural units of the CNS. Although previously thought to only provide oxygen and nutritional demands to the CNS, the crucial role of endothelial cells (ECs) in the proliferation, migration, and network formation of neural components has been demonstrated in recent years. The Hippo pathway is a critical signalling pathway for CNS development that mediates its effect on vascular and neural units individually through its components YAP and TAZ. YAP/TAZ activity in ECs is essential for the development of a proper and functional vasculature. EC-specific YAP/TAZ knockout mice display a defective vascular phenotype in the developing cortex defined by decreased vessel area, junction density and vessel length. Concomitantly, dendritic branching of cortical neurons is altered. With the hypothesis that YAP/TAZ signalling regulates the expression of angiocrine molecules in ECs, we identified Endothelin-1 as an angiocrine factor regulated by YAP/TAZ in CNS ECs that influences neurodevelopment. Endothelin-1 treatment of neuronal cultures reduces dendritic branching and spine density. Interestingly, our results show that Endothelin-1 does not act directly on neurons but its effect is mediated via astrocytes, which then modulate dendritic and spine development through a yet unidentified mechanism. Altogether, we propose a tri-cellular communication model where ECs guide neuronal architecture through astrocytic intermediaries.

Species-specific morphometry of structural compartments of MNTB principal neurons

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In all mammals investigated so far, the medial nucleus of the trapezoid body (MNTB) converts excitatory input originating from its hallmark structure, the calyx of Held, into rapid and temporally precise feed-forward inhibition to local and distal auditory centers indicating an evolutionarily conserved circuit function. Although the input-output function of MNTB neurons is dominated by somatic excitation, the dendritic compartment is implicated in action potential generation and speeding of synaptic potentials. At least in rodents, several cellular features including soma size vary along the MNTB's tonotopic axis. The morphometry of the postsynaptic principle neurons is largely unexplored. Following from the conserved functional similarity, MNTB neuron morphology should be species invariant. A detailed quantitative morphometry and their species-dependency is lacking. We compared the structure of individual MNTB neurons across five mammalian species and gross anatomy in eleven species. We find that significant species-dependent differences in most morphological features are present. Soma size, primary dendrite number, dendritic length and initial dendritic diameter discriminate MNTB neurons from different species most reliably according to discriminant function analysis. Average soma size strongly correlates with brain size and, in nine of eleven species, with tonotopic frequency. Dendritic morphometry appears mostly independent on soma size, tonotopy and brain size. In all species tested, MNTB axons appear targeted by excitatory and inhibitory synaptic inputs. Together, our analysis shows species-dependent differences in MNTB arrangement and morphology that cannot generally be explained by differences in brain size, indicating species-dependent adaptations in this highly conserved brainstem structure.

Analyzing the mechanism of the Ste20-like kinase in the outgrowth of dendrites and the stabilization of inhibitory synapses

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The Ste20-like kinase (SLK) influences morphology and function of neurons by controlling the branching of the distal dendritic tree and stabilizing inhibitory synapses during development. Although interaction partners of SLK have been described in different non-neuronal cells, so far nothing is known about the molecular mechanism underlying SLK function in neurons. To gain insights into SLK-dependent cellular pathways, different experimental strategies were applied. Immunocytochemical analysis of several known SLK downstream targets revealed a reduced amount of F-actin in dendrites of SLK knockdown compared to control neurons, implying that SLK could be involved in actin cytoskeleton dynamics and stability. To identify proteins interacting with or influenced by SLK, a combination of proteomic approaches was conducted. In a phosphoproteomic screen, the phosphorylation profile of neuronal proteins was compared between cultured cortical control and SLK knockdown neurons and specifically proteins associated with the cytoskeleton, involved in neurite outgrowth, or located at postsynaptic densities were differentially phosphorylated. A complementary approach was aimed at uncovering proximal, potentially interacting proteins of SLK by using a proximity-dependent assay (BioID). The proteomic screens were completed by identifying direct SLK-binding proteins through co-immunoprecipitation. Combining these approaches pointed to cytoskeletal and synaptic proteins as interactors of SLK, among them the closely related kinases Mink1 and Tnik that have important functions in neurite outgrowth and cytoskeletal dynamics. In summary, we have obtained the first data set of potential downstream SLK effectors in neurons, which is the basis to resolve the mechanism of how SLK regulates dendritic growth and inhibitory synapse stabilization.

Role of endothelial-derived TIMP-1 in white matter pathology

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Oligodendrocytes (OLs) are glia cells of the central nervous system that produce myelin and wrap around axons to ensure efficient electrical conductivity. OLs originate from oligodendrocytes precursor cells (OPCs). Recent work supports the existence of an oligo-vascular interface at which vessels directly control the specification of OPCs and their maturation. OLs are also observed in contact with the mature vasculature. Still, the contribution of the vasculature to the different steps in the oligodendrocyte lineage in development and disease is not well understood. Multiple sclerosis (MS) is the most common cause of neurological disability in young adults, where myelin sheaths are lost through the injury or death of OLs that results from autoimmune damage. Although myelin sheaths can be regenerated by OPCs that are recruited to lesions and differentiate, endogenous remyelination often fails in MS. In addition, in MS the blood brain barrier is compromised. Using a single-nucleus RNA sequencing dataset from healthy and MS patients, we identified changes in the gene expression profile of ECs from MS patients. In an attempt to characterize mechanisms by which the altered vascular compartment might influence OPCs/OLs, we investigated the effect of such changes. We found that Tissue Inhibitor Metalloprotease 1 (TIMP-1), which has been identified as a factor in several human pathologies, is mainly upregulated in the endothelium in MS patients. Furthermore, we observed that the highest TIMP-1 upregulation is seen in active and chronic active lesions. Interestingly, this EC-specific upregulation seems to be human-specific, as in mouse models of MS upregulation of TIMP-1 does not occur in ECs but instead in astrocytes. Using in vitro assays, we show that TIMP-1 promotes OPC lineage progression. Altogether, our results suggest that EC-derived TIMP-1 can act as a recovery mechanism in demyelinating conditions in humans.

Centripetal integration of past events in hippocampal astrocytes, controlled by the locus coeruleus*

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An essential feature of neurons is their ability to centrally integrate information from their dendrites. The activity of astrocytes, on the other hand, has been described to be mostly uncoordinated across the cellular compartments and therefore without central integration. Here, we describe *conditional centripetal integration*, a principle how astrocytes integrate calcium signals from their distal processes. We found that global astrocytic activity as recorded with population calcium imaging was well explained as a leaky integration of past neuronal and behavior events on a timescale of seconds, but equally well by the concurrent pupil diameter as a proxy for arousal. We found that upon salient past events calcium signals in distal processes of individual astrocytes propagated to the soma on a timescale of seconds. This centripetal propagation was facilitated by high levels of arousal but impeded when pre-event calcium levels were high. Optogenetic activation of the locus coeruleus, a key player for arousal, reproduced centripetal propagation, and pharmacological inhibition of alpha-1 receptors reduced this effect, indicating a role for the locus coeruleus to control centripetal propagation. Together, our results establish astrocytes as computational units of the brain that slowly and conditionally integrate information about the past.

*Also a short talk

Microtubule retrograde flow retains neuronal polarization in a fluctuating state*

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In developing vertebrate neurons, a neurite is formed by more than a hundred microtubules. While individual microtubules are dynamic, the microtubule array has been regarded as stationary. Using live-cell imaging of neurons in culture or in brain slices, combined with photoconversion techniques and pharmacological manipulations, we uncovered that the microtubule array flows retrogradely within neurites to the soma. This flow drives cycles of microtubule density, a hallmark of the fluctuating state before axon formation, thereby inhibiting neurite growth. The motor protein dynein fuels this process. Shortly after axon formation, microtubule retrograde flow slows down in the axon, reducing microtubule density cycles and enabling axon extension. Thus, keeping neurites short is an active process. Microtubule retrograde flow is a previously unknown type of cytoskeletal dynamics, which changes the hitherto axon-centric view of neuronal polarization.

*Also a short talk

Frequency integration in the intermediate nucleus of the lateral lemniscus is based on a biophysically heterogeneous cell population

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Cross-frequency processing is considered to generate important information about the acoustic environment. Yet, sound frequencies are mostly represented tonotopically organized in the auditory brainstem, an organisation principle insufficient to explain frequency perception. The intermediate nucleus of the lateral lemniscus (INLL) might be a suitable candidate for cross-frequency integration, as a fraction of INLL neurons have been suggested to be involved in this task. So far, the biophysical, synaptic and morphological features of this neuronal population remain largely uncharacterized. To characterize the INLL, we use in vitro whole-cell and patchSeq recordings of single neurons, to investigate their biophysical properties, synaptic inputs, morphology and transcriptome. The membrane properties and firing behaviours of INLL neurons display a vast heterogeneity, with a continuum of membrane time constants (τ_{mem}) defining their integration time window. Synaptic time constants and paired pulse ratios matched this cellular integration time. The relation between physiological parameters and τ_{mem} are reflected in varying gene expression of voltage gated ion channels and transmitter receptors. The biophysical differences are not regional specific and do not correlate with morphological features, indicating that the heterogeneity is mainly driven by the differing molecular properties and expression patterns across the continuum. Our data shows that the neuronal population in the INLL exhibits a biophysically based wide continuum of integration time scales. Since no spatial organisation principle was apparent the temporal integration is not linked to a strict anatomically defined tonotopy. This neuronal population rather provides a wide continuum for temporal integration of sound across frequencies.

Neural stem cells regulate vascular properties in the adult subventricular zone neurogenic niche

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The neurovascular unit (NVU) and the blood-brain barrier (BBB) are unique features of the CNS vasculature. NVU properties in different brain regions seem to be different. However, the mechanistic insight leading to such differences less understood. In this study, we characterize vessel heterogeneity and NVU properties in neurogenic and non-neurogenic regions, both at the morphological level as well as at the transcriptomic level. Vessels in the SVZ presents a more planar and less branched structure, which gives rise to a reduction in the blood vessel density compared to the vasculature from the cortex. Additionally, the proportion of vessels completely covered by astrocytic endfeet is lower in the SVZ compared to the cortex, while the pericyte coverage is increased. Using the mTmG pericyte reporter mouse model we have demonstrated that the increase in the coverage is due a change in pericyte morphology from the SVZ compared to the cortex. In order to investigate whether NSCs can control the properties of vessels located within their own neurogenic niche, we characterize the SVZ vasculature upon NSC depletion *in vivo*. While no differences in blood vessel density or permeability of a small tracer molecule (NaF) are detected, an increase in pericyte coverage is specifically observed in the SVZ vasculature (and not in other brain regions) upon NSC depletion. To gain insight into the molecular mechanisms underlying the potential crosstalk between NSCs and vascular cells, we analyze ligand-receptor interactions between NSCs, ECs and perivascular cells using single cell RNA sequencing of cells in the SVZ. With a focus on signals deriving from the NSC compartment, we identified molecules like pleiotrophin (Ptn) and midkine (Mdk) highly expressed in NSCs and their respective receptors in ECs and mural cells, suggesting that there might be instructive signaling from NSCs that may induce and maintain vascular cell properties (and in turn NVU characteristics) within their niche, to assure the optimal niche environment for maintaining neurogenesis.

Computational, Modeling, and Technology

Behavioral quantification and multi-photon microscopy during gap crossing in freely moving rodents

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Measuring behavior in the freely moving animal allows for the quantification of temporal patterns of neural activity during specific behaviors such as decision making tasks all while maintaining the complete mosaic and integrity of sensory input within an ethologically relevant context. Here, we present a gap crossing task, performed by rodents in either light and dark conditions, in which an animal must evaluate a gap of varying distances and choose to cross or not using its vision, whiskers, or both. To quantify whisker strikes on the target platform, we developed a detection system based on expanded and collimated IR laser light. Here we show that both whisker and vision based gap crossing behaviors can be distinguished and quantified using this approach. Cross attempt times (i.e. time taken to reach the decision to cross or to refuse) tracked in parity with animals crossing psychometrics. Attempt times rose significantly at the limit of whisking range, most notably in the absence of visual cues (dark), and remained elevated for vision only gap distances. We additionally show differences in the whisking behavior of mice and rats, e.g. whisking frequency. Further, utilizing head-mounted three-photon excitation-based microscopy, we correlated neuronal activity from discrete cortical layers with single-cell resolution to crossing behaviors.

Minimal energy principles determine spatial localization and turn-over of molecules*

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Neuronal dendrites carry thousands of synaptic spines, which harbor hundreds of protein species each. The necessary number of proteins must be maintained at all spines and times to keep synaptic homeostasis and to provide the molecular basis for synaptic plasticity. At the same time, distributing molecules with limited lifespans along the widespread dendritic morphologies is highly demanding. Today, it is unclear which rules determine how neurons approach this task. Here, we use a biologically plausible and robust mathematical model to show that energy minimization is a strong candidate for such a rule. Our model reveals significant energy benefits when moving certain classes of mRNAs out of the soma and into dendrites. Furthermore, it correctly predicts mRNA and protein copy numbers in neurons. We can also reproduce experimentally observed intracellular protein and mRNA dynamics in dendrites and spines on smaller scales. We support our model predictions with data from six large-scale transcriptomics and proteomics studies covering thousands of molecular species.

*Also a short talk

Phase-to-rate recoding: how phase information is conserved in sparse, synchronous population-rate-codes

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Neural computation is often traced in terms of either *rate-* or *phase-codes*. However, most circuit operations will simultaneously affect information across both coding schemes. It remains unclear how phase and rate coded information is transmitted, in the face of continuous modification at consecutive processing stages. Here, we study this question in the entorhinal cortex (EC)- dentate gyrus (DG)- CA3 system using three distinct computational models. We demonstrate that DG feedback inhibition leverages EC phase information to improve *rate-coding*, a computation we term *phase-to-rate recoding*. Our results suggest that it i) supports the conservation of phase information within sparse *rate-codes* and ii) enhances the efficiency of plasticity in downstream CA3 via increased synchrony. Given the ubiquity of both *phase-coding* and feedback circuits, our results raise the question whether phase-to-rate recoding is a recurring computational motif, which supports the generation of sparse, synchronous population-*rate-codes* in areas beyond the DG.

A novel approach to explore degenerate ion channel distributions in cortical dendrites

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Pyramidal neurons in the neocortex are morphologically and biophysically highly diverse. Capturing this diversity in biophysically detailed multi-compartmental models poses a major challenge. Here we show a novel approach to explore the space of degenerate ion channel distributions by which pyramidal neurons in layer 5 – the major output cell-type of neocortex characterized by elaborate and biophysically complex dendrites – can achieve their characteristic dendritic, somatic and axonal functional properties. For each morphology, we discover a widespread yet continuous spectrum of possibilities by which this neuron can achieve its characteristic function. The thereby generated database of millions of models provides an ideal starting point to study the interplay of morphology, channel expression and neuronal function.

Sparse connectivity is a prerequisite for efficient learning in recurrent neural networks

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Although artificial neural networks (ANNs) were originally inspired by the brain, they differ notably in their structural network architecture: while ANNs are typically initialised with random, dense connectivity, recent advances in large-scale connectomics have revealed that connectivity in cortex is both non-random and sparse. Even as connectomic datasets become increasingly available, it remains unclear how we may learn about the function of cortical networks based on their structure. To this end, we here investigated ANNs constrained by interpretable features of cortical networks in order to disentangle the effect of structural properties on network function. We find that in large and recurrently connected networks, as are found in the cerebral cortex, sparse connectivity facilitates time- and data-efficient learning, but only when nodes have functional properties reminiscent of neurons. Furthermore, in networks where each node constrained to be either excitatory or inhibitory like a biological neuron, we see a large learning delay in densely connected networks which is prevented by sparse connectivity. Taken together, our findings show that sparse connectivity is a prerequisite for efficient learning given some key constraints from biological networks, and set the stage for the investigation of higher-order features of cortical connectivity.

