UNIVERSITÄT LEIPZIG

Brain Dynamics Graduate School

ABSTRACT BOOK

Brain Dynamics Scientific Day

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Brain Dynamics Graduate School
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Speaker: Prof. Dr. Marc Schönwiesner
Co-Speaker: Prof. Dr. Stefan Hallermann
Scientific Coordinator: Dr. Saša Jovanović

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Keynote lecture I

Exciting complexity: Circuit mechanisms of neurodegeneration

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Neurodegenerative diseases are typically characterized by the selective vulnerability of distinct neuronal populations, which is a frequently studied subject in these disorders. However, there is now ample evidence pointing towards a causal involvement of circuit elements seemingly spared from frank degeneration. Alterations in function and connectivity and thus the otherwise tightly regulated balance between excitation and inhibition within neural circuits are not only at the heart of symptoms typical of a given neurodegenerative disorder, but can also trigger und fuel the degenerative process per se. I will outline two exemplary diseases, namely in Spinocerebellar Ataxia and in Amyotrophic lateral sclerosis, in which we could recently identify critical circuit elements involved in the degeneration of the respective well-known vulnerable neuronal population. Our work strongly relies on in vivo imaging techniques in behaving mice in order to unravel neuronal and network dysfunction together with subsequent selective chemogenetic manipulations of identified key regulatory elements. With our work, we contribute to an urgently needed improved mechanistic insight into neurodegenerative diseases and succeeded in identifying hitherto unrecognized novel therapeutic targets.

Keynote lecture II

Monitoring brain activity - out and about

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Unlike other functional neuroimaging modalities, electroencephalography (EEG) shows promise in capturing human brain activity during natural behaviour and whole-body movements. Since human behaviour is context-dependent, advances in the field of cognitive neuroscience can be expected from mobile EEG research. However, interpreting brain activity recorded in complex, uncontrolled situations is very challenging and requires the availability of contextual information, such as sounds, movements or other physiological signals. Moreover, hardware should be unobtrusive and robust, without compromising signal quality. I will discuss the current state-of-the-art and report several studies using mobile EEG for the investigation of cognitive-motor interference and auditory attention tracking in uncontrolled environments.

Symposium I

S01

Discovery of a peroxisome proliferator-activated receptor d-dependent mechanism for hyperactivity in early life stage zebrafish exposed to structurally similar Per- and Polyfluorylalkyl Substances (PFAS)

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Per- and polyfluorinated alkyl substances (PFAS) are a diverse class of synthetic chemicals widely used in industrial manufacturing. We previously showed that exposure to alkyl sulfonic acid PFAS (perfluorooctanesulfonic acid (PFOS) or perfluorohexanesulfonic acid (PFHxS)) caused dark-phase hyperactivity in zebrafish. To determine whether PFAS-dependent behavioral effects result from a developmental perturbation of the central nervous system, swimming behavior was assessed in 5- day post fertilization (dpf) zebrafish following either developmental (1-4 dpf) or acute (5 dpf) exposure to 0.43-7.86 µM PFOS, 7.87-120 µM PFHxS, or 0.4% dimethyl sulfoxide (DMSO). Relative to DMSO, dark phase hyperactivity occurred both developmentally and acutely in PFAS-exposed zebrafish. In contrast, visual startle response hyperactivity only occurred following developmental exposure. Phenotypic persistence was evaluated following chemical removal. Developmental exposure to PFOS or PFHxS from 1-4 dpf triggered irreversible concentration-dependent visual startle response hyperactivity at 6-9 dpf. To identify potential underlying mechanisms, RNA sequencing was performed in head tissue obtained 1-2 days prior to the onset of hyperactivity. Projection of differentially expressed genes onto a self-organizing map showed that both chemicals produced similar global transcriptomic profiles. Peroxisome proliferator-activated receptors (ppara, ppard, pparg) were identified as putative upstream regulators. To test the hypothesis that ppars are required for PFAS-dependent visual startle response hyperactivity, CRISPR/Cas9-based gene editing was used to knockdown pparaa/ab, pparda/db, or pparg at day 0. As a control, two sets of crispant larvae were generated that contained multiple different mutations in the same target gene(s). A negative control that lacked mutations in target gene(s) was also generated. Gene knockdown was molecularly confirmed. Crispants were exposed to 7.86 µM PFOS, 80 µM PFHxS, or 0.4% DMSO from 1-4 dpf and locomotor activity was assessed at 5 dpf.

pparaa/ab or pparg knockdown did not affect the ability of PFOS or PFHxS to provoke visual startle response hyperactivity. In contrast, pparda/db knockdown blunted PFOS and PFHxS-dependent hyperactivity as compared to the exposed negative control. Orthogonal confirmation using the ppard antagonist GSK3787 confirmed that ppard is required for PFOS-dependent visual startle hyperactivity. This work identified two distinct phenomena. One, exposure to PFOS caused acute and transient dark-phase hyperactivity. Two, developmental, but not acute, exposure to the same chemical triggered persistent visual startle response hyperactivity that required the activity of ppard. Taken together, we identified a novel molecular mechanism for hyperactivity in the visual startle response elicited by PFOS exposure. More broadly, this work shows how gene editing can be used for rapid and neurotoxicity-related hypothesis testing in early life stage zebrafish.

This abstract does not necessarily reflect EPA policy

S02

Effects of congenital toxoplasmosis on the developing brain of the guinea pig

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Toxoplasmosis, caused by the protozoan parasite Toxoplasma (T.) gondii, is among the most prevalent zoonoses. If primary infections occur during pregnancy, T. gondii is able to infect the fetus, which may cause lesions in its brain. Until now, detailed data on the course of the infection and the specific host cells in the developing brain are lacking. This project aims to establish the guinea pig as a model for congenital toxoplasmosis and to characterize the alterations and host cells in the fetal brain.

Time-mated guinea pigs were orally infected with 100 T. gondii-oocysts on gestation day 23 and euthanized on gestation day 33, 40, and 48. Parasite loads in offspring organs were determined by qPCR. Alterations in the offspring brain were assessed by pathomorphological examination. Data of cell tropism were obtained by immunohistochemistry.

T. gondii DNA was detected in the majority of offspring brains, which were marked by necrotizing encephalitis. Tachyzoites were observed in neurons and neural stem cells of the offspring brain.

Our results show that T. gondii is vertically transmitted in guinea pigs and infects the offspring brain, demonstrating the suitability for studying congenital toxoplasmosis. By establishing an appropriate model for congenital toxoplasmosis and providing data on cell tropism of T. gondii this model expands our understanding of the pathogenesis of toxoplasmosis in the developing brain.

All animal experiments were performed in accordance with German animal welfare legislation and were approved by the Landesdirektion Sachsen (TVV 45/17, DD24.1-5131./390/47).

S03

LTD increases the coupling distance between Ca^{2+} channels and release sensors at neocortical pyramidal neuron synapses

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The strengthening and weakening of synapses based on spiking patterns, the cellular mechanism underlying synaptic learning habits, has been well documented. (Feldman, 2012) This plasticity is induced via postsynaptic NMDARs and expressed in either pre- or postsynaptic neuronal pathways. The expression of timing dependent long-term depression (tLTD) has previously been identified to be presynaptic, resulting in reduced release probability (Sjöström et al., 2003). the presynaptic mechanisms that ultimately reduce the vesicular release probability (p_v) are largely unclear.

At the presynaptic active zone, the physical coupling distance (CD) between voltage-gated Ca^{2+} channels and the Ca^{2+} sensors triggering fusion of a synaptic vesicle (SV) is a major determinant of p_v. The CD has previously been found to be regulated ontogenetically at different synapses, which altered their release characteristics during postnatal development (Bornschein & Schmidt, 2019). We hypothesized that the CD might also be regulated use-depended, thereby, giving rise to reduced p_v during tLTD.

We addressed this hypothesis at synapses connecting pyramidal neurons in layer 2/3 and layer 5 in the primary somatosensory cortex (S_1) of mature mice. Synapses in S_1 are known to express presynaptic tLTD (Sjöström et al., 2006) and to operate at high p_v with tight nanodomain coupling (Bornschein et al., 2019). To test for activity-dependent changes in the CD we investigated the effects of EGTA on synaptic transmission following induction of tLTD by a spike-timing-dependent plasticity protocol. The slow Ca^{2+} chelator EGTA is a standard indicator of loose coupling since it is far more effective in interfering with release in loose than in tight coupling regimes. We found that our tLTD protocol reduced EPSC amplitudes to 57.72% (IQR = 55.62/61.41, P = 0.008) in 77% of the cells (n=13). In the remainder of cells, amplitudes were not reduced. Interestingly, in those cells showing tLTD, the application of EGTA further reduced the EPSC amplitudes by 52.4%. This data supports our hypothesis and indicates that tLTD indeed reduces p_v by breaking the tight Ca^{2+} influx to SV coupling in S_1 pyramidal neuron synapses.

S04

Selective loss of excitation and p53 activation cause cerebellar circuit pathology in spinal muscular atrophy

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Background: Spinal muscular atrophy (SMA) is caused by the reduction of the SMN protein and characterized by the degeneration of spinal motor circuits resulting in impaired voluntary movement and muscle atrophy. The contribution of motor circuits in the brain to the SMA pathology is largely unknown. The motor circuits in the cerebellum are critical for motor learning and voluntary movements by processing proprioceptive input and modulating motor output, of which both are affected in SMA. A few studies reported alteration of Purkinje cells (PC) - the sole functional output of the cerebellar cortex - in SMA patients, implicating a cerebellar contribution to the disease pathology. In this study, we investigated the extent and mechanisms of cerebellar pathology in SMA patient and mouse models.

Results: We performed immunofluorescence, confocal and super-resolution microscopy on sagittal vermis sections of end-stage mutants and control cerebelli. Our results showed a significantly underdeveloped cerebellum including a reduced size of all cerebellar layers and PC lacking dendritic trees in Taiwanese SMA mice. SMNΔ7 mutant mice also exhibited a slightly reduced cerebellum, but, while some lobules remained resistant, others exhibited smaller cerebellar layers with vast PC death. Importantly, cerebellum tissue from SMA patients also exhibited a consistent reduction of PC compared to controls. Subsequent analysis presented smaller PC dendritic trees and a reduction of excitatory synaptic inputs onto PC in SMNΔ7 mutant mice, indicating reduced activation of PC in SMA by granule cells and the inferior olive. Remaining excitatory synapses revealed a postsynaptic reduction of glutamate receptors, suggesting an impaired cerebellar circuitry. To gain insight into the pathomechanisms, we investigated the p53 pathway which has been shown to induce motor neuron death in SMA. Importantly, vulnerable PC exhibited a robust p53 upregulation prior to their death and viral inhibition of p53 prevents PC death, demonstrating p53-dependent neurodegeneration in the cerebellum. Finally, available viral SMN–restoring treatments prevented PC death, but did not restore cerebellar circuit pathology.

Conclusion: Our results demonstrate a cerebellar circuit pathology comprising of selective PC death and reduced excitatory synaptic input in SMA mouse model and humans. This demonstrates a similar pathology in the cerebellum as currently reported in the spinal cord of SMA mice and suggests the cerebellum as a contributor to SMA pathology.

All work was supported by grants from the DFG (SI 1969/3-1, SI 1969/2-1) and SMA Europe 2020.

Symposium II

S05

Synaptic alterations in motor circuits relevant to the peripheral nerve disease Charcot-Marie-Tooth type 1A

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Charcot-Marie-Tooth type 1A (CMT1A) is a disease in which a duplication of the gene PMP22 predominantly affects the myelinating cells of the peripheral nervous system, Schwann cells, and causes dysmyelination that results in motor and sensory symptoms. Even though the peripheral disease mechanisms have been extensively studied, little attention has been paid to the alterations at the level of the entire motor circuitry, which might contribute to pathology. Indeed, using a mouse model for CMT1A, we found synaptic alterations on the lumbar motor neurons starting early in development. Such alterations could be mediated by an erroneous communication between the motoneurons and microglia, the main synaptic remodelers of the central nervous system. We hypothesize that such alterations can contribute to the early establishment of the disease and that preventing them could thus be a potential therapeutical target for this untreatable disease.

S06

Young children protect rule-breakers to repay a favour - even though they know better

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Direct reciprocity, the mutually beneficial exchange of favours over time, is an important evolutionary catalyst and cornerstone of human cooperation. The propensity to repay those who have intentionally benefitted us emerges in early childhood, opening avenues for sustained profitable relationships and joint cooperative endeavours. A largely unexplored question, however, is the extent to which direct reciprocity compromises children's tendency to engage in another cooperative behaviour: to uphold social norms by sanctioning transgressors. Enforcing norms unequally, dependent on one's relationship with the transgressor, is a central facet of corruption with potentially detrimental effects for society. In a preregistered behavioural experiment, we placed 5- to 7-year-old children (N = 85) in a game context where they witnessed their game partner (a research assistant) cheat. This partner had either previously done them a favour (reciprocity condition) or behaved neutrally (control condition). Across ages, children were slower and less likely to tattle on the transgressor in the reciprocity than the control condition, both spontaneously and when asked directly, necessitating a lie. This behaviour was in stark contrast to children's judgments in a third-party context: A) During a short vignette immediately after the behavioural task, over 80% of children advised a story protagonist to tattle on a transgressor who had previously helped them, including children who had not tattled themselves, revealing an intriguing knowledge-behaviour gap. B) In another pre-registered vignette study (N = 48), children from five years of age also disapproved of inconsistent norm enforcement for cheaters (even though they were not generally averse to unequal treatment and approved of this when justified). These findings showcase a previously disregarded "dark side" of reciprocity: From a young age children are willing to make an exception, look the other way and even lie for rule-breakers who have previously benefitted them, even though they know better.

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S07

Developing Brain Signatures for Self- & Other-Referential Mentalizing

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Thinking about self and others are fundamental processes for adaptive navigation through the social world. It has been shown that these processes overlap substantially both in terms of cognition and their neural representations. Key regions of self-related thought, including mPFC, PCC, and precuneus, are also involved in other-related thought, and an open question remains about how these two processes are differentiated in the brain. Here, we aimed at developing brain markers for self- and other-referential thought by using a brain signatures approach (Kragel, et al., 2018). Brain signatures are multivariate models of brain activity trained to predict mental states and/or behavior across individuals and datasets.

We first trained whole-brain support vector machine (SVM) classifiers of self, and other-referential thought in a training dataset (Koban, Pichon & Vuilleumier, unpublished) of n=21 adult participants who completed a trait-evaluation task with self, other, and control conditions. Using a 10-fold cross-validation procedure, both classifiers showed excellent accuracy (100% two-choice correct out-of-sample prediction, $p<0.001$). Brain regions with significant positive voxel weights for the "self"-classifier included vmPFC, ACC, thalamus, caudate nucleus, insula, and striatum. For the "other"-classifier, significant positive weights were found in the left vlPFC, precuneus, TPJ, and left STS.