Three-photon in vivo imaging: access to previously unreachable brain regions*

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Multiphoton imaging in live and awake animals led to many groundbreaking discoveries since its establishment in 1991. Recently developed three-photon *in vivo* imaging enables deeper tissue penetration than two-photon microscopy and holds the potential for similar breakthroughs. Here we provide a resource to apply high resolution structural and functional three-photon microscopy in different brain regions of adult mice. Specifically, we access the medial prefrontal cortex (mPFC, 1400 µm) and perform GCaMP Ca^{2+}-imaging in awake head-fixed mice for the first time. Furthermore, we longitudinally image dendritic spines of layer 5 mPFC pyramidal neurons over a week at a depth >1100 µm. In addition, we excite red fluorescent microglia with 1650 nm wavelength and monitor their process motility at previously unreachable depths (1100 µm). We also confirm imaging of neurons in the hippocampus of adult mice through intact cortex and functional imaging of dentate gyrus. Implementation of three-photon imaging in many neuroscience labs, like two-photon imaging in the past, will pave the way for novel discoveries in several brain regions at previously unprecedented depths.

*Also a short talk

Studying sensation and perception utilizing biologically realizable ANNs

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How the brain transforms sensory information into perception remains unsolved. However, it has become increasingly clear that, in addition to primary sensory areas, both higher-order and subcortical regions play a role in the generation of a percept. Recently, modeling components of the brain via artificial neural networks (ANNs) has shown to be a powerful method for providing initial validation for hypotheses and leading future experimental endeavors to more fruitful insights. Here, I propose to utilize ANNs to investigate the interaction between cortical areas responsible for perception. In particular, I will model the way in which sensory and non-sensory information schemes integrate at the cellular and circuit levels to determine how this coupling affects cortical dynamics within and across these areas. For this purpose, I will construct ANNs with increasing empirically observed anatomical and physiological detail. I will first test these models against standard machine learning benchmarks and then demonstrate the developed models on functional data acquired from the rodent vibrissal system, a well-studied model for perception. Preliminary results suggest the value in utilizing the distinct toolkit offered by the field of AI for describing higher-order mechanisms found within the brain. This work sets the stage for a novel approach to modeling the cortical processing leading to perception via ANNs, and intends to shine a light on important features to be investigated empirically.

Spiking activity in a Scn2a p.A263V epilepsy model

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Various developmental disorders, such as epilepsy and autism spectrum disorder, have been associated with mutations in *SCN2A* encoding the voltage-gated Nav1.2 sodium channel alpha subunit. Specifically, the missense mutation p.A263V in *SCN2A* was associated with neonatal epilepsy. To investigate the disease pathophysiology and test potential treatment approaches, a knockin mouse line carrying the p.A263V mutation was generated. Heterozygous and homozygous mutant mice of this line exhibited spontaneous hippocampal seizures already at the ages of P3-P7. To characterize seizure and interictal activity in hippocampus and cortex, we conducted silicon probe recordings in awake mouse pups. We used a stereotaxic setup for the pups and a Mobile HomeCage setup with an air-floating arena for awake, head-fixed adult mice. Along with examining the dynamics of seizures, our research on this SCN2A-mouse line aimed to identify and characterize behavioural and cognitive comorbidities similar to those observed in human patients. We focused on specific Local Field Potential events in the hippocampus called Sharp Waves in neonatal animals and Sharp Wave Ripples in adult animals, which are known to be strongly associated with processes of memory reconsolidation. We analysed and compared the dynamics of these events as well as the dynamics of spiking activity associated with these events across different genotypes. Our findings showed changes in the distribution and amplitude of certain types of Sharp Waves in neonatal mutant mice. We identified differences in spiking activity between mutant mice and control mice in neonatal and adult animals.

Human single-neuron dynamics during saccadic and smooth-pursuit eye movements

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During eye movements, corollary discharge signals facilitate spatial remapping and stable visual perception. However, it is unclear whether neurons in the medial temporal lobe (MTL) rely on such signals. Here we asked if the dynamics in the single unit activity of these regions indicate corollary discharge. Furthermore, we explored if and how units represent the speed of gaze. Up to now, we recorded neurons in the MTL from 7 neurosurgical patients implanted with hybrid Behnke-Fried depth electrodes, and performed simultaneous eye-tracking. Subjects smoothly pursued a target moving sinusoidally along the horizontal axis with their eyes and saccaded intermittently. Units in the human MTL fired less during saccades and smooth pursuit than during fixations. The highest proportions of units modulated by eye-event types were found in the parahippocampal cortex and hippocampus. A largely separate group of units exhibited firing rates correlated with gaze speed during smooth pursuit. Most of these units increased their firing rates with speed of gaze. The decrease of activity during movement in the first group of neurons is indicative of corollary discharge in MTL. The results imply a corollary discharge signal not only during saccades, but also during smooth-pursuit eye movements. In addition, we found cells increasing their firing rate with gaze speed during smooth pursuit, which are reminiscent of speed cells in rodents.

Elucidating the role of white matter hyperintensity in cognitive impairment with whole-brain models

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White matter hyperintensities (WMH) were shown to lower functional connectivity (FC) between regions of the default mode network, possibly explaining faster cognitive decline observed in cognitively unimpaired (CU) and mild cognitively impaired (MCI) subjects with WMH. Using whole-brain modeling (WBM), we aimed to mechanistically elucidate WMH's contribution in altering brain dynamics, ultimately yielding to cognitive dysfunction. Twenty-seven patients from the ADNI Database were divided into groups according to cognitive function and presence of WMH. Timeseries were modeled with the normal form of a supercritical Hopf bifurcation. We estimated the activity dynamics of each region (a), by either a grid search in subjects without WMH(baseline models) or by varying a as a function of global WMH volume(WMH-weighted models) in subjects with WMH and compared simulated and empirical FC and the distribution of FC dynamics of phases(phFCD) with Pearson's correlation coefficient(PCC) and Kolmogorov-Smirnov distance(KSD), respectively. MCI with and without WMH had more negative a-values(<= -0.027) than CU without WMH. a-values in CU with WMH were also shifted to more negative values compared to CU without WMH(-.028, versus -0.02). WMH-weighted models yielded better fits for phFCD and FC, although statistically significant only for FC in CU with WMH(PCC=0.26 versus 0.24, p=0.041). WMH-weighted models resulted in a more realistic representation of brain dynamics influenced by vascular dysfunction. CU subjects with WMH showed a shift towards noisier brain dynamics that may contribute to alterations in FC, possibly yielding to cognitive impairment. Considering WMH in WBM provides opportunities to evaluate treatment strategies in-silico.

Unraveling altered protein turnover dynamics in Huntington's disease: insights into transcription, translation, and degradation processes

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Regulating transcription, translation, and degradation processes is crucial for proper neuronal function, ensuring required protein levels. However, in conditions like Huntington's Disease (HD), these processes may exhibit aberrant behavior due to altered signaling pathways, resulting in either elevated or reduced mRNA and protein levels in affected animals. Understanding modifications in these processes and their collective impact on mRNA and protein dynamics is essential for unraveling the molecular mechanisms underlying HD pathology. Our study introduces a novel approach to investigate how mutant Huntington mRNA and protein disrupt protein synthesis processes and explores their combined effects under diseased conditions. Initially, we constructed a comprehensive biochemical model that incorporated the influence of multiple signaling pathways on the translation process, considering both wild-type and mutant scenarios. Remarkably, the model accurately replicated previous findings, demonstrating that mutant mRNA leads to increased levels of mutant Huntington protein. Subsequently, the model predicted a decrease in transcription, which we then confirmed through follow-up experiments in mice, revealing a significant 20% reduction in mRNA levels. However, measurements of mRNA in later life stages of mice and humans exhibited a significant increase. Based on this, we propose that the degradation rate of Huntington mRNA is diminished, leading to an accumulation of mRNA. By elucidating the intricate interplay between transcription, translation, and degradation processes in Huntington's Disease, our study provides valuable insights into the mechanisms by which mutant Huntington mRNA and protein influence protein synthesis. These findings highlight the importance of considering multiple aspects of the cellular machinery in disease pathology.

Characterizing cellular computation in terms of biophysical expression

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Morphology and the distribution of ion channels on the dendrite are major determinants of cellular computation. How the interplay of spatially distributed ion channels affects somatic responses remains poorly understood. In general, similar cellular dynamics can be achieved with vastly different ionic currents, while minor variations in ionic currents can yield vastly different cellular dynamics. Here, we generate millions of biophysically detailed models of layer 5 pyramidal tract (L5PT) neurons, which map out the spectrum of possibilities of how channels can be distributed to achieve the characteristic dendritic and somatic electrophysiology of this celltype. We show how to utilise Explainable Artificial Intelligence (XAI) to reveal nonlinear multidimensional relationships between the distribution of channels and somatic output that can be empirically tested. Our approach thereby is an important step towards linking electrophysiological responses to their mechanistic origin.

DeepFisFis: a novel tool for real-time USV detection

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Ultrasonic Vocalizations (USVs) are integral in the subtle and complex world of rodent communication and social interaction. However, these essential communicative cues have remained elusive for a long time, hidden beyond the human range of hearing. Only in the mid-1950s were these crucial vocalizations detected and brought to the forefront of scientific curiosity. Recognizing the potential and importance of USVs, researchers found them to be reliable biomarkers for various neurological conditions including Autism Spectrum Disorder and Parkinson's Disease. Yet, the process of manually identifying USVs proved challenging and demanded significant resources. The landscape of USV study transformed with the recent advances in Digital Signal Processing and Machine Learning. These advances allowed the development of novel tools offering automatic and semi-automatic identification and analysis of USVs, revolutionizing the field. Within this context, we will introduce the novel DeepFisFis USV Detector (DeepFisFis), an end-to-end solution for real-time USV detection, utilizing a 1-Dimensional Convolutional Neural Network (1D-CNN). We'll be demonstrating the functionality of DeepFisFis by analyzing the USV pattern of mice during a social habituation/discrimination paradigm monitored in a purpose build multi-modal experimental setup. Our analysis revealed that DeepFisFis was able to reliably detect ultrasonic vocalizations of mice that might serve as a facile indicator of specific social interactions. Interestingly, the number of USVs decreased as a function of repeated social interactions, indicating a previously undescribed social habituation phenomenon. These findings are supported by automated postural analysis using A-SOiD, revealing a correlation between specific types of behavior and vocalization in a social context.

Deep learning based 3D-segmentation of dendritic spines recorded with two-photon in vivo imaging

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The automatic detection of dendritic spines in 3D is still a challenging and yet not fully resolved problem with regard to two-photon *in-vivo* imaging. The emergence of convolutional neural networks (CNN) like U-Nets enabled the development of deep learning based segmentation pipelines for biomedical images in general and for dendritic spines in particular. While these pipelines are most suitable for *in-vitro* confocal image data, they provide lower prediction accuracy when applied to volumetric *in-vivo* two-photon images that have a lower signal-to-noise ratio and larger motion artifacts. Thus, researchers of this field still tend to analyze dendritic spines manually, which is time-consuming and prone to human bias. We therefore developed a pipeline for multi-class semantic image segmentation based on a fully convolutional neural network, that specifically targets 3D two-photon *in-vivo* image data. By choosing U-Net as the underlying network architecture, only a few labeled training images (< 50) are required. The U-Net processes 2D images to reduce computation time. A post-hoc 3D connectivity analysis merges the classified spine pixels and reconstructs the 3D morphology. Our pipeline is capable to segment spines from its associated dendrite with 85% accuracy and enables the further analysis of, e.g., spine morphology and spine density.

Simulating cortical dynamics in anatomically detailed network models

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Classic population rate models describing cortical networks consider a single excitatory and a single inhibitory (E-I) population network. Biological cortical networks, in contrast, often contain multiple excitatory and inhibitory cell types with asymmetrical connectivity patterns. Due to such asymmetrical connectivity, each cell type can show significant divergence in cortical network dynamics. However, the mechanisms of how cell-type-specific asymmetrical connectivity impact network dynamics are not fully understood. We asked whether a rate model can help understand the consequences of asymmetrical connectivity for cortical network dynamics. To answer this question, we simulated the stabilized supralinear network (SSN) rate model and the spiking network activity of approximately 4000 leaky-integrate-and-fire (LIF) neurons. The parameters of SSN and LIF neurons were based on biologically plausible data for each specific cell type reported for the barrel cortex L5. We found that the SSN rate model and LIF network activity were consistent with each other, confirming that our approximation of population rate is biologically constrained. In the activity regime characterized by supersaturation, the asymmetric connectivity from the excitatory neural population changed the amplitude of the dynamics. In contrast, asymmetric connectivity from the inhibitory neural population changed the dynamic's amplitude and width. We conclude that the SSN rate model can help interpret the role of the asymmetrical connectivity of the network dynamics. Furthermore, the asymmetrical connectivity from excitatory and inhibitory neural populations has a distinct impact on the network dynamics. We can now use the model to derive predictions for future experiments and identify cell-type-specific activity patterns that contribute to sensory perception.

A protein-driven heterosynaptic rule for spiking neural networks

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Conditions for synaptic plasticity have been extensively studied throughout the last decades, attempting to gain more insight into the elusive mechanisms that underlie superior cognitive phenomena like memory and learning. However, a unifying functional principle with clear biological grounding explaining these manifestations has not yet been fully formulated, and many state-of-the-art modelizations still partially rely on purely heuristic rules. To address this complex question, we explore the molecular machinery underlying heterosynaptic plasticity evolving on minute-hour timescales. Inspired by the results from one of our previous collaborations, we investigate the spatiotemporal dynamics of peri-synaptic calcium, indirectly reconstructing its fingerprint from the behavior of primary synaptic plasticity driving molecules (calmodulin, calcineurin, CaMKII). From the activation profile of these effectors, we construct a reaction-diffusion model that supports emerging properties like inter-spine competition and cooperation. Accounting for the correct timescales, we find a closed-form approximation for the model, obtaining a clean, explainable, and light functional form, the parameters of which we then fit using the experimental data in our possession. We then implement the newly found plasticity rule in a computationally simulated dendritic branch and validate its behaviour on several previous experimental findings. Finally, we investigate the computational properties of the linear dendritic branch system, focusing, in particular, on its information-encoding features (e.g., spine clustering, stimulus discrimination) and their impairment deriving from molecular alterations.

Sensory encoding in cortical output populations

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The cortex transforms sensory input into cortical output to enable perception. A common feature of all sensory systems is the broadening of the cells receptive fields along the sensory pathway. Here we study the relevance of this broadening for sensory information processing using the rat barrel cortex as a model system. We used detailed computational models that are representative of in-vivo morphological, biophysical and network variability to simulate the response of the major cortical output population to sensory stimulation, and assessed its encoding ability. Our results indicate that broadness of the cells' receptive fields and cell-to-cell variability enables them to reliably encode the stimulated whisker from any small population of cells across the barrel cortex. We conclude that labeled-line input to barrel cortex gets transformed into a parallel and redundant output where populations of cortical output cells in each column can encode all whiskers.