Next, we tested these SVM classifiers in several completely independent datasets that used similar trait-evaluation tasks. These validation datasets included two samples of healthy adults, two adolescent samples, and three clinical (schizophrenia and bipolar disorder) samples. In these datasets, the average prediction accuracy was 77% for the "self"-classifier, and 76% for the "other"-classifier, suggesting good generalizability. In conclusion, we have trained and validated two new classifiers that predict self- and other-referential thought across different studies and different (incl. clinical and developmental) samples. Beyond informing us about the functional neural organization, these brain markers can be used as neurophysiological measures of self- and other-related mentalizing in multiple tasks and across different contexts.

Poster presentations

P01

Presynaptic mechanisms underlying the functional differentiation of olfactory sensory neurons in *Drosophila*

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Olfactory sensory neurons (OSNs) detect odors at a wide range of intensities. In insects, volatile compounds are perceived by odorant receptors (ORs), which are made up of an odorspecific protein (OrX) and the ubiquitous odorant co-receptor Orco. In principle, ORs tune the sensitivity of odor detection, with some OSNs exhibiting exceptionally high sensitivity. To test whether additional mechanisms underlie odour-specific neuronal processing, we investigated synapses between OSNs and projection neurons in the antennal lobe, the first relay station of the olfactory pathway. Here, studied the molecular structure and plasticity of the presynaptic active zone (AZ), the specialized site of neurotransmitter release. We focused on a highly sensitive OSN type that expresses the receptor Or56a and exclusively detects geosmin, an odorant signaling ecologically harmful stimuli. Using confocal microscopy, our results uncover a differential arrangement of the AZ proteins Bruchpilot (Brp) and Unc13A at Or56a and conventional OSNs. Interestingly, our recent findings also show that Or56a-OSNs display a limited capacity to undergo homeostatic plasticity in response to a genetic reduction of presynaptic release probability. We hypothesise that this difference to conventional OSNs reflects the basal tuning of geosmin-sensing neurons to maximum levels of performance.

P02

When do youth generalize representations of parents to peers: The role of maltreatment

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Unfamiliar individuals may trigger strong emotions in us upon first contact. One mechanism to explain this is representation generalization (Andersen & Chen, 2002), whereby a cue observed on unfamiliar individual triggers the mental representations of a significant other which in turn are generalized back to the individual in the form of expectations, emotions, etc. Mental representations of significant others guiding one's social world in this way is indeed the core idea of many clinical and developmental theories of psychology (Bowlby, 1973; Beebe & Lachmann, 2002).

However, the boundary conditions of this process are underspecified to date. Specifically, it is unclear how selectively individuals generalize and relatedly what conditions may drive one to generalize „despite absence of fit" (Bowlby, 1988). The latter is especially relevant for people experiencing psychological difficulties, as people learn to rely under extreme stress on negatively biased, automatic processing of the social environment (Luyten & Blatt, 2016). Therefore, this study aims to fill these gaps by selectively activating parental representations of maltreated adolescents during a virtual ball-tossing paradigm to examine how similarity vs non-similarity to a parent influences their encounters with new peers.

To this end, participants (N ≈ 200) describe one of their parents using open-ended statements via an online survey a week before their arrival at the lab. These statements are then used by the researchers to create idiosyncratic „Target" blurbs that contain ten descriptive sentences, six of which are paraphrased parental descriptive items and four of which are filler.

During their appointment, adolescents play "Cyberball" (Williams & Jarvis, 2006) with two co-players who are supposedly connected online and are introduced to the participant before the game via these descriptive blurbs: One of these co-players (Target) resembles their parent personality-wise, while the other co-player constitutes the Control.

While Cyberball is originally a social-exclusion paradigm, our design only contains an inclusion phase (33% of ball tosses to the participant) to create a social context as neutral as possible. An electrocardiogram (ECG) simultaneously records participants' heart rate during Cyberball. After the game, participants complete a recognition-memory test on previously seen blurbs of two co-players. Meanwhile in another room, the parent completes the Maternal Maltreatment Classification Interview (MMCI; Cicchetti, Toth, & Manly, 2003) with which adolescents are classified as either maltreated or non-maltreated based on the presence of any maltreatment events.

We expect to find that maltreated and non-maltreated adolescents exhibit differential mnemonic and cardiac responses to these two co-players as a function of parental resemblance and that

these differences index their relational expectations. Specifically, our main hypothesis is that maltreated adolescents will display less pronounced immediate heart-rate deceleration following not-receiving-the-ball from Target vs Control. This hypothesis rests on the premises that heart-rate deceleration is a correlate of heightened attention to salient negative stimuli (Bradley, 2009) and that not receiving the ball from Target vs Control will be less unexpected for the maltreated participants, as their parental expectations are already negatively biased.

P03

Rab3 is required for olfactory learning

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Associative olfactory conditioning in *Drosophila* has been used to study learning and memory for over half a century. The mushroom body (MB) is the main learning centre in the *Drosophila* brain. It has been established that memory formation involves plastic changes at presynapses of Kenyon cells, the intrinsic MB neurons. Neurotransmitter release from the presynapse occurs at so-called active zones (AZs), highly specialized sub-cellular signaling compartments. However, the molecular mechanisms of AZ plasticity and how these mediate learning processes remain unknown. AZ function depends on the precise arrangement and the interactions of specific proteins, such as Bruchpilot (Brp), voltage-gated Ca^{2+} channels, the small GTPase Rab3, RIM (Rab3 interacting molecule), Unc13s, and RBP (RIM binding protein). Previous studies at the neuromuscular junction demonstrated that loss of Rab3 triggers a reorganization of Brp, leading to a decrease in total AZ number and an increase in individual AZ size. Moreover, our recent findings uncovered impaired cAMP-dependent presynaptic plasticity in *rab3* mutants. It is therefore of great interest to explore the behavioural consequences of these changes. By evaluating *rab3* null mutants and cell-specific knockout via CRISPR/Cas9, we show that Rab3 expression in a subset of Kenyon cells is necessary for aversive learning on a timescale of minutes. These findings provide a new entry point to improve our mechanistic understanding of the molecular processes that mediate memory formation.

P04

Task-evoked high frequency oscillations (>400Hz) in cortex and spinal cord

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High frequency oscillations (HFOs) in response to somatosensory stimulation were first observed in the 1970s in human EEG recordings as small notches on the earliest cortical somatosensory evoked potential (SEP). Recent concurrent EEG and single-unit recordings in primates suggest HFOs may be a non-invasive marker for cortical population spiking. Here, we examined whether HFOs can be non-invasively recorded not only in cortex, but also the spinal cord, potentially providing a novel window into neuronal activity across the central nervous system.

The data was acquired during an experiment in which 36 participants received electrical stimulation of the upper and lower limb. Electrospinography was recorded from 40 electrodes arranged in two patches over the cervical and lumbar spine, while EEG was simultaneously recorded from 64 scalp channels. To extract HFOs from these surface recordings, canonical correlation analysis (CCA) was applied to find spatial filters that maximise the correlation between single-trial data and the trial-averaged signal.