Role of CKAMP44 in retinogeniculate synapse

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The dorsolateral geniculate nucleus (dLGN) in the thalamus is the first relay station of visual information. Relay cells (RCs) in the dLGN receive excitatory inputs from retinal ganglion cells (RGCs) via retinogeniculate synapses and cortical neurons via corticogeniculate synapses. Previous studies from our group (von Engelhardt et al., 2010; Khodosevich et al., Chen at al., 2018) showed that CKAMP44 influences surface trafficking and gating kinetics of AMPA receptors (AMPARs) in RCs, downregulates the recovery from desensitization of AMPARs and reduces short-term depression in retinogeniculate synapses. In vivo recordings showed that the alteration in short-term plasticity affects relay cell spike probability in response to visual input. To investigate in more detail how deletion of CKAMP44 alters computational properties of RCs, I performed in-vivo tetrode recordings of RCs activity in awake head-fixed mice in response to diverse visual stimuli. The results indicate that the influence of CKAMP44 on RCs is particularly high when RGCs respond to external input with high firing frequency. Furthermore, to understand the relevance of main mechanisms involved in short-term plasticity of retinogeniculate synapses and information processing in RCs, I generated a computational model of RCs with retinogeniculate synapses based on realistic anatomical and physiological parameters. Results from simulations revealed that synaptic inputs integration at retinogeniculate synapses is strongly dependent not only on the long-lasting recovery from desensitization of AMPARs, but also on glutamate spillover. Taken together these finding indicate that, CKAMP44 acts as a low pass filter in retinogeniculate synapses.

Models of neocortical pyramidal neurons with well constrained sub- and suprathreshold dendritic and somatic properties

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Layer 5 pyramidal neurons have elaborate dendrites spanning all layers. They express a plethora of ion channels, enabling the dendrites to perform nonlinear computations. Generating biophysically detailed multi-compartmental models that capture both their sub- and suprathreshold electrophysiological properties remains a major challenge. Here, we show how to generate models that capture both dendritic and perisomatic properties for both sub- and subthreshold stimuli. Specifically, the resulting models reproduce backpropagating APs, dendritic Ca2+ APs, burst firing and critical frequencies as well as steady state subthreshold attenuation along the dendrites, dendritic and somatic input resistance and characteristic responses to chirp stimuli. Thereby, the models accurately capture a wide range of features describing the dendritic and perisomatic physiology and will be instrumental in investigating information processing in the L5 pyramidal neurons.

A-SOiD: an active learning platform for expert-guided, data-efficient discovery of behavior*

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The identification and quantification of animal behavior from video recordings have rapidly developed with the rise of easy-access, markerless pose estimation. In particular, unsupervised algorithms have enabled the unbiased discovery of movement motifs and simultaneously reduced effort costs. However, despite these considerable benefits, user refinement is often needed to align motifs with scientific nomenclature, particularly for specific social behaviors that coarse descriptions may only define. Although some supervised methods exist that aim to reproduce human classification directly, their inflexibility and training costs are prohibitive and lack the benefits of unsupervised behavioral identification. Our algorithm overcomes these challenges by incorporating an active-learning component into the behavioral discovery process, considerably improving accuracy and training costs. In addition, the developed pipeline allows the unsupervised discovery of latent behaviors (B-SOiD, Nat Comm) within the dataset to identify subtypes within known groups or disentangle unlabeled data. These newly found groups can then be integrated into the active learning process to build a balanced, high-quality dataset for the robust classification of social behavior, combining user-defined actions with unsupervised pattern discovery in a single classifier. We show its capabilities by investigating a sizeable human-annotated data set of social behavior in mice (CalMS21; Sun et al. 2021) and extending the range of detected behavioral expressions. Moreover, we illustrate that the algorithm is agnostic to animal models in an independent single monkey dataset.

*Also a short talk

Astrocytes implement a reward-mediated BCM rule giving rise to flexible learning

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Recent experiments have uncovered an astrocytes-mediated feedback loop in CA1 pyramidal neurons, initiated by the release of endocannabinoids by post-synaptic neurons and closed by astrocytic regulation of the D-serine levels at the dendrites. D-serine is a co-agonist for the NMDA receptor, and it exerts regulatory effects on synaptic plasticity. The existence of a regulatory feedback loop was already conjectured in the well-known model of synaptic plasticity proposed by Bienenstock, Cooper, and Munro (BCM). Here, we explore the relationship between this theoretical model and the newly available biological data. Based on the experimental findings, we present a formal derivation of the BCM plasticity rule. The new formulation allows us to reproduce the behavioral effects of CB1Rs knockout and the subsequent disruption of the feedback loop on mice during a place avoidance task. Moreover, we could make testable predictions on the dynamics of synaptic D-serine during learning and on the effects of astrocytic CBRs manipulation on synaptic plasticity. The proposed biological formulation of the BCM model provides new validation for the theory and new experimental questions. It paves the way for developing a more biologically plausible theory and understanding the role of astrocytes in synaptic plasticity.

A biophysical model for neuronal dynamics during energy deprivation with glutamate recycling

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Introduction Cerebral ischemia is a condition in which insufficient blood flow to the brain leads to metabolic stress. This can result in synaptic transmission failure, disturbances in ion homeostasis and eventually, cell swelling or cell death. Previous research has shown a tipping point beyond which return to physiological behaviour is impossible. We study neuronal dynamics during energy deprivation to identify the transition from reversible to irreversible damage, focusing on excitatory synaptic transmission. Methods We have constructed a biophysical model of a presynaptic neuron within a finite extracellular space calibrated with experimental data. To maintain ion homeostasis, the transmembrane ion fluxes are counteracted by ion transporters, such as the energy-dependent sodium-potassium ATPase (NKA). To model glutamate endocytosis and exocytosis, we combine calcium-dependent glutamate release and uptake by the excitatory amino acid transporter. To simulate energy deprivation, we deactivate the NKA. Using our model, we study the transition from physiological to pathological behaviour. Furthermore, we analyze the different recovery time scales for ion homeostasis and glutamate clearance. Results Our model faithfully reproduces baseline physiological behaviour. During ischemia, ion homeostasis and glutamate clearance are disturbed, and cell swelling occurs due to imbalanced osmotic pressure. Upon restoring energy supply, the neuron returns to the physiological state or approaches an irreversible pathological state in which the neuron is not excitable anymore. Recovery of ion homeostasis and synaptic transmission are not simultaneous. The neuron may return to physiological levels while excitotoxicity remains. In conclusion, our model allows simulation of neuronal dynamics during ischemia and during recovery.

Connectomics, Circuits

Mapping functional to anatomical connectivity of circuits in the macaque retina*

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Understanding computation in a neural circuit requires correlating its structure and function. Here we dissect the visual computations performed by retinal ganglion cell (RGC) circuitry in macaque retina by estimating and comparing cone inputs to RGCs using physiological and anatomical data. By recording the responses of RGCs to white-noise stimuli using a 512-electrode array, we identified receptive field mosaics of the five major RGC types ON and OFF parasol, ON and OFF midget, small bistratified cells (SBC), and several unknown RGC and polyaxonal amacrine cell types. Single-cone resolution stimulation revealed the inputs of many individual cones in the receptive field of each RGC. Multi-beam serial section electron microscopy was used to image this functionally characterized retina over a volume spanning 750x1000x150 μm^3. After aligning the physiological and anatomical data, we anatomically identified many functionally characterized RGCs of the five major types. To determine the anatomical connectivity of one SBC, we reconstructed S-cone bipolar cells that provide ribbon input to it, and the S-cone synapses that provide ribbon inputs to the corresponding bipolar cells. The anatomical weights of the cone inputs are then approximated as the product of the cone-bipolar cell and the bipolar cell-SBC connectivity matrices. To determine the functional connectivity, we estimated the weights of the individual S-cone contributions to the receptive field of this SBC. Direct cone-by-cone comparison revealed a strong positive correlation between functional and anatomical cone weights. Similar analysis is currently underway with L/M-cone inputs to midget and parasol RGCs.

*Only a short talk

Alone in the Dark: Illuminating Phototaxis in single Danionella translucida

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Research on larval Zebrafish has yielded valuable insights into the mechanics of phototaxis within a vertebrate species. However, the extent to which this structure is maintained through brain rewiring during fish maturation remains an open question. Adult fish of the Danionella translucida species are of particular interest for this topic, having among the smallest brain sizes among vertebrates and exhibiting optic transparency. Assessing phototaxis in single Danionella, however, has been challenging due to their irritability. Nevertheless, our recent findings demonstrate a remarkable phototaxis response can be elicited in single, free-swimming adult Danionella. Notably, we show that even minor differences in luminance induce a spatial bias away from the light source. In the future, we aim to employ calcium imaging techniques with these fish to examine neural circuits involved in the phototaxis process in an adult vertebrate species

Thalamus drives active dendritic computations in the cortex

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Perception relies on a calcium-dependent spiking mechanism by which the dendrites of layer 5 pyramidal tract neurons - the major output cells of the cerebral cortex - combine inputs from different information streams. Which circuits activate this mechanism upon sensory input is unclear. Here we found that thalamocortical axons, which provide sensory input to cortex, target specifically the dendritic domain in pyramidal tract neurons that initiates calcium spikes. Sensory input thereby enables distal dendritic inputs preceding the stimulus to transform the first responses that leave cortex into bursts of action potentials. Thus, thalamus can drive active dendritic coupling of sensory with nonsensory information streams to modulate cortical output. Our findings indicate that thalamocortical coupling is first in a cascade of mechanisms that transform sensory input into perception.

Axon initial segment dynamics during associative fear learning*

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The axon initial segment (AIS) is the site of action potential initiation and plays a crucial role in the generation of neuronal activity and the maintenance of network function during sensory processing and learning. While previous *ex vivo* studies identified the AIS as a site of homeostatic plasticity, the occurrence of structural changes of AIS *in vivo* and their implication in learning remains unknown. By performing *in vivo* longitudinal two-photon imaging of live-stained AIS in the mouse medial prefrontal cortex, we reveal dynamic AIS length remodelling during associative fear learning and extinction. Notably, we observed distinct bidirectional AIS plasticity mechanisms that may balance the excitability of neuronal subnetworks with distinct functions in memory formation and extinction, which could ultimately influence behavior and memory recall. Our findings suggest that axon initial segment dynamics are not only crucial for homeostatic adaptation but also act as a hallmark of memory formation.

*Also a short talk

Optogenetic manipulation of freely-flying flies

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The flight of *Drosophila melanogaster* comprises two distinctive stages: straight flight patterns which are punctuated by fast and sharp turns known as saccades. These saccades can be triggered either due to a threat (like collision avoidance), or as part of environmental exploration. To gain a better understanding of the neuronal mechanisms that control these saccades, we use a 3D tracking system in combination with optogenetic light stimulation. This method allows us to examine the role of specific descending neurons in the turning behaviors of flies during free-flight. Focusing on the novel DNaX neuron, we showed a marked turning response in flies expressing CsChrimson both uni- and bi-laterally. However, a closer examination of their behaviors using high-speed cameras showed a more complex behavior, which we are now looking into in more detail. We additionally started looking at other DNs, which are known to be involved in the flight control of Drosophila.

Biased connectivities underlie the different odour preference and odour discrimination capabilities of the Drosophila mushroom body*

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The mushroom body (MB), the centre for olfactory associative learning in the insect brain, performs pattern separation to discriminate even similar odours. We examined mechanisms underlying odour discrimination in the *Drosophila* MB by combining connectome analysis, network modelling and functional imaging. We revealed that different MB neuron (MBNs) subtypes sample the olfactory space differently, deviating from the theoretical optimum for odour discrimination. MBNs receive combinatorial input from different types of olfactory projection neurons (PNs). In the presence of inhibition, MBNs display sparse population coding. The PNs-to-MBNs connectivity determines the MBN's pattern separation ability: it is theoretically optimal if MBNs receive random inputs from the PNs. Contrary to the common belief that MBNs subtypes receive similar information about the odour for various memory processes, our analysis of multiple connectomics datasets revealed that α/β and α'/β' MBNs receive highly biased inputs from food-odour-responding PNs, while γ MBNs receive slightly biased inputs from mating-odour-responding PNs. By building an MB network model that incorporates realistic PN-to-MBN-subtype connectivities, we showed that biased connectivity of α/β and α'/β' MBNs to food-odour-responding PNs could increase their response overlap between food odours. In contrast, the biased connectivity of the γ MBNs to mating-odour-responding PNs resulted in further decorrelation of the MBNs response. These predictions were supported by our functional imaging experiments. Altogether, these results suggested that connectivities of α/β and α'/β' MBNs favour generalisation of novel food odours, while γ MBNs have reduced response overlap among food odours, enhancing food odour discrimination.

*Also a short talk

The role of inputs from the hippocampus to the prefrontal cortex in spatial memory impairments in a mouse model of Alzheimer's disease

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Alzheimer's disease (AD) is characterized by memory deficits including spatial working memory. The hippocampus (HPC) and the prefrontal cortex (PFC), highly impaired by AD pathology, are relevant brain regions for spatial working memory. The HPC and the PFC are highly connected and operate in synergy to consolidate and retrieve memories. Both excitatory and long-range inhibitory projections between the HPC and the PFC have been poorly characterized in AD. In fact, it remains unknown whether structural and functional connectivity deficits between these brain areas are causally linked to spatial working memory deficits under AD-like conditions. To investigate this, we utilized APP/PS1 transgenic mice, a mouse model for amyloidosis that recapitulates important aspects of AD. We used a Cre-driver mouse line to target somatostatin-positive interneurons by AAV-mediated Cre-dependent fluorophore and chemogenetic tool expression to conduct neuronal tracings and manipulate neuronal activity, respectively. Moreover, we will perform microprism-based in vivo two-photon Ca2+ imaging in the PFC of awake, head-fixed mice to functionally characterize PFC inputs. Our data show the accumulation of amyloid β plaques in the PFC. Furthermore, we observed a novel somatostatin-positive axonal -projection originating in the ventral HPC and targeting the PFC. These projections showed amyloid pathology-associated structural alterations and a decreased density. These results indicate that structural connectivity impairments between HPC and PFC in a mouse model of AD-like pathology might underlie spatial working memory deficits.

Differential contribution of CA1 and EC to spatial and velocity coding of subicular pyramidal neurons

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The subiculum is the major output structure of the hippocampal formation and plays an important role in learning, memory and spatial navigation. Two major inputs to the subiculum arise from the CA1 region and the entorhinal cortex (EC). It is not known, how this input is integrated by individual neurons and converted into their spatially tuned output. To address this question, we first performed whole cell voltage clamp recordings of individual subicular neurons while mice were running on a circular track. Second, we investigated the synaptic distribution in the dendritic tree of subicular neurons for both input regions using Chronos-assisted circuit mapping (CRACM). Finally, we examined the influence of both input paths to the spatial tuning of subicular neurons by performing 2 photon calcium imaging together with DREADD-mediated silencing of axonal fibers from either CA1 or EC while mice were running on a linear track. Our whole-cell voltage clamp recordings revealed that subicular pyramidal neurons receive spatially- and velocity-tuned excitatory synaptic input during spatial navigation. Furthermore, our CRACM data showed that CA1 input is located in the perisomatic region, while entorhinal cortex input is integrated more distally in the dendritic tree of subicular neurons. The DREADD-mediated pathway dependent reduction of this input demonstrated that the two major inputs from CA1 and EC had differential effects on the spatial- and velocity-tuning of subicular neurons on the output level. Taken together, we provide experimental evidence for differential contribution of EC and CA1 input to spatially-tuned output of subicular pyramidal neurons.

Modality specificity of multisensory integration and decision-making in the frontal cortex and superior colliculus

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The integration of inputs from different senses is a crucial aspect of sensory perception and the production of corresponding behavioral decisions. However, whether such multisensory integration occurs at specific stages of neural processing, for example between unisensory information processing and choice formation, remains unclear. Two brain regions, the anterolateral motor cortex (ALM) and the superior colliculus (SC) have been implicated as important structures for multisensory integration and decision-making, suggesting that they are part of a loop transforming multisensory inputs into behavioral decisions. To study the role of these areas in multisensory decision-making, we trained mice in a visuo-tactile discrimination task, where animals had to integrate sensory 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). Moreover, ALM and dSC showed strong choice-predictive activity during stimulus presentation and a subsequent delay period. Interestingly, visual and tactile choice were differently encoded in ALM, with no clear relation between visual and tactile choice neurons. Additionally, the neurons encoding multisensory choice were distinct from both unisensory populations. In contrast, dSC neurons encoded choice signals independently of the sensory modality. 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.

Decoding modality-specific function and neuromodulation in the Drosophila nociceptive network

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Fast and efficient escape behavior in response to noxious stimuli is essential for the protection and survival of all animals. In *Drosophila* larvae, specific sensory neurons, so-called nociceptors, detect noxious stimuli including heat and touch triggering a rolling escape response. Despite the extensive characterization of nociceptors across organisms, how noxious stimuli including harsh touch and heat are processed at the neuronal network level remains poorly understood. The recently reconstructed central nervous system of the *Drosophila* larva now provides insight into the organization of the circuits underlying nociceptive behavior. In addition, neuromodulatory peptidergic signals play an important role in shaping stimulus-specific network responses by influencing neuronal activity. In the larval nociceptive circuit, short Neuropeptide F (sNPF) and its receptor (sNPF-R) are required for both, mechano- and thermo-nociception, yet sNPF signaling seems to occur in different neurons for each modality. We are investigating the diverging circuits and sNPF function underlying thermo- and mechano nociception at the behavioral and functional level in this network. We found that distinct 2nd order neurons downstream of the nociceptors are required for escape responses to noxious mechanical or thermal stimulation. However, at higher-order levels, both modalities rely on the same neuron(s) suggesting divergent and convergent sensory processing by the underlying network. By mapping the expression of sNPF and its receptor in combination with their genetic manipulation and functional imaging, we aim to assess the modality-specific requirement of peptidergic signaling in these circuits. Our study thus provides the basis for a detailed understanding of the divergent mechano- and thermonociceptive circuitry and neuromodulatory signaling underlying larval escape behavior.

Organization of ascending pathways from the ventral nerve cord to the brain of adult Drosophila melanogaster

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The organization of the somatosensory system presents conserved principles across taxa; the mammalian and insect nerve cords are organized in similar modality-specific sensory layers. From there, information is conveyed to the brain by ascending neurons (ANs), but little is known about their precise projections and neuron types. We used the fruit fly *Drosophila* as a model to investigate the organizational principles of ANs. Combining sparse driver lines and stochastic single-cell labeling enables us to investigate the morphology of individual ANs. We searched 69,548 image sets of single-neuron labeling and identified XXX images ANs. Using this dataset, we identified 709 putative AN types based on their specific projection patterns. These neuron types were further categorized into 416 "families" and 270 "orders" based on the common projection patterns and shared key features.. We overlaid the AN images with identified primary sensory neuron images to determine the likely modality of sensory input, and with motoneuron images to identify AN types that may encode efference copies. About 70%, 10%, 9% and 7% of AN types receive inputs in the leg neuropils, wing and haltere neuropils, and abdominal ganglia, respectively. The brain regions targeted by the most AN types are the gnathal ganglion, saddle, anterior ventrolateral protocerebrum (AVLP) and wedge. The AVLP mainly receives leg neuropil ANs. ANs from the chordotonal (movement) and campaniform (load) proprioceptive sensory layers terminate in specific AVLP subregions. Our systematic map of fly ANs lays the groundwork for understanding the neuronal circuit functions underlying sensorimotor control.

Neural correlates of trace eyeblink conditioning in long-range CA3 projections

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Activity in the mammalian hippocampus encodes sequences of events and is necessary in order to learn associations involving discontinuous stimuli. It has been proposed that the recurrent network of the hippocampal area CA3 may be necessary to bridge the temporal gap between paired stimuli. However, so far there is little direct evidence while at the same time alternative explanations are lacking. The hippocampus is an elongated structure with a functional differentiation along its dorso-ventral axis and encompasses three major subregions – DG, CA3, and CA1. Among them, the Pyramidal cells of area CA3 compose the major source of intrahippocampal short- and long-range connectivity. In this project we therefore investigated neural activity of CA3 long-range projections during trace eyeblink conditioning (CS-trace-US), complementing previous work in the DG and CA1. To do that, we performed 2-photon Calcium imaging in head-fixed mice. Interestingly, we could not find learning-related correlates of neural activity in CA3 projections, although the conditioned response was acquired by all animals. Furthermore, the fraction of CS-trace responsive projections was very low and their activity did not result in a temporal tiling of the trace period. However, we observed a few projections with highly consistent activity 1 – 2s after the US presentation. A similar phenomenon was found in somatic imaging data of DG and CA1 Pyramidal cells during the same paradigm. In how far this phenomenon contributes to the acquisition of the CS-US association and which role, if any, CA3 plays in this task is therefore still an open question.

Multimodal sensory cue-based novelty detection in CA1

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The detection of novelty within a given environment and its evaluation is crucial for survival. Novelty is an attribute of a stimulus that lacks a pre-existing representation and novelty detection involves a series of interrelated processes, each playing a unique role. Different medial temporal lobe structures including the hippocampus have been highly implicated to play a major role in novelty detection. However, the underlying neuronal circuit is not described. In this project, we aim to understand how hippocampal CA1 does encode novelty and familiarity signals induced by unimodal and multimodal sensory stimuli and what are the processes that mediate the transition between novelty and familiarity. We plan to use a multisensory cue delivery setup including a novel behavioural paradigm for head-fixed mice where we can introduce different individual sensory modalities in a fast and quantifiable way, coupled with 2-photon imaging of CA1 neuronal cell bodies and axonal inputs from Entorhinal Cortex (EC) and CA3 to CA1. We aim at shedding light on how CA1 responds to distinct perceptual novelty processing stages like (1) absolute novelty processing, (2) novelty due to recency, and (3) sensory surprise and at determining the threshold of change in different sensory stimuli that induce novelty signals in CA1.

Dentate granule cell activity and slow gamma oscillations support the formation of precise memories*

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Animals constantly form memories and often, also very subtle differences in their environment are worth remembering. How does the brain compute these very similar, overlapping inputs and converts them into more distinct and memorable patterns? In the mammalian brain, a candidate area for this process, termed pattern separation, is the dentate gyrus. It is part of the hippocampal formation and receives strong inputs from the entorhinal cortex, a major hub for multisensory information in the brain. We used a dentate-specific optogenetic inhibition strategy combined with a spatial object pattern test, to show that in mice, unperturbed dentate activity during the acquisition phase is required for successful memory formation. Adding in-vivo electrophysiology to that, we found that dentate granule cell inhibition, however, does not alter the LFP profile in CA1, a major output area of the hippocampus. A computational model of the hippocampus suggested that dentate granule cells might perform pattern separation best when they receive input from the entorhinal cortex in the slow gamma range. To test this, we used optogenetic stimulation of PV-positive neurons in the medial septum to entrain the brain to different oscillations. We found that slow gamma, but not slow or fast theta stimulation, led to an improved performance in the object pattern separation test. In addition, mice that received slow gamma stimulation moved more and faster, and explored more, suggesting that entraining the brain to slow gamma frequencies puts the mouse in an “exploratory state”, where pattern separation is best performed when most necessary.

*Also a short talk

Ensemble state changes in sensory thalamus represent learned outcomes

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Subcortical thalamic brain areas play an important role in adaptive behaviors. Nevertheless, the neural population dynamics and plasticity of thalamic sensory relays during behavioral learning across sensory modalities remains unknown. Using a cross-modal sensory reversal learning paradigm in combination with longitudinal deep brain two-photon calcium imaging of large populations of auditory thalamus neurons (medial geniculate body, MGB), we identifiedfunctional classes of MGB neurons that align with distinct task periods and behavioral outcomes both, dependent and independent of sensory modality. In addition, during non-sensory delay periods, MGB ensembles developed coherentneuronal representation as well as distinct co-activity network states reflecting task outcome. Our results demonstrate the flexible cross-modal ensemble coding capacity of auditory thalamus during adaptive learning and highlight its importance in brain-wide computations for complex behavior.

Deciphering the role of the locus coeruleus for hippocampus-dependent learning and its impairment in a mouse model of Alzheimer's disease

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Locus Coeruleus (LC) is a neuromodulatory system that is heavily and early affected during Alzheimer´s Disease (AD). This is observed as the loss of neurons, especially in the part that projects to the hippocampus (HPC). The goals of this project are to examine the influence of the LC on HPC-dependent mnemonic processes and on their impairment in AD. To achieve this, we explored whether the structural and functional connectivity between the LC and the HPC is altered in a APPswe/PSEN1dE9 mouse model of AD. A structural analysis of LC-HPC projections was performed by conducting neural tracing of LC-originating fibers, as well as, by immunohistochemically staining brain tissue of APPswe/PSEN1dE9 mice for a norepinephrine marker protein. For the functional analysis of LC-HPC projections we plan to record axonal projections in hippocampal CA1 that originate from the LC while the mouse is performing tasks designed to test spatial and working memory. For this, we achieved precise expression of an axonal GCaMP in LC-originating fibers by a complementary neural tracing approach. We are also going to examine the role of the LC in APPswe/PSEN1dE9 mice for novel context recognition. A structural analysis of LC-HPC projections revealed axonal dystrophies of LC-originating neurites in the HPC of APPswe/PSEN1dE9 mice. This result supports our hypothesis that AD-mouse models show morphological impairments of LC-HPC axonal projections. We expect to gain further insights about potential morphological and functional impairments of LC-HPC projections in mouse models of AD-like pathology.

Plasticity of amygdala interneurons in associative learning

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The basolateral amygdala (BLA) is a cortex-like structure known to be involved in simple forms of emotional learning such as fear conditioning. It is the main entry site for sensory information to the amygdala complex, and local plasticity in the BLA is crucial for associative memory formation. Plastic changes of glutamatergic projection neurons (PNs) induced during learning have been well characterized, while little is known about the contribution of GABAergic interneurons. Although inhibitory interneurons only constitute about 20% of the neuronal population in the BLA, they tightly control PN activity and plasticity. Nonetheless, the behavioral relevance of different interneuron subtypes and their plasticity upon learning remain largely unexplored. Thus, the present study aimed to assess how the activity of local interneurons in the BLA is modulated across fear conditioning and extinction. To address this, we performed deep-brain calcium imaging with miniature microscopes in freely behaving mice during an associative fear learning paradigm. We selectively targeted discrete interneuron subtypes by injecting a cre-dependent GCaMP6 into the BLA of VIP-Cre, SST-Cre, or PV-Cre mice, followed by an implantation of a gradient-index (GRIN) lens. Following the same neuronal populations across days, we found that interneuron subtypes show diverse activity patterns during the conditioning paradigm, with distinct plastic responses upon fear and extinction learning. Overall, our results suggest that BLA inhibitory interneuron plasticity can contribute to the acquisition and expression of associative fear memories.

Modulation of thalamocortical transmission in active vision

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Brain activity, including visual processing, is profoundly shaped by self-motion and behavioral state. Locomotion under head-restrained conditions prominently influences the gain of visual responses in both primary visual cortex and visual thalamus. However, the interplay of proprioceptive, vestibular, and motor command signals with retinal input remains unclear in the context of natural vision as part of unrestrained, self-paced, and goal-directed behavior. Our study seeks to illuminate how rich movements during naturalistic orienting behavior influence visual processing in primary visual cortex and different neuronal populations in primary thalamus, namely dorsal lateral geniculate nucleus. In the project, we examine how the response properties of thalamocortical afferents and layer 2/3 pyramidal neurons in murine primary visual cortex are modulated by complex motion during unrestrained behavior in comparison with passive head-fixed stimulation. For this, we develop a closed-loop paradigm when mice pursue visual projection in the open arena. The task mimics rodent insect hunting but provides coarse retinotopic stimulus control and allows for an increased trial count. Animal behavior is densely quantified by multicamera tracking and inertial motion sensors. To record neuronal and axonal projection activity under both head-fixed and freely moving conditions we establish miniature two-photon imaging of genetically encoded Ca2+ indicators. Together with input-specific viral transduction methods, we can investigate the differences in motion modulation of visual responses in segregated tecto-thalamo-cortical visual circuits. Here we present the current state of the project.

Representational stability in the mouse visual system

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Our brain is in constant change and at every moment our neuronal system alters its circuit, cellular and subcellular structures. However, our visual perception remains stable over time, evoking the question of how reliable cognition can arise from unstable circuits. Here we study the (in)stability of sensory representations on single neuron level in the visual system of the mouse. Numerous studies observed varying degrees of so-called "representational drift". However, the extent to which this drift is caused by changes in behavior and brain states, as well as the cellular and circuit mechanisms that influence representational stability, remain unknown. Theories of dynamic neural networks suggest that error feedback from higher-order neuronal populations can stabilize representations in lower-order circuits. We test this hypothesis on the reciprocal connections between the dorsal lateral geniculate nucleus (dLGN) and primary visual cortex (V1), which offer the opportunity to identify and alter these connections, specifically. By utilizing two-photon Ca^{2+}-imaging of dLGN afferents and V1 neurons, we conduct chronic measurements of neuronal activity in awake mice to evaluate the stability of visual representations. We subsequently apply (chemo)genetic tools to disrupt V1 dLGN feedback and investigate whether this intervention alters the stability of previously observed neurons. Furthermore, we employ closed-loop experimental paradigms combined with machine learning models to effectively disentangle behavioral and sensory influences on neuronal responses. We thereby aim to bridge findings of stability and plasticity in the brain and provide insight into how stable perception arises in dynamic networks.

Projection specific information coding in frontal cortical networks

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Perceptual decision making involves processing of sensory information to select appropriate motor responses. A known hub for such complex process is the anterior lateral motor cortex (ALM), which is relevant for integrating diverse sensory information to drive associated behavioural outcome. ALM pyramidal neurons form a heterogeneous population, and can be distinguished by their molecular profiles and projection patterns. Primary target regions of the ALM are subcortical areas as striatum, thalamus and superior colliculus (SC), which are known to be involved in sensorimotor transformation, working memory and decision making. The ability of building associations between stimuli and actions emerges with experience and training, and therefore through a learning process. While pyramidal neurons have already been shown to be involved in learning of goal-directed actions, the specific role of distinct projection pathways in learning is still under active study. Here, we implemented a series of behavioral tasks with increasing cognitive demand, as distinct stages of learning. In order to understand the specific role of distinct ALM neuronal populations, we performed pathway-specific two-photon imaging over the course of training. Moreover, we used the optogenetic synaptic silencer eOPN3 to inactive different projection pathways and dissect their respective role during different paradims. Two-photon imaging of ALM-to-striatum (CStr) projecting neurons revealed distinct tuning properties of CStr neurons during the tasks and their change over learning. Furthermore, our optogenetics experiments showed specific impact of inhibiting CStr, ALM-to-thalamus and ALM-to-SC projections during each task, pointing to a learning stage-dependency of their relevance in decision making.