First, we replicated previous findings concerning cortical HFOs evoked by upper and lower limb stimulation, revealing clearly visible HFOs in our dataset with the somatotopy corresponding to the stimulated nerves. Next, we tested for the existence of HFOs in the cervical and lumbar spinal cord and detected HFOs in most participants (cervical cord: ~90%, lumbar cord: ~50%). Group-level results in the spinal cord and cortex were then assessed by averaging across the HFO amplitude envelope of single-participant responses. Using this procedure, we obtained evidence for group-level HFOs across both cortex and spinal cord.

Overall, our results demonstrate that HFOs to both upper and lower limb stimulation can be observed in both cortex and spinal cord, and when individual variation in response-latency is considered, robust group-level HFOs can be obtained.

P05

Myelin insulation as a risk factor for axonal degeneration in autoimmune demyelinating disease

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Axonal degeneration determines the clinical outcome of multiple sclerosis and is thought to result from exposure of denuded axons to immune-mediated damage. Therefore, myelin is widely considered to be a protective structure for axons in multiple sclerosis. Myelinated axons also depend on oligodendrocytes, which provide metabolic and structural support to the axonal compartment. Given that axonal pathology in multiple sclerosis is already visible at early disease stages, before overt demyelination, we reasoned that autoimmune inflammation may disrupt oligodendroglial support mechanisms and hence primarily affect axons insulated by myelin. Here, we studied axonal pathology as a function of myelination in human multiple sclerosis and mouse models of autoimmune encephalomyelitis with genetically altered myelination. We demonstrate that myelin ensheathment itself becomes detrimental for axonal survival and increases the risk of axons degenerating in an autoimmune environment. This challenges the view of myelin as a solely protective structure and suggests that axonal dependence on oligodendroglial support can become fatal when myelin is under inflammatory attack.

P06

Microscopic Characterization of Short Association Fibers in the Human Brain

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In our research project, we investigated the white matter of the human neocortex, which is organized into specialized regions that communicate in synchronized networks and are interconnected by long-range projection fibers and short-range association fibers. The connections, along with their microstructural properties and interactions, collectively form the microstructural connectome. Presently, most assumptions about the human structural connectome rely on diffusion-weighted magnetic resonance imaging (DWI). However, these DWI-based measurements suffer from incompleteness and methodological biases. Particularly, short association fibers are significantly underrepresented. Short, weakly myelinated, and highly curved fibers are essentially unmeasurable using DWI or other magnetic resonance imaging (MRI)-based methods. That is why we need histological methods to detect them.

In our study, we determined the length and geometry of superficial white matter fibers in the human brain and characterized their structural fiber properties. To achieve this, we combine the CLARITY method with immunohistochemistry, in addition to employing gold standard histological techniques. The CLARITY method involves converting brain tissue into an optically transparent hydrogel polymer. This takes several months and is still an ongoing process. Finally, electron microscopy was employed to quantify fiber microstructure properties.

By combining these advanced techniques, we characterized various aspects of potential short association fibers in the human visual cortex (V1/V2) and the motor-somatosensory system (M1/S1). These aspects included fiber lengths and diameters, the g-ratio, orientation distribution, as well as myelination levels.

P07

Hyperleptinemia as a driver of obesity-induced neuropathy

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Peripheral neuropathies are chronic conditions that significantly affect the peripheral nervous system, leading to progressive motor and sensory impairments. Neuropathies can have various underlying causes, with metabolic disorders, particularly obesity, emerging as prominent contributors. However, the exact pathological mechanisms and the intricate interactions between different cell types within the nerves, including peripheral axons and Schwann cells, in the context of peripheral neuropathy remain poorly understood. Obesity is closely linked to elevated levels of circulating leptin, a proteohormone produced by adipocytes. Previous research conducted in our laboratory has demonstrated that leptin receptor signaling in Schwann cells is beneficial in promoting nerve repair following acute trauma, by triggering a series of catabolic pro-regenerative processes. Paradoxically, in cases of obesity, a positive correlation between hyperleptinemia and the presentation of neuropathic symptoms has been reported, suggesting a potential adverse causal relationship. We therefore here follow the hypothesis that chronic leptin stimulation may transition from a pro-regenerative signal to a detrimental driving force in obesity-induced neuropathy. To investigate this hypothesis, we have utilized conditional mouse mutants lacking functional leptin receptor signaling in Schwann cells. These mutant mice are exposed to a high-fat diet (HFD) to trigger obesity-induced neuropathy. In line with our hypothesis, preliminary data indicate that neuropathic symptoms, including impaired gait parameters and altered electroneurographic properties, are notably improved in the leptin receptor mutant mice compared to the control group.

P08

Heterogeneity of myelinating Schwann cells

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The peripheral nervous system (PNS) enables movement and sensation through distinct motor and sensory fibers of varying axonal size and myelination status. For unknown reasons, peripheral neuropathies often affect only a subset of fiber types, indicating specific intrinsic vulnerabilities. By combining single-cell RNA sequencing with imaging approaches and myelin proteomics, we delineate a thus far unresolved diversity of PNS fibers. In detail, we discovered that individual Schwann cell transcriptomes do not seem to correlate strongly with motor or sensory nerve modalities, but instead depend strongly on cell size – a finding that is easily lost when analyzing whole nerves in bulk. We identified marker genes and myelin proteins, including 2'3'-cyclic neucleotide-3'-phosphodiesterase (CNP), that specifically define small myelinated fibers and demonstrate, in principle, that myelin composition is functionally relevant. Indeed, CNP is specifically required for the maintenance and integrity of small myelinated fibers, which convey tactile and pain sensations.

Our study sheds new light on the diversity of Schwann cells and underlines that myelin composition is intricately linked to specific Schwann cell types. These findings have important implications for the ongoing research into peripheral nerve disorders that are characterized by distinct symptomatology.

P09

The role of Octβ3R in appetitive learning and memory in larval and adult *Drosophila melanogaster*

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Octopamine is a neuromodulator in the invertebrate nervous system. Similar to Noradrenaline, its equivalent in vertebrates, Octopamine is involved in various physiological and behavioral processes. In this study, the role of Octopamine in reward learning and memory is analyzed. It is known that the reward system in *Drosophila melanogaster* is comprised of dopaminergic PAM neurons that transmit appetitive information onto the memory center, the mushroom body. On the other hand, it has been observed that octopamine, too, plays a role in reward learning and memory. Using an associative learning and memory assay, we identified an octopaminergic receptor, Octβ3R, to be responsible for the aforementioned effects in the larva. On the contrary, this receptor seemed to be not involved in appetitive learning and memory in the adult. Elucidating dynamics of the octopaminergic system in learning and memory can help to understand the different ways of how reward information is integrated in the memory process over developmental stages.