New definition of the whisker motor areas in the rat cerebral cortex

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Which areas of the rodent cerebral cortex control movements of the facial whiskers (i.e., vibrissae)? Here, we address this question by using retrograde transneuronal transport of the rabies virus to identify the cortical areas with disynaptic access to the motoneurons that innervate a single whisker muscle. For comparison, we also examine which cortical areas have disynaptic access to the motoneurons that innervate a single forepaw muscle, extensor digitorum communis (EDC). We find that five major cortical areas in both the contra- and ipsilateral hemispheres contribute to the control of whisker motoneurons: primary motor (M1) and sensory cortex (S1), secondary motor (M2) and sensory cortex (S2), and anterior insular cortex (AI). The proportions of the neurons with disynaptic access to the whisker motoneurons are similar in both hemispheres – 50% originate from M1, 35% from S1, and 5% from M2, S2, and AI, respectively. However, the distributions of the neurons within these major cortical areas differ between hemispheres. The vibrissa-related part of S1, the barrel cortex, and a medial-caudal part of M1 are unilateral – i.e., they have access exclusively to whisker motoneurons on the contralateral side. The remaining parts of M1 and S1, as well as M2, S2, and AI are bilateral – i.e., they have access to whisker motoneurons on both the contra- and ipsilateral sides. Notably, the bilateral whisker area of M1 is subdivided further into two parts, where either the contra- or ipsilateral side dominates. Cortical areas with disynaptic access to EDC motoneurons are restricted to the contralateral hemisphere. The cortical areas representing the forepaw and whisker muscle are disjoint in S1, partially overlap in M1, and fully overlap in M2 and S2. The insular cortex has no disynaptic access to forepaw motoneurons. Thus, descending influences from multiple, functionally distinct cortical areas contribute to the control of whisker motoneurons. In addition, we discovered new regions for differential control of contra- and/or ipsilateral whiskers and for coordination in the control of whiskers and forelimb.

A septal-VTA circuit drives exploratory behavior*

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To survive, animals need to balance their exploratory drive with their need for safety. Subcortical circuits play an important role in initiating and modulating movement based on external demands and the internal state of the animal; but how motivation and onset of locomotion are regulated remains largely unresolved. Here, we show that a glutamatergic pathway from the medial septum and diagonal band of Broca (MSDB) to the ventral tegmental area (VTA) controls exploratory locomotor behavior in mice. Using a self-supervised machine learning approach (VAME; Luxem et al., 2022), we found an overrepresentation of exploratory actions, such as sniffing, whisking, and rearing when this projection is optogenetically activated. Mechanistically, this role relies on glutamatergic MSDB projections that monosynaptically target a subset of both glutamatergic and dopaminergic VTA neurons. Taken together, we identified a novel glutamatergic basal forebrain to midbrain circuit that initiates locomotor activity and contributes to the expression of exploration-associated behavior.

*Also a short talk

Is representational drift regulated by cell-autonomous mechanisms in CA1?

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Recent evidence from calcium imaging and electrophysiology experiments has challenged the notion that stable perception, memories, recall, and behavior rely on consistent neuronal activity. This phenomenon, known as representational drift, has been observed in various brain areas, yet the underlying causes remain unknown. Although theoretical models have proposed connections between drift and Hebbian and homeostatic plasticity, as well as the balance between excitatory and inhibitory neural activity (E/I ratio), robust empirical evidence is necessary to understand the functional significance of drift in cognition and behavior. Therefore we aim to investigate the hypothesis that genetic mechanisms of Hebbian plasticity affect the representational stability of neurons in the hippocampal CA1, which is crucial for learning and memory processes. Using miniature 2-photon microscopy (mini 2p), we will longitudinally monitor and record the activity of wild-type and knockout hippocampal CA1 neurons as animals navigate linear tracks and open fields for food rewards. Specifically, we will conduct sparse and dense knockout experiments of the Grin1 gene, which regulates Hebbian plasticity, and compare CA1 neuronal activity before and after the gene knockout. The mini 2p will enable us to chronically record neurons with subcellular resolution over several weeks, and also to identify affected cells in sparse knockout experiments, allowing for correlation with marker gene expression. This research holds significance in uncovering the factors that govern representational drift and providing insights into its potential role in learning and behavior. By manipulating such drift, we can further explore its impact and implications.

Acquired avoidance of pathogenic bacteria depends on specific antimicrobial peptides in the nervous system and fat body

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Pathogen ingestion can lead to physiological consequences such as infection and tissue damage. However, animals can learn from the consequences of pathogen feeding and modify their behavior to avoid future exposure (Garcia et al., 1955; Wright et al., 2010;). While the fundamental mechanisms seem conserved across species, the mechanisms are not fully understood. Recent work implicates the immune system as a central mechanism (Gonzalez-Santana and Diaz Heijtz, 2020) since bacterial peptidoglycans (PGNs) are recognized by peptidoglycan-recognizing proteins (PGRPs) in various tissues, including the nervous system; PGN recognition can activate the downstream expression of antimicrobial peptides (AMPs) (Pean and Dionne, 2014). Previous studies revealed that AMPs are required for learning and memory (Barajas-Azpeleta et al., 2018). Recently work from our lab showed that the flies acquire the CFA through a mushroom body-dependent neural circuit, AMPs and PGRP-LC in octopaminergic neurons (OANs) are essential parts of this mechanism(Kobler et al., 2020). In this study, we aimed at identifying the involved AMPs and their roles in CFA. To this end, we tested flies mutant for different AMPs or lacking specific AMPs in specific neurons and tissues by testing their preference for two Gram-negative pathogenic bacterial strains, Erwinia Carotovora 15 (Ecc15) and Pseudomonas entomophila (Pe) post-ingestion. First, we identify specific AMPs necessary and sufficient for CFA, specifically in the head fatbody and OANs. Second, RNAseq data confirms that pathogens ingestion leads to the upregulation of AMPs and other candidate genes in the head and fatbody. Finally, our preliminary data suggest that octopamine and dopamine receptors are involved in this behavior. Current experiments aim at characterizing (i) the functional relationship between AMPs, dopamine and octopamine receptors signaling and (ii) the interaction between the fatbody and the nervous system by using a combination of feeding assays, genetics and imaging approaches.

Development

Fat sensory cues in early life program central response to food and obesity*

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Maternal obesity predisposes offspring to metabolic diseases. Insulin resistance and adiposity secondary to maternal calorie-rich high-fat diet consumption are considered key contributors to such developmental programming. Here, we show that non-nutritive sensory components of high-fat diet (HFD), beyond its hypercaloric, obesogenic effects, are sufficient to alter metabolic health in the offspring. To dissociate the caloric and sensory components of HFD, we fed dams a bacon-flavored diet isonutritional to a normal chow diet but enriched with fat-related odors, mimicking the commonly used lard-based HFD. We show that offspring exposed to these fat-related odors during development display in adulthood increased weight gain, adiposity, and insulin resistance in response to HFD feeding independently of maternal metabolic health alterations. Dopamine and calcium fiber photometry imaging revealed that developmental exposure to fat-related odors shifts mesolimbic dopaminergic circuits and Agouti-related peptide (AgRP) hunger neurons' responses to phenocopy those of obese mice, including desensitization of AgRP neurons to ghrelin and dietary fat. Further, neither neonatal optogenetic activation of sensory circuits nor passive exposure to fat-related odors was sufficient to exacerbate obesity, suggesting that the sensory programming of metabolism is contingent on caloric associations. Collectively, we report that fat-related sensory cues during development act as instructive signals to prime central responses to food cues and whole-body metabolism regulation.

*Presented on the second poster session

Changes in striatal blood-brain barrier tightness during early development*

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The striatum coordinates several cognitive and motor functions, which could be hindered by impaired blood-brain barrier (BBB) integrity in early development. Here, *ISMICAP* model was used to assess the integrity of tight junctions connecting endothelial cells of the BBB in striata during development. Striatal acute slices were obtained from embryos (E18), neonates (P0-P2) and pups (P12 and P25) of wildtype mice. In *ISMICAP*, capillaries are perfused with fluorescent molecules, while monitoring their paracellular diffusion with two-photon microscopy. Biocytin-tetramethylrhodamine (TMR), sulforhodamine 101 (SR101) and 7-hydroxycoumarin-3-carboxylic acid (7HCC) were individually perfused in vasculature for 30 min and their extravascular diffusion was quantified. FM1-43 diffusion to the abluminal side of endothelial cells was also assessed. In embryos, the parenchymal diffusion of all molecules was comparable to that at P25. However, a gradual but significant increase of extravascular fluorescence was recorded in neonates, reaching nearly 2-fold, 3-fold and 5-fold that in embryos for TMR, SR101 and 7HCC, respectively. At P12, the extravascular leakage of all molecules dropped to ~1.5-fold that at P25. In neonates, SR101 accumulated in some endothelial cells, while some perivascular cells, likely fibroblasts, took up 7HCC. FM1-43 abluminal diffusion in embryos was 2-fold that at P25, while it significantly increased to ~3 folds in neonates and some pericytes were labelled as well. Our results imply that the BBB in embryonic striata exhibits a comparable tightness to that at P25. Once the embryos are delivered, BBB integrity seems to decline noticeably, while some perivascular cells potentially act as a "second line of defense".

*Also a short talk

Ceramide synthase influences function of astrocytes in Drosophila and mouse

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Ceramide Synthases (CerS) are key enzymes in the sphingolipid pathway acylating a sphingoid long-chain base with a fatty acyl-CoA of variable chain lengths. All CerS consist of a catalytic motif required for enzymatic activity. Whereas *Drosophila* encodes for only one CerS named Schlank, mammals have six CerS homologs, mostly tissue-specific expressed. Schlank is strongly enriched in the central nervous system (CNS), especially in glial cells as well as in nearly all neurons of the ventral and lateral sensory group. In mammals, e.g. CerS2 is expressed in oligodendrocytes, the myelinating cells of the CNS, whereas CerS1 is only found in neurons. Ceramides contribute to lipotoxicity that underlies diabetes, hepatic steatosis and other chronic diseases. Especially the balance between different ceramide subtypes and contribution of the CerS enzymatic activity and its pathological consequences have been addressed in many studies. Surprisingly little is known about relationships between CerS, causing ceramide misregulation, and neurodevelopment, neurodegeneration and glia-neuron communication in healthy or diseased brain. In our group, we established CerS KO or catalytic inactive CerS variants in *Drosophila* and mouse models. In both organisms, we observed an altered astrocyte appearance, impaired survival and developmental delay. In mice, it is not clear to what extent the brain phenotype contributes to this, because these mice also develop severe liver tumors. We want to unravel the contribution of astrocytes to brain development and analyze secreted signals from astrocytes and their impact on glia-neuron crosstalk. Therefore, we established primary astrocyte culture assays from our CerS *Drosophila* and mouse models.

Development of locomotion during metamorphosis in the Western clawed frog, Xenopus tropicalis

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Uncovering the principles underlying the development of spinal locomotor circuits is essential for gaining an integrative understanding of rhythmic motor behavior. Frog metamorphosis involves a gradual transformation of locomotor modes in live and behaving vertebrates. The bilateral alternation of axial muscles that facilitates tail swimming is superseded by slower, synchronous kicking during this developmental transition, which takes place over a period of 6-8 weeks. This transition makes frogs a unique model system for exploring the functional ontogeny of locomotor circuits. In this study, we aim to characterize the ontogeny of movement in the developing frog hindlimb and its coupling with the oscillatory motion of the receding tail during metamorphosis. We achieve this by measuring the three-dimensional kinematics of the limb joints and tail in freely swimming tadpoles of the western clawed frog species, Xenopus tropicalis, at multiple metamorphic stages, using Deeplabcut and multi-camera triangulation techniques. We compare the diversity in locomotor bouts and analyze the pose-trajectories of the animals across development to identify changes in the structure of locomotor activity during metamorphosis. In addition to establishing the behavioral basis of locomotor development across metamorphosis, our three-dimensional kinematic characterization will also facilitate engineering closed loop systems to measure neuronal activity in developing spinal locomotor motifs using in-vivo multi-photon microscopy.

Loss of a cell surface molecule affects dendritic development and interaction with surrounding tissue in Drosophila larvae

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Neuronal function is informed by neuronal morphology, be it to enable basic computation of incoming signals, or to increase the field coverage of the neuron to e.g. broaden the overall input area, as is the case in some sensory neurons. One example of a sensory neuron with an expansive dendritic field is the class 4 dendritic arborization neuron of Drosophila melanogaster larvae, who's dendrites tiles the whole body wall of the animals thereby facilitating detection of strong nociceptive stimuli. Their highly arborized dendritic fields have been successfully used to study dendritic elaboration due to their stereotyped appearance. Yet many of the more dynamic components of dendritic branch establishment, as well as many of the potential interactions guiding these dynamics, remain poorly understood. Here we used a genetic screen to identify cell surface molecules involved in the dynamics of dendritic field establishment. One candidate molecule identified by this screen could be shown to affect the development of the dendritic branches, in context with their surrounding tissues. To further characterize the effect this molecule has on the extension/retraction/stabilization and thereby overall dendritic elaboration we employ a combination of long term in vivo imaging across several developmental stages of the animals as well as neuronal reconstructions for quantification. Combining this with targeted manipulation of the molecules in question during development will enable this work to further elucidate the mechanisms underlying dynamic growth changes upon interaction with external cues throughout dendritic development.

Academic publication of neurodegenerative diseases from a bibliographic perspective: a comparative scientometric analysis

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Background: For measuring the impact in clinical and scientifific research, the citation count of the articles is used in the bibliometric analysis, although there is no comprehensive summary of neurodegenerative disease research. This study intends to provide the neuroscientists and investigators with a practical reference guide to appraise the most important and inflfluential articles written on this subject through a macroscopic view of the research activities on neurodegenerative diseases. Materials and Methods: The Clarivate Analytics Web of Science was searched in July 2020. To ensure the breadth of the search scope, the search terms were confifirmed as "multiple sclerosis" (MS) or "amyotrophic lateral sclerosis" (ALS) or "Parkinson's" or "Alzheimer's" or "Huntington's" or "neurodegenerative." After excluding completely unrelated articles, the top-cited articles were collected and evaluated from special characteristics. The data analysis was performed using SPSS 18.0. The articles were characterized by citation number, publication year, topic, study type, authorship, journal, country, and institute of responding author and foundation. Results: The query identifified 593,050 articles. A total of 45% of the top-cited articles were published during 2000–2009, followed by 30 articles from 1990–1999. Diagnosis and pathology were the main research categories (n = 62). Alzheimer's disease (AD) was the main study topic (n = 43). Meanwhile, the United States confifirmed the tremendous impact on the fifield of neurodegenerative diseases. Notably, 69 of 100 articles were studied in the United States, and the National Institutes of Health sponsored 49 articles. There were only 22 articles that can be divided by evidence level. No article was categorized as level 1 evidence. In the journal list with multiple articles, seven of 15 were general journals. The 58 authors, who contributed to more than one article, have been identifified by VOSviewer, and the clusters of authors reveal the evolution of research focus in neurodegenerative diseases. Conclusions: This study analyzed the bibliometric characteristics and connections of 100 top-cited articles in the fifield of neurodegenerative diseases in the Web of Science. Their main outcomes were as follows: First, the pathology and diagnostic researches took a major role in top-cited articles while the therapy articles are relatively less. Second, the United States confifirmed the tremendous impact on the fifield of neurodegenerative diseases. Third, researchers also submitted their researches to general journals, not just focused on specialty journals.