P10

Discovery of a peroxisome proliferator-activated receptor d-dependent mechanism for hyperactivity in early life stage zebrafish exposed to structurally similar Per- and Polyfluorylalkyl Substances (PFAS)

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Per- and polyfluorinated alkyl substances (PFAS) are a diverse class of synthetic chemicals widely used in industrial manufacturing. We previously showed that exposure to alkyl sulfonic acid PFAS (perfluorooctanesulfonic acid (PFOS) or perfluorohexanesulfonic acid (PFHxS)) caused dark-phase hyperactivity in zebrafish. To determine whether PFAS-dependent behavioral effects result from a developmental perturbation of the central nervous system, swimming behavior was assessed in 5- day post fertilization (dpf) zebrafish following either developmental (1-4 dpf) or acute (5 dpf) exposure to 0.43-7.86 µM PFOS, 7.87-120 µM PFHxS, or 0.4% dimethyl sulfoxide (DMSO). Relative to DMSO, dark phase hyperactivity occurred both developmentally and acutely in PFAS-exposed zebrafish. In contrast, visual startle response hyperactivity only occurred following developmental exposure. Phenotypic persistence was evaluated following chemical removal. Developmental exposure to PFOS or PFHxS from 1-4 dpf triggered irreversible concentration-dependent visual startle response hyperactivity at 6-9 dpf. To identify potential underlying mechanisms, RNA sequencing was performed in head tissue obtained 1-2 days prior to the onset of hyperactivity. Projection of differentially expressed genes onto a self-organizing map showed that both chemicals produced similar global transcriptomic profiles. Peroxisome proliferator-activated receptors (ppara, ppard, pparg) were identified as putative upstream regulators. To test the hypothesis that ppars are required for PFAS-dependent visual startle response hyperactivity, CRISPR/Cas9-based gene editing was used to knockdown pparaa/ab, pparda/db, or pparg at day 0. As a control, two sets of crispant larvae were generated that contained multiple different mutations in the same target gene(s). A negative control that lacked mutations in target gene(s) was also generated. Gene knockdown was molecularly confirmed. Crispants were exposed to 7.86 µM PFOS, 80 µM PFHxS, or 0.4% DMSO from 1-4 dpf and locomotor activity was assessed at 5 dpf. pparaa/ab or pparg knockdown did not affect the ability of PFOS or PFHxS to provoke visual startle response hyperactivity. In contrast, pparda/db knockdown blunted PFOS and PFHxS-dependent hyperactivity as compared to the exposed negative control. Orthogonal confirmation

using the ppard antagonist GSK3787 confirmed that ppard is required for PFOS-dependent visual startle hyperactivity. This work identified two distinct phenomena. One, exposure to PFOS caused acute and transient dark-phase hyperactivity. Two, developmental, but not acute, exposure to the same chemical triggered persistent visual startle response hyperactivity that required the activity of ppard. Taken together, we identified a novel molecular mechanism for hyperactivity in the visual startle response elicited by PFOS exposure. More broadly, this work shows how gene editing can be used for rapid and neurotoxicity-related hypothesis testing in early life stage zebrafish.

This abstract does not necessarily reflect EPA policy.

P11

Development of an acute toxicity fingerprinting system to identify neuroactive environmental chemicals in larval zebrafish

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Exposure to an increasing number of environmental chemicals has been associated with acute neurotoxicity (ANT) or developmental neurotoxicity (DNT) in humans. The majority of chemicals in commerce have not been evaluated for ANT/DNT because of a lack of next-generation toxicity tests. We developed an 18-endpoint behavioral battery to assess ANT/ DNT in larval zebrafish. This new approach method (NAM) contains endpoints for the light-dark transition test, acoustic and visual startle responses, habituation learning and potentiation, and memory retention. Our current work outlines the progressive development of a toxicity fingerprinting framework designed to unveil potential underlying mechanisms through which exposure to environmental chemicals triggers instances of ANT/DNT. For the purpose of constructing acute neurotoxicity fingerprints, zebrafish larvae at 5 days post fertilization (dpf) were subjected to 55 compounds (0.8–120 µM) targeting neurodevelopmental receptors, such as GABA-B (CGP13501, CPG35348), NO-cGMP (ODQ), and NMDA (MK-801) receptors, for a duration of 60 min prior to behavioral assessment. Acute behavioral fingerprints for the toxicologically relevant receptors PPARa (GW7647, GW6471) and PPARγ (T0070907, Pioglitazone hydrochloride) were generated. Five compounds predicted to be non-neurotoxic (fluconazole, sodium benzoate, sodium saccharine hydrate) and three putative non-specific toxicants, predicted to cause membrane disruption based on physicochemical properties (Diphenylamine, N-methylaniline, Butoxyethanol) were also assessed. All fingerprints were generated relative to the vehicle control (0.4% DMSO). The outcomes encompassed a wide range of toxicity fingerprints, encompassing chemicals that resulted in declines in habituation learning (MK-801, SC79, LY294.002) or alterations in visual (GW7647, CGP13501, T0070907, ODQ) and/or acoustic (GW7647, CP673451, ODQ, CGP13501, T0070907) startle responses, with varying directionality. Interestingly, exposure to Diphenylamine, predicted to cause non-specific toxicity at 50-200 uM, produced impairment of habituation learning, dark period hypoactivity, and increased startle and acoustic responses, beginning at 22 uM. In summary, this study employed an innovative zebrafish NAM to generate acute toxicity fingerprints. Collectively, these findings establish the sensitivity of the fingerprinting system and its ability to distinguish phenotypic differences among a wide range of neurotoxicological modes of action that remain consistent across various taxa. Ultimately, the ANT/DNT fingerprinting system will be deployed to identify potential mechanisms underlying neurotoxic outcomes resulting from exposure to environmental chemicals, utilizing an alternative test system compliant with the principles of the 3Rs (Replacement, Reduction, Refinement).

P12

Molecular mechanisms of GABAergic network integration and myelination

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Fast-spiking parvalbumin-expressing interneurons (PV-IN) provide robust GABAergic inhibition in cortical networks, a major prerequisite for synchronized network activity tightly linked to learning and memory. PV-INs follow a protracted trajectory during brain development, including pairing with oligodendrocyte precursor cells (OPC) and (partial) axonal myelination. In combination with a substantial energy demand due to their fast-spiking behavior, these features render PV-IN vulnerable to various cell-autonomous and external insults during neurological disease conditions. However, the underlying molecular and cellular (patho)mechanisms are largely unexplored.

This research project is based on the hypothesis that the EGF-like growth factor Neuregulin (NRG) 1, a key regulator of peripheral myelination, also regulates processes that coordinate PV-IN intrinsic, axonal, and synaptic activity with (adaptive) myelination under normal and disease conditions in the brain. To address this concept, we are currently investigating mouse mutants with a conditional disruption of NRG1 signaling in PV-IN using electrophysiological and immunohistochemical analysis. Our preliminary data suggest NRG1 signaling functions in PV-IN excitability, network integration, and oscillatory network activity.

Taken together, a more detailed mechanistic insight into NRG1-mediated signaling functions in PV-IN will be instrumental for a better understanding of GABAergic network functions in the healthy brain and the development of more potent treatment strategies for epilepsy and myelin disorders.

P13

Young children protect rule-breakers to repay a favour - even though they know better

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Direct reciprocity, the mutually beneficial exchange of favours over time, is an important evolutionary catalyst and cornerstone of human cooperation. The propensity to repay those who have intentionally benefitted us emerges in early childhood, opening avenues for sustained profitable relationships and joint cooperative endeavours. A largely unexplored question, however, is the extent to which direct reciprocity compromises children's tendency to engage in another cooperative behaviour: to uphold social norms by sanctioning transgressors. Enforcing norms unequally, dependent on one's relationship with the transgressor, is a central facet of corruption with potentially detrimental effects for society. In a preregistered behavioural experiment, we placed 5- to 7-year-old children (N = 85) in a game context where they witnessed their game partner (a research assistant) cheat. This partner had either previously done them a favour (reciprocity condition) or behaved neutrally (control condition). Across ages, children were slower and less likely to tattle on the transgressor in the reciprocity than the control condition, both spontaneously and when asked directly, necessitating a lie. This behaviour was in stark contrast to children's judgments in a third-party context: A) During a short vignette immediately after the behavioural task, over 80% of children advised a story protagonist to tattle on a transgressor who had previously helped them, including children who had not tattled themselves, revealing an intriguing knowledge-behaviour gap. B) In another pre-registered vignette study (N = 48), children from five years of age also disapproved of inconsistent norm enforcement for cheaters (even though they were not generally averse to unequal treatment and approved of this when justified). These findings showcase a previously disregarded "dark side" of reciprocity: From a young age children are willing to make an exception, look the other way and even lie for rule-breakers who have previously benefitted them, even though they know better.

P14

Thalamocortical structural connectivity and its relation to microstructure

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Here, we studied the link between thalamocortical structural connectivity, intra-thalamic microstructure, and thalamocortical microstructural covariance.

Using diffusion-weighted imaging and quantitative T1 (qT1) data from the MICA-MICs dataset (N=50, age 29.54±5.62y), probabilistic tractography from thalamic seeds to 100 ipsilateral cortical parcels was computed and averaged to create a structural connectivity matrix. To extract two main axes of thalamocortical structural connectivity, we performed diffusion map embedding. To contextualize with intra-thalamic microstructural features, the thalamic axes were correlated with a core-matrix gene-expression map, and intra-thalamic qT1 as a proxy for myelin, while correcting for spatial autocorrelation. Next, we extracted qT1 values of thalamic voxels, and qT1 intensity profiles for cortical parcels sampled perpendicular to the cortical surface. Depth-specific structural covariance matrices were generated by correlating thalamic and cortical qT1 measures. The correlation between gradient loadings and structural covariance was studied using the cross-depth average and according to depth-specificity.

The principal gradient (G1) of thalamocortical structural connectivity reflected a medial to lateral-central transitional axis, while for the secondary gradient (G2) one apex was located at the medial-anterior and medial-posterior thalamic pole, and the opposite apex intersected the thalamus anterior-laterally to central-medially. Projected onto the cortex, G1 revealed a paralimbic-to-somatosensory axis, while G2 dissociates posterior-to-anterior regions. G2 was correlated with the distribution of core-matrix cells in the left thalamus (Pearson's r=0.57, p_{SA}=0.03). We further found G1 correlated with intra-thalamic qT1-intensity (L: r=-0.49, p_{SA}=0.026; R: r=-0.54, p_{SA}=0.008). For G1, structural covariance correlated negatively with the inferior-anterior cortex regions and positively with superior-posterior regions. Moreover, the association between G1 and thalamocortical structural covariance varied across cortical depths.

We characterized variations of thalamocortical connectivity patterns across the thalamus and showed its links to intra-thalamic microstructural and cellular variations. Further, we demonstrated that thalamocortical structural connectivity is linked to structural covariance in a depth-varying manner.

P15

Fishing for mechanisms: Using zebrafish behavioral phenotyping to unravel the molecular mechanisms of neuroactive environmental chemicals

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Approximately one in six children is diagnosed with a developmental disability including learning and intellectual disability. While causes are often elusive, chemical exposure can interfere with neurodevelopment. However, due to extensive time, cost, and animal number requirements for rodent neurotoxicity guideline studies, the potential hazard of most chemicals remains unknown. To fill this gap, we devised a battery of eleven automated behavior assays in larval zebrafish, a 3R-compliant model amenable to higher-throughput chemical screens. The battery captures stereotypical visual and acoustic behaviors including habituation, a form of non-associative learning. We hypothesized that behavior-rich phenotyping of chemicals provides a comprehensive readout of potential adverse outcome pathways (AOPs) that dictate a broad range of events and interactions that orchestrate nervous system development and function. According to the AOP "Impairment of Learning and Memory", we focused on the NMDA receptor (NMDAR) as a target of chemical exposure. In an initial step, the assay battery was evaluated against seven drugs with distinct mechanisms including NMDA and $GABA_A$ receptor ($GABA_AR$) antagonism. In line with effects reported in rats, exposure to the prototypical NMDAR antagonists APV and MK-801 caused a deficit in habituation learning. Multivariate analyses revealed distinct alterations in visual behaviors, indicating off-target activity. The battery was further evaluated against a set of ten US Environmental Protection Agency ToxCast chemicals positive for *ex vivo* NMDAR modulation. Acute exposure to chlorophene, a biocide and preservative in cosmetics, was confirmed to affect habituation learning in zebrafish. In addition to defective habituation learning, chlorophene caused 'paradoxical excitation', a phenomenon linked to $GABA_AR$ agonism. Pharmacological intervention using $GABA_AR$ antagonist picrotoxin blocked chlorophene-induced sedation, substantiating its GABAergic interaction. Orthogonal, electrophysiological validation in cultured mouse cortical neurons confirmed $GABA_AR$ activation, whereas no direct interaction with NMDARs was found. In addition, chlorophene exposure reduced the firing rate of GABAergic and glutamatergic units in 3D BrainSpheres comprised of human-induced pluripotent stem cells. In summary, this work highlights the capacity of the multibehavioral phenotyping approach to illuminate chemical modes of action. Ultimately, the alternative assay can reduce or replace rodent-based testing approaches to accelerate neurotoxicity research and better protect human health and the environment.

P16

Microscopic Characterization of Short Association Fibers in the Human Brain

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Hibernation is an outstanding physiological phenomenon by which animals survive under extreme environmental conditions like lack of food or cold ambient temperatures. The body adapts by lowering the heartbeat, breath rate and body temperature to a species dependent minimum (torpor state). After arousal normal body function returns (euthermic state) within hours. The role of the brain in induction, maintenance and arousal from torpor remains unsolved. Nevertheless, acoustic stimulation can lead to an arousal from torpor indicating residual neuronal networks still being active in torpor state. In this work, the ascending auditory pathway in brains of Syrian hamsters (Mesocricetus auratus) in euthermic and torpor state was used to analyse differences in synaptic input and neuronal activity depending on the brain region. It is hypothesized that the relation of inhibitory and excitatory synaptic input may change, eventually controlling neuronal activity. For this purpose, synaptic input and neuronal activity in brain slices of six regions (anteroventral cochlear nucleus, medial nucleus of the trapezoid body, lateral lemniscus, inferior colliculus, medial geniculate body, auditory cortex) of the auditory pathway were examined using immunohistochemistry and the Western blotting technique. The immunohistochemically probes stained for synapse marker proteins were analysed by LSM to semi-quantify protein level by measuring the emitted fluorescence signal. Additionally, immunohistochemical staining of the transcription factor c-fos and analysis of the respective signal intensity was used as a measure of neuronal activity. Western Blotting was used to quantify the amount of synapse marker proteins. The results demonstrate changes in the number of synapses as well as neuronal activity in torpor state with differences across brain regions. In conclusion, our data suggest that neurons are not inactivated per se in torpor state, but structural and physiological adaptations lead to defined changes in neuronal networks which potentially still enable the neuronal processing of relevant external stimuli.

P17

LTD increases the coupling distance between Ca^{2+} channels and release sensors at neocortical pyramidal neuron synapses

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The strengthening and weakening of synapses based on spiking patterns, the cellular mechanism underlying synaptic learning habits, has been well documented. (Feldman, 2012) This plasticity is induced via postsynaptic NMDARs and expressed in either pre- or postsynaptic neuronal pathways. The expression of timing dependent long-term depression (tLTD) has previously been identified to be presynaptic, resulting in reduced release probability (Sjöström et al., 2003). the presynaptic mechanisms that ultimately reduce the vesicular release probability (p_v) are largely unclear.