Deregulated translational control and somatosensory function in genetic models of autism spectrum disorders in Drosophila

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Autism spectrum disorders (ASDs) are a group of heterogeneous early onset neuropsychiatric disorders with symptoms including impaired social interaction and repetitive stereotyped behavior. Recent evidence showed that ASDs are highly inheritable developmental synaptopathies affecting dendritic development, synaptogenesis and excitatory/inhibitory balance. However, the molecular and circuit mechanisms contributing to autism are not fully understood. Recent studies in genetic mouse models showed that developmental defects in the somatosensory system can drive ASD-like changes including in social behavior. This suggests that abnormal somatosensory development might be a key driver for functional and behavioral changes observed in ASD patients. Mechanistically, altered translational control resulting in exaggerated protein synthesis was hypothesized to contribute to the development of ASDs as observed for Fragile X syndrome, which is linked to the function of FMR1 and its genetic network. To unravel the potential link between protein translation and ASD-linked gene function in the somatosensory system, we investigate the role of the conserved ASD-linked genes Tao/TAOK2, FMR1 and Cyfip/Cyfip1 in the somatosensory system of Drosophila larvae. Similarly as FMR1 and Cyfip1, Tao kinase is a conserved regulator of neuronal growth and cytoskeletal dynamics, which might be a common feature linked to ASDs. We established cellular readouts to monitor translation in larval somatosensory neurons and found that besides Fmr1 and Cyfip, Tao can also regulate translation. We further identified somatosensory functions and genetic links between these three genes suggesting translational control might be a common mechanism affecting somatosensory development and function. We are further investigating the combinatorial impact of these ASD risk genes on translation to identify key downstream targets driving functional and behavioral changes in this system.

Göttingen Meeting of the German Neuroscience Society 2023: the axonal vesicle release machinery and myelination

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Myelination of axons, necessary for saltatory conduction of action potentials, requires the development of mature oligodendrocytes from oligodendrocyte precursor cells, also called NG2 cells, which can be newly generated at any stage of the central nervous system (CNS) development. It has been suggested that myelin formation is plastic and might be regulated by neuronal activity, for example during learning and memory. Neurons directly communicate with NG2 cells via synapses which are functionally and structurally similar to classical synapses between neurons. NG2 cells express glutamate receptors and voltage-gated ion channels and are able to transform synaptic input into intracellular calcium signals. However, whereas several studies have addressed the relevance of postsynaptic signal processing in NG2 cells, the role of neuronal glutamate release on myelination is still not fully elucidated. Here, we bi-directionally manipulated axonal neurotransmitter release by targeting, components of the presynaptic release machinery. To this end, we introduced plasmids expressing specific shRNAs, mutated proteins and Tetanus toxin into excitatory neurons in layer II/III of the cortex WT mice at E14.5 by in utero electroporation. These mice were then perfused at distinct postnatal time points and coronal brain slices were prepared for analysis of myelination by electron microscopy via immunogold labelling against the aforementioned DNA. At an early postnatal timepoint of preparation various parameters of myelination, such as myelin thickness, g-ratios, axon diameter and number of myelinated axons, seem not to be affected by the first set of introduced plasmids. However, at this timepoint only a small number of axons is myelinated. Effects might differ at a later developmental timepoint, at which myelination in the corpus callosum reaches its peak. This possibility as well as potential effects of targeting other components of the presynaptic release machinery are still being investigated.

Disease

Altered dendritic excitability and cell maturation of CA3 pyramidal neurons during development in the Scn2aA263V genetic epilepsy model

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Gain-of-function (GOF) variants of the Nav1.2 sodium channel are strongly associated with various developmental disorders, with epilepsy as a common feature. Although previous studies in heterologous expression systems have identified the biophysical mechanism underlying the GOF, not well understood is how a GOF mutation alters cellular and synaptic properties during development. In this study, we studied the cellular excitability and dendritic integration in CA3 pyramidal neurons during early (PN10-PN14) and later (PN24-PN28) developmental stages in the Scn2aA263V mouse model of genetic epilepsy using patch-clamp recordings and simultaneous glutamate iontophoresis. At PN10-PN14, the data show an abnormal transient somatic hyperexcitability in Scn2aA263V mutant animals. During early development, CA3 dendrites from wt animals exhibited largely linear increases in EPSP amplitudes, whereas CA3 dendrites from Scn2aA263V+/wt animals were capable of aberrant dendritic spikes (d-spikes). Next, we examined how the maturation of dendritic morphology and excitability changed following these aberrant dendritic spikes. At PN24-PN28, dendritic spikes maturated with a distinctive fast-rising phase in wt mice. In addition, most CA3 cells switched from a 'athorny' to a 'thorny' phenotype. However, CA3 dendrites in Scn2a mutant animals did not develop the characteristic fast d-spikes and remained primarily 'athorny.' These data suggest that aberrant dendritic hyperexcitability during early developmental stages alters the maturation of CA3 pyramidal neurons in the Scn2aA263V model of genetic epilepsy.

Neuron-microglia communication modulates sensorimotor function and resilience to chronic stress*

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Major depression is a major medical, social, and economic challenge. Chronic psychological and physical stressors are important pathogenic factors. Preclinical models link stress effects to dysfunction of neurons and microglia in regions such as the prefrontal cortex and hippocampus. Other important brain regions, such as the sensorimotor cortex, are understudied. We have recently shown that chronic stress impairs motor cortical function and alters microglia-neuron interaction. Fractalkine signaling is a highly specific mode of neuron-microglia communication and modulates neuroplasticity and stress response. In addition, exercise has been shown to increase stress resilience and counteract negative effects of stress. It is unknown how gradual disruption of the fractalkine pathway affects the interaction between stress resilience and the motor/somatosensory domains and whether this can be modulated by preventive physical training. Male mice with intact, partial, or complete deficiency of the fractalkine receptor on microglia were assessed at baseline, after treadmill training, and after chronic restraint stress with a wide range of tests of affective, motor, and somatosensory behavior. In addition, we measured sensory fibers with non-invasive somatosensory evoked potentials (SSEP). Our data show that the disruption of the fractalkine pathway produces distinct phenotypes with increased stress vulnerability and impaired somatosensory function in complete receptor deficiency. Unexpectedly, partially deficient mice displayed signs of increased stress resilience. Next, we plan to investigate underlying molecular mechanisms in the brain and periphery as a function of genotype, training, and stress exposure. Taken together, our data may provide new targets for the diagnosis and treatment of stress-related disorders.

*Also a short talk

Released factors from malignant neuroepithelial glioneuronal tumors enhance neuronal network synchronicity

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Introduction: Glioneuronal tumors (GNTs) are neuroepithelial tumors strongly associated with epilepsy. Despite being generally benign, they can progress to malignant neoplasms, for which seizures can be pharmacoresistant and difficult to control. Thus, understanding the mechanisms underlying epilepsy appears critical to find new anticonvulsant treatments. Our group has recently established a murine model of malignant GNTs ($BRAF^{V600E}$/pAkt/$Trp53^{KO}$) and here we aimed to assess the effect of the tumor-secreted factors on neuronal activity patterns and network maturation in-vitro. **Methods:** Tumor cells were cultured for 5 days and the supernatants were collected and used as conditioned media (CM) for plating primary cortical neurons in multielectrode-arrays (MEA) plates. As controls, fresh naïve media or neuronal CMs were used. The number of spikes, percentage of bursts and synchrony index (SI) were recorded over time. **Results and discussion:** Our results showed that the percentage of bursts and SI were enhanced in primary neurons incubated with tumor-CM compared to control media at the early stages of neuronal maturation. These electrophysiological effects could not be reversed by washing out the CM, indicating the ability of tumor-CM to foster plastic/permanent effects on the neuronal networks. The subsequent analysis of the tumor-CM by Mass Spectrometry detected proteins associated with neurogenesis, including App, Apoe, Sod1, Prdx2 or Tnc. Overall, we demonstrate that GNT cells release factors that can alter the electrophysiological features of the neurons. These data offer a substantial gain of knowledge as a basis to find new targetable molecules for dealing with seizures in GNTs.

NLRP3 contributes to microglial morphological changes, ASC aggregation and long-term behavioural phenotypes after traumatic brain injury*

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Traumatic brain injury (TBI) is one of the leading causes of death and permanent disability in the economically active population, and one of the most important risk factors for developing dementia. We hypothesized that the NLRP3 inflammasome pathway is essential to induce microglia morphological responses, and its genetic removal or pharmacological inhibition could prevent neurobehavioral impairment. We evaluated NLRP3 inflammasome-related proteins and key inflammatory markers in different time point after controlled cortical impact (CCI). Additionally, we performed molecular, histological, and neurobehavioral evaluation of *Nlrp3*$^{-/-}$ mice after CCI. Lastly, we evaluated the effect of administering two new oral NLRP3 inhibitors on behavioural outcomes after CCI. NLRP3 inflammasome-related proteins and key inflammatory markers appear upregulated in the first 7 days and normalize at 30 days post CCI. *Nlrp3*$^{-/-}$ microglia retain a ramified morphology and reduced ASC-aggregates, indicating lower activation and persistence of homeostatic functions. Furthermore, injured *Nlrp3*$^{-/-}$ mice or mice treated with NLRP3 inhibitors show a faster neurological recovery in neurobehavioral tests in comparison to injured WT treated with vehicle. Moreover, using *in vivo* intravital repeated 2-photon microscopy, we confirmed a reduced aggregation of ASC and conserved microglia morphology in mice treated with an NLRP3-inhibitor. Our results showed that upregulation of the NLRP3 pathway is an acute hallmark after CCI, and contributes to morphological and functional changes of microglia, and neurocognitive impairment after TBI. Our translational research shows that NLRP3 inflammasome is a feasible target for clinical trials in patients that suffered or are at risk of neurocognitive decline after TBI.

*Also a short talk

A quantitative model of sporadic axonal degeneration in the Drosophila visual system

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In human neurodegenerative diseases, neurons undergo axonal degeneration before they die. Therefore, for potential intervention and to better understand early phases of neurodegeneration, defining the initiation of axon damage is of great importance. Invertebrate models, have significantly contributed to our understanding of neurodegenerative disorders. However, these models mainly rely on manipulation of genes identified in familial cases of neurodegenerative diseases. Nonetheless, the vast majority of cases of neurodegenerative diseases are thought to be sporadic. We developed a system modelling early degenerative events in *Drosophila* adult photoreceptor cells, in which mild constant light stimulation for several days overcame the intrinsic resilience of R7 photoreceptors and led to progressive axonal degeneration in the absence of cell death. Aged flies displayed an accelerated and increased vulnerability in this system and loss of synaptic integrity between R7 and its postsynaptic partner preceded axonal degeneration, thus recapitulating important features of human neurodegenerative diseases. Furthermore, we defined precisely the time window in which the axonal damage becomes irreversible. We will present our ongoing work towards a dissection of the cellular circuit mechanisms involved in the early events of axonal degeneration, allowing for a better understanding of how neurons cope with stress and lose their resilience capacities.

Analysis of neuroinflammation in CCL17-DTR mice post DT-treatment

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The chemokine CCL17 is best known for its role in facilitating interactions between T cells and dendritic cells. Previous research in our lab demonstrated homeostatic expression of CCL17 in murine hippocampal neurons. Analysis of the tissue-resident macrophages of the brain, the microglia, showed that CCL17 is required to maintain the homeostatic morphology of these cells. Ablation of CCL17-expressing cells by intraperitoneal injection of Diphtheria toxin (DT) in mice expressing the DT receptor (DTR) under the control of the *Ccl17* promotor (CCL17-DTR) results in the development of epileptic seizures. The brains of CCL17-DTR mice undergoing seizures are characterized by a loss of hippocampal neurons as well as inflammatory responses of brain resident cell populations, particularly microglia and astrocytes. In addition, strong fluctuations in body weight were observed in these mice. Onset and development of neuroinflammation following the DT-mediated ablation of $CCL17^+$ neurons in CCL17-DTR mice was monitored by immunohistology. Neuronal cell loss was analyzed by H&E and FJC staining. The integrity of the blood-brain-barrier (BBB) was analyzed by Evans Blue dye extravasation. Ablation of $CCL17^+$ cells induced extensive neuronal cell loss in the hippocampus as well as micro- and astrogliosis in the first two weeks post-DT. Shortly after DT-injection, the BBB remained intact in CCL17-DTR mice following DT-treatment. Administration of DT induces neuroinflammation and spontaneous recurrent seizures in CCL17-DTR mice. This model is less invasive than common epilepsy mouse models, e.g. the intracranial kainic acid model and could, in accordance with the 3R principle, reduce the harm inflicted on animals.

Investigation of hippocampal alterations in mouse models of spinal muscular atrophy and Parkinson's disease

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Mutations or deletions of the Survival of Motoneuron 1 (*SMN1*) gene lead to low amounts of Survival of motoneuron (SMN) protein and cause progressive muscle atrophy resulting in death during early childhood of spinal muscular atrophy (SMA) patients. Recent achievements in research have led to three effective SMN-restoring, life-prolonging therapies. However, the amount of SMN protein restauration is tissue-specific and therefore, the long-term clinical outcome in patients is not predictable. In particular, a potential cognitive decline during aging needs to be addressed. Beside the classical motor symptoms, Parkinson's disease (PD) also causes early non-motor symptoms, e.g., mild cognitive dysfunction, and late cognitive decline. Alpha-synuclein, the protein that forms insoluble aggregates in the brain of PD patients, enhances the survival of SMA mice after administration. Therefore, we aimed at investigating potential common mechanisms between SMA and PD regarding potential treatment targets and focused on the hippocampus with its role in memory formation and neurogenesis. We investigated molecular alterations in the hippocampus of a severe mouse model for SMA and of a PD mouse model. We found a significant upregulation of the mRNA for BDNF in the PD mouse model compared to control animals. This could indicate a compensatory mechanism to avoid further neurodegeneration and needs therefore to be further addressed.

Developmental and epileptic encephalopathy-linked HCN1 mutations cause epilepsy and affect interictal hippocampal and cortical network activities in mice

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Inherited and *de novo* mutations in voltage- and ligand-gated ion channels underlie many developmental and epileptic encephalopathies (DEEs). Affected patients' phenotypes are often resistant to antiseizure medications or even show paradoxical responses. The key to developing effective therapies that also prevent behavioral comorbidities is to understand the general or gene-specific disease mechanisms behind these DEEs. Here, we examined two knock-in mouse models that each carry one patient-derived pathogenic *HCN1* (hyperpolarization-activated cyclic nucleotide-gated cation channel) sequence variation. The human mutations p.G391D and p.M153I (*Hcn1*$^{G380D/+}$ and *Hcn1*$^{M142I/+}$ in mice) are associated with severe neonatal- and early infantile-onset epileptic encephalopathies with daily seizures, high mortality, and neurodevelopmental comorbidities like intellectual disabilities. The corresponding mouse models both display spontaneous generalized tonic-clonic epileptic seizures and comorbidities including locomotor hyperactivity, reduced motor coordination, and deficits in spatial working memory, which were overall more severe in *Hcn1*$^{G380D/+}$ animals. Analysis of HCN1 immunoreactivity in the hippocampus and cerebellum revealed pronounced alterations in the distribution and the levels of HCN1 channels, specifically disrupted targeting to the axon terminals of basket cell interneurons. Treatment with the sodium channel blockers lamotrigine and phenytoin induced epileptic seizures, which was also reported for patients carrying pathogenic *HCN1* sequence variants. Analysis of interictal electrocorticogram (ECoG) recordings revealed reduced low and high gamma power during rapid-eye-movement (REM) sleep. Moreover, hippocampal CA1 local field potentials recorded from awake head-fixed mice in the Mobile Homecage showed a reduced frequency of ripple oscillations. Together, these findings are indicative of impairment in inhibitory neuron function.

Effects of neural synchronization on hippocampal representations in healthy and Aβ-pathology conditions

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Disrupted neural oscillations and memory are common features of Alzheimer's disease (AD). Our previous studies have demonstrated the potential to restore the temporal organization of theta oscillations by utilizing LFP-guided closed-loop optogenetic stimulation of local parvalbumin-positive (PV+) interneurons in the CA1 region of the hippocampus in APP/PS1 mice. Furthermore, employing the same protocol, we successfully enhanced the performance of transgenic mice in a spatial recognition memory task. Building upon these findings, our objective is to investigate the impact of PV+ stimulation on circuit function within the CA1 region. To achieve this, we utilize the same mouse model for Aβ-pathology, specifically expressing an excitatory opsin in local PV+ interneurons and a functional Ca2+ reporter in CA1 pyramidal neurons. We subject head-fixed mice to a spatial reward-learning and memory task on a linear treadmill while simultaneously evaluating neural population coding. In this context, our goal is to identify behavioral disparities, as well as cellular and cognitive deficits, in APP/PS1 transgenic animals. Subsequently, we aim to determine if temporally and spatially controlled PV+ interneuron-mediated synchronization of population activity affects spatial representation. It will be investigated, if this modulation ultimately can be employed to ameliorate the identified deficits. Overall, the objective of this project is to establish a connection between observations associated with disturbed theta oscillations under conditions of Aβ-pathology and underlying cellular correlates.

Inhalative budesonide for treatment of Alzheimer's disease

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Various clinical and epidemiological observations suggest that immune activation contributes to neuronal dysfunction and death in many neurodegenerative diseases, particularly in Alzheimer´s disease (AD). However, the most potent inflammation suppressing drug category, glucocorticosteroids (GCCs), was never investigated in clinical trials. Analyzing German health claim data, we found that inhalative and intranasal GCC treatment was associated with a decreased dementia incidence. We hypothesized that intranasally applied GCCs will reach the brain directly via the nasal epithelium without causing severe adverse systemic effects and ameliorate neuroinflammation and AD pathology. We tested this theory by treating a murine AD model (5xFAD) with inhaled budesonide, a GCC drug commonly prescribed in asthma therapy. We saw a prevention of cognitive impairment in a preclinical paradigm and a rescue of cognitive function in a mild cognitive impairment paradigm. This was paralleled by a prevention of pathology-induced hippocampal atrophy and synaptic loss. Interestingly, single-cell sequencing data of microglia revealed no expression changes of classical cytokines, but a reduction of complement associated genes by GCC treatment. On top, we found a reduced number of infiltrating T-cells in GCC treated 5xFAD as well as a healthier transcriptomic profile of their neurons and oligodendrocytes. We conclude that inhaled GCCs show a broad range of positive effects on brain health making them suitable candidates for clinical trials in AD.

Sex- and region-specific cortical and hippocampal whole genome transcriptome profiles from control and APP/PS1 Alzheimer's disease mice

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Alzheimer's disease (AD) poses a significant challenge due to its multifaceted etiopathogenesis characterized by progressive cognitive decline. To gain an integrative understanding of this intricate neurodegenerative disorder, comprehensive transcriptome studies have become indispensable. In this investigation, we conducted an in-depth whole genome transcriptome analysis, leveraging microarray data derived from retrosplenial cortex and hippocampus of age-matched male and female APP/PS1 AD mice, alongside control counterparts. Our transcriptome analysis divulged novel, detailed insights into sex-specific gene expression profiles and the associated fold changes within the distinct APP/PS1 subgroups. Gene ontology and Venn analysis unveiled a predominance of (co)-upregulated genes, primarily related to the activation of microglial, astrocytic and neutrophilic cells, the innate immune response, the synaptic transmission. Conversely, the number of (co)-downregulated genes exhibited notable disparity across the various subgroups, encompassing mainly the synaptic vesicle docking/fusion machinery, the rRNA processing, the proteasome degradation. Significantly, this study represents the foremost systematic endeavor to untangle sex-specific and brain region-specific transcriptome fingerprints in the APP/PS1 mouse model. These pioneering findings hold paramount importance for future preclinical and clinical investigations in the realm of AD, and advance personalised approaches in AD research and management.

Network dynamics and early intervention in a Scn2a mouse model of developmental and epileptic encephalopathy

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Developmental insults during early postnatal development can have profound consequences on brain function, particularly in the context of developmental and epileptic encephalopathies (DEEs). Ion channel dysfunction, which disrupts the balance between excitation and inhibition, could cause severe forms of DEEs. In this study, we employed a DEE mouse model with a pathogenic patient-derived gain-of-function variant of Nav1.2 sodium channel alpha subunit encoded by *Scn2a* to investigate the potential therapeutic efficacy of early intervention. Our primary objective was to comprehensively characterize the network activity changes of the model. Both heterozygous and homozygous mutants develop seizures shortly after birth, the latter as early as postnatal day P3, which we assessed by hippocampal *in vivo* depth local field potential (LFP) recordings. Electrocorticographic (ECoG) recordings in adult mice revealed that seizures persist into adulthood only in homozygous mutants. Preliminary results did not show significant changes in interictal activity, consistent with the observed mild behavioral phenotype, but not excluding the possibility of more subtle changes in neuronal firing rates or phase coherence of theta and gamma oscillations. Having characterized the model's key features, our next steps involve treatment interventions during the early postnatal period to evaluate their impact on long-term outcomes in adulthood. Specifically, we are currently assessing the efficacy of chronic phenytoin administration from P1 to P21. By elucidating the seizure phenotype and network dynamics in the *Scn2a* mouse model, this study contributes to our understanding of the underlying mechanisms of DEEs and provides a foundation for future research on novel treatment strategies.

Antisense oligonucleotides (ASOs) and CRISPRa/i for the study of SCN2A-associated genetic epilepsies

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Mutations in SCN2A are associated with a wide spectrum of epilepsy syndromes, ranging from benign familial neonatal-infantile seizures (BFNIS) to epileptic encephalopathies. In fact, *SCN2A* variants are of common findings among children with epilepsy referred for genetic testing, including neonates. Mice deficient for Nav1.2 $Scn2a^{-/-}$ (Knockout animals (KO)) die at perinatal stages while haplodefiency for SCN2A, such as $SCN1A^{KO/+}$ develop non-convulsive absence-like seizures with spike-wave discharges (SWDs) beyond the age of postnatal day (PN) 70. On the other hand, animal models with gain-of-function mutations such as $Scn2a^{A263V/A263V}$ develop electrographic seizure activity localized to the hippocampus as early as PN3. The phenotype of early seizure onset seen in SCN2A related epilepsies in patients as well as in the different animal models strongly suggests an important role of Nav1.2 during embryonic development and reveals an important time window for treatment intervention. Here, we established the use of antisense oligonucleotides (ASOs), CRISPR-activation (CRISPRa) and CRISPR-inhibition (CRISPRi) for manipulating the expression of Nav1.2 in vitro with the future prospective of investigating these therapeutical time windows in SCN2A-animal models.

Accelerated prion-like spreading of protein aggregation by exogenous and endogenous viruses

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Tauopathies are a heterogeneous group of neurodegenerative diseases which are associated with the accumulation of aberrantly folded tau protein. Evidence accumulates that viral infections can contribute to disease onset and progression by inducing neurotoxicity and neuroinflammation. Here we uncover that viral gene products also accelerate the cell-to-cell spreading and propagation of pathologic protein aggregates such as tau. Using cell-based assays to study the dissemination of several pathologic protein aggregates, we demonstrate that viral gene products can increase transmission of protein aggregates by direct cell contact or via extracellular vesicles. We show that viral envelope proteins sorted onto cell surfaces and extracellular vesicles mediate contact with cognate receptors and efficient endosomal escape of aberrant protein aggregates into the cytosol of recipient cells. Importantly, the capacity of viral proteins to increase protein aggregate dissemination is not restricted to exogenous viruses, as epigenetic de-repression of endogenous retroviruses also accelerates protein aggregate spreading. Therapeutic targeting of viral envelope proteins inhibits protein aggregate spreading and might thus represent a promising strategy for disease intervention.

Glia

Microglia-complement interactions mediate synaptic dysfunctions in a mouse model of schizophrenia

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Synaptic pruning during brain development has been found to be mediated by microglia-complement interactions. Interestingly, convergent studies point to a loss of dendritic spines in the cortex of schizophrenic patients, which could reflect disturbances of the normal synaptic pruning processes. High-expression variants of complement component 4 (C4) genes constitute a major risk factor of schizophrenia. Likewise, over-expression of C4 in the mouse cortex leads to several schizophrenia-associated phenotypes, including synapse loss. To further assess the role of microglia-complement interactions in this C4 overexpression mouse model, we used two transgenic mouse lines lacking either the CR3 or the C3aR receptor, which are both expressed on microglia. We examined morphological properties, as well as electrophysiological properties of pyramidal neurons in the PFC. Additionally, microglia-neuron interactions were inspected in vivo via two-photon imaging, as well as microglia morphology. Our results show that the absence of CR3, but not C3aR, leads to significantly elevated spine density in both C4 over-expression and control conditions. Additionally, we found alterations in functional excitatory transmission after CR3 knock out. In vivo results show active involvement of microglia processes in spine refinement by altered contact and motility rates. Furthermore, microglia morphology changes are normalized with either knock out. These results suggest that the effects of C4 over-expression might be regulated by a microglia-complement interaction, where microglia recognize complement-tagged synapses by CR3 and are activated by C3aR. This pathway could influence the development of neural circuits and alterations in the prefrontal cortex and could consequently trigger cognitive disorders such as schizophrenia.

Characterization of cultivation methods to improve induced pluripotent stem cell-derived microglia culture

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Microglia, the brain-resident macrophages, shape many processes in the brain and have a major impact on brain development, homeostasis, and neurological diseases. Previous efforts to study microglia *in vitro* in the human context have only been possible through post-mortem brain tissue or neurosurgical resections. With the development of human induced pluripotent stem cells (iPSCs), the differentiation of various cell types, including microglia-like cells (iMGLs), has become available enabling to mimic their unique developmental biology. Since microglia are highly motile cells that adapt to their environment, we here compare cultivation approaches of iPSC-derived iMGLs by 2D and 3D cultivation in monoculture and within cerebral organoids. Using MotiQ, an open-source toolbox, we investigate morphological differences of the different cultivation types. In addition, we determine differences in their motility and phagocytic capacity using fluorescent beads. Using flow cytometry, we determine whether the cultivation method influences the differentiation and maturation of iMGLs. This study has the potential to further improve *in vitro* culture conditions for iMGLs for future studies in the field of neuroinflammation in order to converge experimental settings towards *in vivo* conditions.

Remission after stress via enriched environment increases hippocampal dendritic spine density independent of microglia

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Major depressive disorder (MDD) is a common disease of the central nervous system that leads to high socio-economic and clinical challenges. Chronic stress is suggested to be a major risk factor for MDD, but little is known about the underlying mechanisms. Accumulating studies suggest that microglia are implicated in synaptic dysfunction, that plays a crucial role in the etiology and course of depression. We focus on the role of hippocampal microglia-synapse interaction during both the induction and remission after stress in a mouse model. We behaviorally phenotype a depression-like mouse model by inducing chronic mild stress to adult male mice in the automated *IntelliCage* system. We further assess the effect of an enriched environment on the remission from stress. Simultaneously, we monitor structural changes of dendritic spines and microglia in the hippocampus using chronic *in vivo* two-photon microscopy. To assess the role of microglia on changes in spine density or behavior, we deplete them via the CSF1-R antagonist BLZ945. Stress led to an increased spine density on hippocampal CA1-neurons dependent on the presence of microglia. As previously shown, enriched environment resulted in increased spine density and was able to ameliorate stress-dependent behavior phenotypes. Surprisingly, enriched environment related dendritic spine increase was independent of microglia. The microglia fine process motility decreased upon stress induction and increased upon exposure to the enriched environment. Our multimodal approach of chronic *in vivo* imaging in behaving mice enabled us, to investigate the relationship of dendritic spine changes and microglia under stress conditions in the hippocampus.

The cytoskeletal proteins ADF/cofilin1 and their role in microglia morphology and function

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Microglia are the resident immune cells of the central nervous system in which they play a crucial role in homeostasis and disease. Moreover, they are implicated in brain development, neuronal interactions, and higher brain functions such as learning mechanisms. Microglia are highly motile cells that constantly scan the brain parenchyma with their fine processes. However, the cellular mechanisms underlying these dynamic changes in shape remain to be further elucidated. The cytoskeleton proteins actin depolymerizing factor and cofilin 1 (ADF/Cfl1) have been shown to be crucially involved in neuronal development, function and cell cycle control by actin filament organization. However, their role in microglia remains unknown. We generated a mouse line that conditionally lacks ADF/Cfl1 in microglia in the adult brain. Chronic *in vivo* two photon imaging of ADF/Cfl1-KO microglia in the cortex revealed altered microglia morphology and motility. Additionally, long-term monitoring of microglia migration towards a laser-lesion site was affected in absence of ADF/Cfl1 in microglia. Furthermore, an associative learning task indicated a role of microglial ADF/Cfl1 in learning and memory. Results from mRNA-Sequencing revealed distinct regulations in global gene expression upon knockout of ADF/Cfl1, affecting for example the postsynaptic actin cytoskeleton, or actin filament-based processes. Our results assign a crucial role of ADF/Cfl1 to the diverse microglia functions in health and disease. Moreover, our data suggest that microglial integrity in presence of ADF/Cfl1 is important for higher brain network processes that impact cognition.

The neurovascular unit repair process in an animal model of Alzheimer's disease

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Alzheimer's disease (AD) is the most common form of dementia, and is linked to the accumulation of amyloid-ß in the parenchyma (amyloid plaques) and around blood vessels (cerebral amyloid angiopathy). The latter has been correlated with dysfunction as well as increased fragility of cerebral blood vessels, resulting in cerebral microbleeds (CMBs) seen on MRI and in post-mortem examinations in AD. Parenchymal damage induced by these vessel ruptures leads to an activation of surrounding glial cells, in particular microglia and astrocytes, but the pathways underlying protective vs. potentially detrimental effects of this glial activation in the context of AD have remained incompletely understood. We hypothesized that microglia and astrocyte-based repair mechanisms of damaged blood vessels in the AD brain are impaired, potentially exacerbating dysfunction/loss of vessels that could lead to impaired neuronal circuits and behavioral deficits. To this end, we explored microglial and astroglial responses to CMBs in an AD model, and how this affects vessel integrity (leakiness) and repair (survival). Moreover, we aimed to characterize the molecular pathways underlying these changes. To this end, we assessed microglial dynamics and accumulation as well as vessel integrity and repair longitudinally in APP/PS1::Cx3cr1-GFP and age-matched controls at three stages of age-related amyloid accumulation. Mice were studied using *in vivo* multi-photon imaging through a chronic cranial window. CMBs were induced by high-power femtosecond laser ablation. Cortical areas were imaged acutely (30 min) and longitudinally (days-weeks) after CMB induction. In a second set of experiments, mice received an astrocyte-targeted adeno-associated virus expressing the calcium indicator GCaMP6f, allowing for simultaneous imaging of astrocyte activity. We found that microglia show rapid responses by extending their processes around the CMB, and that this perivascular microglial accumulation returns to baseline levels by day 14. Vessels that were not repaired (i.e. did not regain flow) showed significantly stronger microglial responses early after CMB compared to repaired vessels. CMBs also triggered sustained astroglial hyperactive perivascular network activity. Interestingly, APP/PS1 mice showed more vessel loss, increased microglial accumulation as well as increased astroglial calcium activity compared to controls. Hence, our data suggest that AD-related amyloidosis leads to sustained and distinct activity patterns of microglia and astrocytes in response to CMBs, and that these cellular changes may affect pathophysiologically relevant vascular elimination and repair mechanisms. Keywords: Alzheimer's disease, Two photon imaging

Synapses

Neuronal extracellular vesicles and associated microRNAs induce circuit connectivity downstream of BDNF*

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Extracellular vesicles (EV) are secretory vesicles that have emerged as important regulators of inter-cellular communication, in part by delivering their cargo, including proteins, lipids and microRNA. Despite collective evidence implicating EV in several neurodegenerative diseases, their physiological significance in inter-neuronal communication remains largely unexplored. We show that brain-derived neurotrophic factor (BDNF) specifically alters the microRNA composition of neuronal EVs. BDNF-induced EVs in turn induce synaptic vesicle clustering at excitatory synapses via the transfer of specific microRNAs, thus increasing synaptic transmission and synchronous neuronal network activity in naïve hippocampal neurons in vitro. Furthermore, we observe the retrograde synaptic spreading of EV, similarly to some viruses. Our ongoing work aims to investigate whether EVs influence the connectivity of neuronal circuits in vivo, and whether they are implicated in learning and memory. Overall, this work provides evidence for a novel constituent of trans-synaptic signaling that may be highly relevant to neurological diseases characterized by aberrant BDNF signaling.