At the presynaptic active zone, the physical coupling distance (CD) between voltage-gated Ca^{2+} channels and the Ca^{2+} sensors triggering fusion of a synaptic vesicle (SV) is a major determinant of p_v. The CD has previously been found to be regulated ontogenetically at different synapses, which altered their release characteristics during postnatal development (Bornschein & Schmidt, 2019). We hypothesized that the CD might also be regulated use-depended, thereby, giving rise to reduced p_v during tLTD.

We addressed this hypothesis at synapses connecting pyramidal neurons in layer 2/3 and layer 5 in the primary somatosensory cortex (S_1) of mature mice. Synapses in S_1 are known to express presynaptic tLTD (Sjöström et al., 2006) and to operate at high p_v with tight nanodomain coupling (Bornschein et al., 2019). To test for activity-dependent changes in the CD we investigated the effects of EGTA on synaptic transmission following induction of tLTD by a spike-timing-dependent plasticity protocol. The slow Ca^{2+} chelator EGTA is a standard indicator of loose coupling since it is far more effective in interfering with release in loose than in tight coupling regimes. We found that our tLTD protocol reduced EPSC amplitudes to 57.72% (IQR = 55.62/61.41, P = 0.008) in 77% of the cells (n=13). In the remainder of cells, amplitudes were not reduced. Interestingly, in those cells showing tLTD, the application of EGTA further reduced the EPSC amplitudes by 52.4%. This data supports our hypothesis and indicates that tLTD indeed reduces p_v by breaking the tight Ca^{2+} influx to SV coupling in S_1 pyramidal neuron synapses.

P18

Fully-primed slowly-recovering vesicles mediate presynaptic LTP at neocortical neurons

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Pre- and postsynaptic forms of long-term potentiation (LTP) are candidate synaptic mechanisms underlying learning and memory. A classical form of presynaptic LTP, referred to as synaptic redistribution, has been described for layer 5 pyramidal neurons. However, how this apparent increase in the release probability relates to recent advances in the understanding of priming of synaptic vesicles remains unclear. We therefore performed whole-cell recordings from layer 5 pyramidal neurons in acute cortical slices of rats in combination with extracellular stimulation of local excitatory inputs and analyzed the presynaptic function before and after the induction of LTP. LTP increased the EPSC amplitude by a median factor of 1.5 in half of the synapses. In these responder synapses, LTP increased synaptic depression during high-frequency transmission and slowed the recovery from depression by adding a second slow component to the time course of recovery. Analysis with a recently established two-step vesicle priming model indicates an increase in the number of fully-primed vesicles that recover slowly following stimulation. To further test this hypothesis, we pharmacologically stimulated the cyclic adenosine monophosphate (cAMP) and diacylglycerol (DAG) pathways, which are both known to promote synaptic vesicle priming. Both pharmacological manipulations indeed mimicked all features of electrically-induced LTP. Comparing presynaptic plasticity at various synapses revealed a general correlation that stronger synapses recover slower from synaptic depression, indicating that fully-primed vesicles rely on a slowly maturating release machinery. Our data show that LTP at layer 5 pyramidal neurons increases the synaptic strength primarily by enlarging a subpool of fully-primed slowly-recovering vesicles.

P19

Perceptual Learning and Integration of Auditory Spatial Cues in Three Dimensions

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Auditory spatial cues along the three spatial axes differ greatly in their acoustic properties, representation in the brain and the time during the sound at which they become available for decoding. Sound direction can be decoded from brain responses at different time intervals after stimulus onset for each of the three axes, however the way the acoustic cues are integrated into a unified sound object is yet unknown. In the following study we focus on the cortical encoding of sound distance: several monaural cues contribute to the perception of sound source distance, including intensity, direct-to-reverberant sound energy, and vocal effort. Binaural cues (interaural time and intensity differences) play little role in this percept. Several of the distance cues are thought to be extracted in the cortex. We measured the cortical encoding of auditory distance through a series of EEG experiments employing different acoustic features as distance cues. Two sets of intensity equalised stimuli were generated: 1) unidentifiable sound objects compiled from anechoic recordings of impact sounds of everyday objects which were rendered at different distances in a simulated rectangular room (10x30x3m), 2) anechoically recorded vocalisations with different levels of vocal effort. The two sets of stimuli were designed and selected such that acoustically non-overlapping features evoke perceptions of distance: in set 1) direct-to-reverb sound energy ratio, in set 2) exerted vocal effort of a given vocalisation. These sets of stimuli were then presented in a within-subject EEG experimental design to 24 participants. After the adequate pre-processing steps the evoked responses were computed as a function of perceived distance. We report systematic covariations of EEG source components with sound source distance. Reverberation and vocal effort as distance cues evoke comparable event related potentials at P2 and P3 components. There is a linear relationship between the level of vocal effort and the corresponding event related amplitudes around the P2 component. The comparison across stimulus conditions allows us to differentiate genuine representations of distance from representations of the underlying acoustic cues.

P20

Pharyngeal sense organs of *Drosophila* larvae

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Drosophila melanogaster larvae have become a favored model organism to study the principles of sensory perception. Of all senses, assessing food quality and innocuousness is one of the most crucial for survival, especially for *Drosophila* larvae but for all feeding species in general. Recent studies (Richter et al., 2023) have described the larval sensory system on an ultrastructural level in detail, including the terminal organ, which is the major external taste organ in *Drosophila* larvae. However, the major internal taste or pharyngeal organs were only described in publications from the 1980s with limited ultrastructural detail (Singh and Singh 1984) or without cellular resolution (Kwon et al. 2011, Miroschnikow et al. 2018). To fill this gap, we analyzed the four major pharyngeal sense organs (or compound sensilla, respectively) on an ultrastructural level and used this knowledge to make well-grounded predictions about the function of their sensory neurons. These organs are the ventral pharyngeal sensilla (VPS), the dorsal pharyngeal sensilla (DPS), the pharyngeal organ (DPO) and the posterior pharyngeal sensilla (PPS). In addition, not all sensory structures are described and named in the pharyngeal region. Therefore, we aimed to identify all undescribed sensory neurons associated with the pharynx and the feeding process and to classify them according to their ultrastructure. A precise classification and nomenclature of the different types of sensilla across the entire larval body will be beneficial for future anatomical and functional studies.

P21

Automated Analysis of Myelinated Fibers in Electron Microscopic Data of Human White Matter

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Diffusion weighted imaging (DWI) is a central method for the interrogation of white matter connectivity in the human brain. However, this indirect method of mapping tractography has several shortcomings, such as low spatial resolution, inability to resolve fiber crossings, and low reliability in thin white matter sections. Therefore, it fails to accurately map short association fibers, which constitute the majority (>90%) of white matter connections in the human brain. We aim to augment DWI-based models of brain connectivity by including results from invasive analysis techniques that enable mapping of neural connectivity on (sub-)cellular level. We employ a combination of classical histological methods (immunohistochemical staining, lipophilic tracers) with novel clearing techniques (e.g. CLARITY) and three-dimensional electron microscopy (serial block-face scanning electron microscopy). These allow extraction of measures such as fiber diameter, curvature, myelination thickness, g ratio, and fiber orientation from tissue slices and blocks. We demonstrate proof of concept for the extraction of these measurements from histological data. We aim to include them in models of short white matter tract connectivity in M1/S1 and visual cortex.

P22

Impact of pesticides on the gut microbiome of mammalian wildlife foraging on palm-oil plantations

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The expansion of the oil palm as a cash crop has fueled the economy in Southeast Asia, with palm oil representing the most traded vegetable oil worldwide and being used in virtually all industries and households. However, the expansion of oil palm plantations and following unsustainable management strategies comes at significant costs to the region's tropical rainforests as well as native animal and plant biodiversity through habitat loss and fragmentation. The usage of chemical pesticides is a vital part of production on most plantations by guaranteeing higher yields. Wildlife forced to forage on plantations due to habitat shrinking is therefore exposed to pesticides by, e.g., ingesting chemical residues on fruits or leaves or by drinking water. Recently, studies were able to show a negative impact of pesticide exposure on the gut microbiome. The gut microbiome consists of a plethora of different microorganisms, like bacteria, viruses and fungi. Diet, social interactions and habitat are among a variety of other factors that can influence the composition of the respective microbial community. More and more studies link the composition and general 'health' of the gut microbiome to diseases, and a disturbed microbiome may suppress the immune response of its host towards pathogens, facilitating infections and decrease the general fitness of the respective organism. The safety classification of pesticides for different organisms was long based on whether or not the specific pathways targeted by the pesticide is present within the respective organisms not being the target of the pesticide (e.g., wildlife). However, this completely ignored the possibility of these pathways being present in the microbiome of the host, which could still affect the host negatively. Studies on these sublethal effects of pesticides on wild populations still remain rare. In the planned dissertation project, I therefore aim to investigate the influence of pesticide exposure on i) the gut microbiome, ii) the parasite load and iii) the cortisol level of a primate species, the Southern pig-tailed macaques (*Macaca nemestrina*), that is foraging in the forest–oil palm matrix in Malaysia. Pig-tailed macaque troops in the Segari Melintang Forest Reserve have been observed entering adjacent plantations frequently to feed on oil palm fruits, seeds, arthropods, fungi and plants, but also on rats which are known to be the major pest species of these plantations. However, they simultaneously show high infant mortality, facial malformations and a steady population decline, which could potentially be caused at least in part by pesticide exposure. The pressing issue of a decreasing population of this endangered species led to the drafting of this dissertation project and the focus on the gut microbiome was chosen as a non-invasive method which may help gain a better understanding on the influence of pesticides on wildlife.

P23

N1 suppression for self-generated sounds is unaffected by predictability of sound identity and occurrence

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Recent theories describe perception as an inferential process based on internal predictive models that are adjusted by prediction violations (prediction error). Two modulations of the auditory N1 event-related brain potential component have been interpreted as reduced or enhanced prediction error for predictable sensory input: The sound-related N1 component is attenuated for self-generated sounds compared to the N1 elicited by externally generated sounds (N1 suppression). An omission-related component in the N1 time-range is elicited when the self-generated sounds are occasionally omitted (omission N1). Interestingly, in a previous study we did not observe a modulation of N1 suppression by manipulating the predictability of sound occurrence, but a modulation of omission N1. Here, we wanted to confirm that both N1 suppression and omission N1 are both sensitive to the predictability of sound identity, as reported in the literature. We manipulated the predictability of sound identity in a self-generation paradigm in which button presses in one condition always produced the same sound or in another condition produced a sound randomly selected from a large set of sounds, thereby inducing a strong or a weak expectation for a specific sound. Surprisingly, omission N1 was modulated by manipulating the predictability of sound identity but not N1 suppression. This contradicts previous reports, further challenges prediction-related interpretations of the N1 suppression and supports alternative explanations like action-related unspecific suppression of sensory processing.

P24

Facilitation by spatial attention precedes facilitation by object-based attention in a spatial attentional shifting task with a probability cueing

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Effects of spatial and object-based attention on sensory processing were mostly studied in isolation leaving the relation between those types of attention and its temporal dynamics on a lesser studied level. Here, temporal dynamics of spatial and object-based attention were investigated with a spatial probability cueing task. Stimuli consisted of two vertical rectangles stimuli with a white noise pattern. Each rectangle was superimposed by two flickering clouds of dots to elicit steady-state-visual evoked potentials (SSVEPs), that tagged two distinct positions on each object. A central cue indicated either one (single cue) or two (double cue) task relevant positions where a defined target event could occur. Targets occurred with a higher probability on the cued position (valid targets), and with a lower probability on uncued positions. Those were located equidistant to the cued position either on the attended object (unattended same) or the unattended object (unattended different). Changes in amplitude of SSVEPs in a post-cue time window compared to a pre-cue baseline represented early sensory gain modulation by attention. Facilitation by spatial attention (on the attended position) preceded the facilitation by object-based attention (on the unattended same position), if only one position on that object was cued. The temporal difference between attended positions was substantially smaller, if both positions of an object were cued. SSVEPs in this task were modulated by spatial and object-based attention. A probable relation between the temporal dynamics of SSVEPs and a search priority map are discussed.

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Selective loss of excitation and p53 activation cause cerebellar circuit pathology in spinal muscular atrophy

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Background: Spinal muscular atrophy (SMA) is caused by the reduction of the SMN protein and characterized by the degeneration of spinal motor circuits resulting in impaired voluntary movement and muscle atrophy. The contribution of motor circuits in the brain to the SMA pathology is largely unknown. The motor circuits in the cerebellum are critical for motor learning and voluntary movements by processing proprioceptive input and modulating motor output, of which both are affected in SMA. A few studies reported alteration of Purkinje cells (PC) - the sole functional output of the cerebellar cortex - in SMA patients, implicating a cerebellar contribution to the disease pathology. In this study, we investigated the extent and mechanisms of cerebellar pathology in SMA patient and mouse models.

Results: We performed immunofluorescence, confocal and super-resolution microscopy on sagittal vermis sections of end-stage mutants and control cerebelli. Our results showed a significantly underdeveloped cerebellum including a reduced size of all cerebellar layers and PC lacking dendritic trees in Taiwanese SMA mice. SMNΔ7 mutant mice also exhibited a slightly reduced cerebellum, but, while some lobules remained resistant, others exhibited smaller cerebellar layers with vast PC death. Importantly, cerebellum tissue from SMA patients also exhibited a consistent reduction of PC compared to controls. Subsequent analysis presented smaller PC dendritic trees and a reduction of excitatory synaptic inputs onto PC in SMNΔ7 mutant mice, indicating reduced activation of PC in SMA by granule cells and the inferior olive. Remaining excitatory synapses revealed a postsynaptic reduction of glutamate receptors, suggesting an impaired cerebellar circuitry. To gain insight into the pathomechanisms, we investigated the p53 pathway which has been shown to induce motor neuron death in SMA. Importantly, vulnerable PC exhibited a robust p53 upregulation prior to their death and viral inhibition of p53 prevents PC death, demonstrating p53-dependent neurodegeneration in the cerebellum. Finally, available viral SMN–restoring treatments prevented PC death, but did not restore cerebellar circuit pathology.

Conclusion: Our results demonstrate a cerebellar circuit pathology comprising of selective PC death and reduced excitatory synaptic input in SMA mouse model and humans. This demonstrates a similar pathology in the cerebellum as currently reported in the spinal cord of SMA mice and suggests the cerebellum as a contributor to SMA pathology.

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