*Also a short talk

Using a deconvolution strategy to analyze high-resolution microscopic time-lapse imaging data of neurons expressing optical reporter proteins to quantitatively reveal neurotransmitter release events

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Neurons are cells specialized in the reception, transmission and processing of information in wide networks through the formation of synapses that connect them. Synaptic signals are transmitted unidirectionally from one neuron to the next through the liberation of neurotransmitters. Inside the neurons the signals move though electrical currents, but since the neurons have no contact between them, the signals passage occurs though the vesicular release of neurotransmitters. The understanding of the brain complexity involves the study of synapse signaling, their regulation and their role in storing information. In this work we identify the intensity and occurrence of glutamate release synapses reported by iGluSnFr, a novel biosensor capable of reacting to the presence of glutamate with an increase of fluorescence in microscopical imaging. Procedures developed in Dr. Dietrich and Dr. Schoch Labs have shown that single vesicular release can be monitored from the presynaptic to the postsynaptic neurons using iGluSnFr, opening the door to study the nature and mechanics of spontaneous single vesicle release in synapses, phenomena widely known but which physiological implications still unclear. Many gigabytes of experimental images containing triggered and spontaneous release events in neural cultures *in vivo* are being quantitatively analyzed using an image deconvolution approach. This approach uses deconvolution and clustering algorithms to locate the signals using a kernel template based on real signals data. Operating this kernel over the set of images, the signals are reduced to single points revealing their position and intensity. A correct clustering of responses allows us also to identify the single vesicular release signals and clearly differentiate them from the multivesicular ones, harder to analyze and track in the study of neuron interactions.

The spatial organization of synaptic proteins in mammalian synapses and Drosophila neuromuscular junctions detected by two-photon polarization microscopy

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In recent years, the progress has been made in resolving the ultrastructure of the presynaptic cytomatrix. However, we still lack a detailed understanding of the 3-D organization of individual active zone (AZ) members. In this study, we aimed to investigate the spatial orientation of AZ proteins using the effect of linear dichroism (LD) detected by two-photon polarization microscopy. We validated our approach by demonstrating high LD in methoxy-X04 stained amyloid plaques and in a membrane-bound eGFP variant expressed in HEK293T cells and primary neurons. We also fused the actin filament reporter LifeAct to eGFP via several linkers and found that even non-structured linkers provide clear LD. Moreover, we next inserted the membrane-bound eGFP in neurexin1α to target the construct to synapses and showed that the reliable LD can be registered in the presynaptic membrane as well. Then LD was measured in synapses of cultured mouse primary neurons and in Drosophila larval NMJs expressing key AZ proteins fused with eGFP via non-structured linkers. We did not observe LD for the tested AZ proteins and also proved that LD is not disturbed by the eGFP-tagged protein molecules that are not integrated into the AZ cytomatrix. Thus, the regular spatial orientation of the AZ proteins can be excluded. However, certain radial regularities of their arrangements would remain undetected. Our data form the basis for further analyses aiming at resolving the spatial orientation of AZ members.

Characterization of the synaptic ultrastructure in human epileptic tissue*

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During epileptogenesis a stable brain network transitions into a functionally altered state that leads to the development of spontaneously recurring seizures. This process is associated with structural rearrangements contributing to the imbalance between excitation and inhibition. Loss of synapses, in particular in the hippocampal CA1 region has been observed in human biopsy specimen and tissue from animal models. However, so far the changes of synaptic ultrastructure of the remaining synapses in the human epileptic hippocampus has not been resolved. Here, we have employed high resolution ultrastructural FIB-SEM imaging to generate 3D EM data of the hippocampal CA1 and Hilus of the dentate gyrus regions from human brain tissue obtained during surgery for seizure control. We have compared freshly fixed tissue from patients suffering from temporal lobe epilepsy (TLE) with Ammons Horn sclerosis (AHS) to a control group undergoing hippocampectomy for other indications (lesion group). We have performed a comprehensive quantitative analysis of various parameter, e.g. synapse density, astrocytic coverage and volume, ratio between asymmetric and symmetric synapses, number of synaptic vesicles, active zone length and number of docked vesicles in 3D of synapses in these two regions. Our analysis revealed that in CA1 asymmetric synapses have more overall and docked vesicles as well as larger active zones. We also found an altered ratio between asymmetric and symmetric synapses towards more symmetric synapses. In the Hilus we observed a severe loss of synaptic density in the AHS samples as well as astrocytic growth, however no significant alterations were found on the individual synaptic level. Taken together, our results show that synapses in the hippocampus of TLE patients show multiple alterations in their ultrastructure when compared to a control group (lesion) that support an increase in excitation in these regions. As synaptic ultrastructure is tightly linked to function these findings will contribute to our understanding of the mechanisms underlying epileptogenesis.

*Also a short talk

Dephosphorylation of the RIM1α C2B domain impedes homodimerization

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The cytomatrix of the presynaptic active zone (AZ) is enriched in several proteins pivotal to vesicular release and integral to AZ architecture and function, including the Rab3-interacting molecule (RIM) protein family. The RIM1α isoform was previously shown to be involved in regulating synaptic transmission, and plays a significant role in synaptic vesicle priming and localizing calcium channels to the AZ. RIM1α knockout (KO) synapses exhibit reduced transmitter release and changes in multiple forms of presynaptic plasticity. Amongst other mechanisms, RIM1α function is modulated by post-translational modifications, including phosphorylation. Phosphorylation of RIM1α is required for the induction of presynaptic plasticity, and multiple phosphodeficient mutants of RIM1α cannot rescue the reduced release probability observed in KO synapses. Using protein purification and size exclusion chromatography, we here show that phosphorylation of two C-terminal serines of RIM1, S1600 and S1603, regulates homodimerization of the C2B domain of RIM1α, thereby potentially impacting its function in the release process. Through comparison of the molecular weight of purified RIM1α C2B domain harboring phospho-mimetic (S to E) and phospho-deficient (S to A) mutations, we show that phospho-deficient S1600A and S1603A sites induce an exclusively monomeric state for the C2B domain, thus interfering with its ability to form homodimers. Using the glutamate sensor iGluSnFR, we also show that these phospho-deficient RIM1 mutants potentiate the synaptic release of glutamate from the synapse. We hereby propose a mechanism whereby the functional role of RIM1α in synaptic release is controlled, through regulation of its homodimeric state, using phosphorylation.

More efficient synaptic vesicle release with smaller active zone protein complexes

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Neurotransmission efficiency depends on a complex interaction of presynaptic proteins in the so-called presynaptic active zone. Details of those interactions determine the fundamental properties of neurotransmission and information storage in the brain. Interactions of active zone proteins are still incompletely understood as their spatial arrangement and stoichiometry in the mammalian synapse are only partially revealed. In this work, we compare the distribution and amount of presynaptic proteins between functionally very distinct cerebellar synapses: very efficient climbing fiber synapses (cfs) showing a high release probability and low efficient parallel fiber synapses (pfs) showing a low release probability. We established quantitative volume electron microscopy (FIB-SEM) of phosphotungstic acid (PTA)-stained tissue. PTA is known to reveal a regular grid of protein complexes, so-called dense projections, likely containing high concentrations of active zone proteins. Our results show that dense projections strikingly differ between cfs and pfs: The more efficient cfs contain thinner, smaller, and fewer (per active zone area) dense projections compared to the less efficient pfs. We estimate that each dense projection of cfs contain ~25 MDa of active zone proteins whereas individual dense projections of less efficient pfs show a ~2-fold higher protein content (~50 MDa). The data show that dense projections substantially differ between types of synapses and indicate that their composition and/or stoichiometry reflects functional specialization. Moreover, the results shine new light on the role of the amount and distribution of presynaptic proteins as they suggest that highly efficient neurotransmission correlates with a smaller amount of active zone proteins.

Unravelling the impact of synaptic cleft composition on glutamate signalling dynamics

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Glutamate, a predominant excitatory neurotransmitter in the vertebrate central nervous system, plays a crucial role in synaptic communication. Utilizing a recently developed glutamate fluorescent sensor iGluSnFr, our laboratory demonstrated that glutamate released from individual hippocampal synapses can activate extracellular iGluSnFr molecules at distances greater than 1.5μm. In parallel, the use of the activity-dependent NMDA receptor inhibitor MK-801 revealed that release events from one synapse can activate distinct sets of NMDA receptors within the synaptic cleft. This observation suggests that the spatial separation of release sites within an active zone and the potentially uneven distribution of synaptic cleft material may partition the cleft into distinct compartments. However, previous studies based on existing synaptic cleft material data failed to identify a significant influence of the cleft material on signal transduction. To address this discrepancy, we conducted theoretical studies where our investigations focused on the release and diffusion of glutamate in the presence of synaptic cleft material within a single synapse. By exploring extreme conditions, including variations in the size, shape, and density of cleft obstacles, we aimed to determine the specific concentration and positioning of cleft proteins that significantly impact signal transmission. Our findings demonstrate that biologically plausible protein concentrations in the cleft can reduce amplitudes of AMPA receptor activation by more than 50%. These results provide valuable insights into a possible role of synaptic cleft material in modulating neurotransmitter transmission.

Three-dimensional neddylation modification identified from cofilin1

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Reversible post-translational modifications are essential for the regulation of eukaryotic cell functions. One possible modification is the addition of ubiquitin and ubiquitin-like proteins. We focus on neddylation, which, like ubiquitylation, involves three sequential reaction steps. During neddylation, a specific lysine in the target protein is linked by an isopeptide bond to a carboxyl group of the C-terminal glycine of Nedd8. This is achieved by the heterodimeric E1-activating enzyme NAE (Nedd8 Activating Enzyme), the conjugating enzyme Ubc12 (ubiquitin carrier protein 12) and as yet unknown E3 ligases. We have previously shown that neddylation is involved in the post-translational modification of synaptic proteins. For example, immunoblots of synaptosomes demonstrate that both pre- and postsynaptic proteins are neddylated. Previous studies have shown that neddylation increases during postnatal brain development and regulates synaptogenesis during neuronal development. In mature neurons, neddylation promotes spine stability and spine maturation. To further elucidate the role of neddylation in synapse function, we aim to identify synaptic proteins modified by neddylation. To this end, we have analysed several synaptic proteins for neddylation by co-immunoprecipitation. We used an avidin-labelled NEDD8 co-expressed with the GFP-tagged candidate protein in HEK-293 cells. The neddylated proteins were then co-immunoprecipitated using magnetic beads attached to streptavidin. Neddylated proteins were then detected by Western blotting against the GFP tag. Our experiments show that cofilin1 is neddylated. Following validation of these results, the neddylated lysine residues and the binding side of the Nedd8-conjugating enzyme Ubc12 were identified. From these data, we propose a three-dimensional binding motif for Ubc12, which we have also found in other neddylated proteins.

Effects of temperature on neuronal functionality and the number of synapses in cultured human neuronal networks exposed to hypoxia

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The application of mild therapeutic hypothermia has emerged as a potential neuroprotective intervention for mitigating the effects of transient cerebral ischemia. Preclinical investigations conducted in animal models have suggested its efficacy. Nonetheless, conflicting outcomes from clinical trials have cast uncertainty upon the therapeutic benefits of hypothermia. Furthermore, the precise mechanisms underlying the neuroprotective actions of hypothermia remain largely enigmatic. Here, we investigated the effects of hypo- and hyperthermia during hypoxia on neuronal functionality and synaptic connections in human induced pluripotent stem cell (hiPSC)-derived neuronal networks. HiPSC-derived neurons were differentiated and cultured on micro-electrode arrays (MEAs) or glass coverslips. Controlled hypoxia and target temperatures (normo- (37 ^{0}C), hypo- (34 ^{0}C), or hyperthermia (39 ^{0}C)) were achieved by climate chambers and maintained for 48h. Outcome measures included spontaneous and synchronous neuronal network activity (derived from MEAs) and number of synaptic puncta (derived from microscopic assessment) during and after hypoxia. Hypothermia-treated neuronal networks showed lower spontaneous and higher synchronous activity during hypoxia than neuronal networks under normothermia. Hyperthermia-treated neuronal networks showed an initial increase in spontaneous and synchronous activity followed by a rapid decrease. After reoxygenation and re-establishment of normothermia, hypothermia-treated neuronal networks showed mild recovery in spontaneous and synchronous activity; there was no such recovery in untreated or hyperthermia-treated neuronal networks. Recovery after hypothermia was associated with preservation of synapses. Hypothermia exerts neuroprotection during 48 hours of hypoxia in hiPSC-derived neuronal networks, yielding preservation of electrophysiological neuronal network functioning. Preservation of synapses is a possible protective mechanism. Hyperthermia exerts opposite effects.

Quantification of the number and mobility of presynaptic RIM1 molecules using single-synapse spectroscopy

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RIM1 proteins are integral regulators of synaptic function. However, little is known about their abundance and mobility within a presynapse's active zone (AZ). We addressed this question by applying 2-photon fluorescence correlation spectroscopy (2ph-FCS) in the context of single cortical synapses expressing RIM1 or single RIM1 domains fused to the fluorescent reporter GFP. We directed our Ti:Sa IR laser on top of single synapses, enclosing the putative AZ in a tiny observation volume. As the GFP-fused RIM1 proteins traverse this observational volume, they give rise to fluorescent fluctuations. Through autocorrelation analysis and fitting of a "3D-diffusion model", we could estimate the average number of RIM1 molecules per synapse and AZ. Moreover, we often found two RIM1 fractions with diverging mobilities. One generally smaller fraction, with a lower diffusion coefficient, that we think is due to a tight integration of RIM1 molecules into the AZ and a larger more mobile fraction that we assume represents the cytoplasmic pool of RIM1 proteins. First, we probed if one of RIM1's domains mediates the tight integration into the cytomatrix at the AZ. Therefore, we examined the properties of RIM1 truncation mutants, each containing one of the domains, namely ZN, PDZ, C2A and C2B. We found that, especially the RIM1 C2B domain appears to be a likely candidate for AZ anchoring because it had a relatively high fraction of low-mobility entities. Combining these findings with unpublished STORM data from our lab, we could estimate the number of RIM1 molecules per release site.