International Neuroscience Meeting, Budapest 2022

IBRO Workshop

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Abstract book

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General information

International Neuroscience Meeting, Budapest 2022
IBRO Workshop organized by the Hungarian Neuroscience Society
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Plenary Lectures

L I Brainstem circuits that control locomotion in the healthy and diseased brain

Prof. Ole Kiehn[1,2]

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Locomotion is a universal motor behavior that is expressed as the output of many integrated brain functions. Locomotion is organized at several levels of the nervous system, with brainstem circuits acting as the gate between brain areas regulating innate, emotional, or motivational locomotion and executive spinal circuits. To be executed, locomotion requires dynamic initiation and termination and appropriate directionally. This lecture will focus on recent advances that has elucidated the functional organization of brainstem motor circuits in mammals needed to perform these roles. It will show that designated command pathways in the brainstem control the episodic expression of locomotion and that directionality of locomotion is controlled by activity in discrete brainstem circuits. The lecture will also show how these brainstem circuits are linked to higher brain centers and address how locomotor disturbances following e.g. basal ganglia disorders may be alleviated by targeted manipulation brainstem command pathways.

L II Brainstem control of fear memories

Dr. Gábor Nyiri[1]

[1] Institute of Experimental Medicine, Budapest, Hungary

Encoding, recalling and, if necessary, efficient forgetting the memory of negative experience is essential for survival. Malfunctions of these memory processes can lead to mental health issues, cognitive deficits or dementia. Our recent discoveries suggest that key interconnected cell populations in the brainstem play a previously unrecognized yet crucial role in these processes.

L III What we know and what we need to know about peptidergic signaling in the brain. Oxytocin as an example

Prof. Valery Grinevich[1,2]

[1] Central Institute of Mental Health, University of Heidelberg, Department of Neuropeptide Research in Psychiatry, Heidelberg, Germany
[2] Georgia State University, Center for Neuroinflammation and Cardiometabolic Research, Atlanta, United States

Neuropeptides represent a new class of non-canonical neurotransmitters, which dramatically challenge a plethora behavioral and homeostatic functions. Among a hundred of identified neuropeptides, oxytocin remains the best studied molecule due to a great attention of the general public, basic neuroscience researchers, psychologists and psychiatrists based on its profound pro-social and anxiolytic effects. During the last decade, a substantial progress has been achieved in understanding the complex neurobiology of the brain oxytocin system. However, the picture of oxytocin actions remains far from being complete, and the central question remains: “How does a single neuropeptide exert such pleiotropic actions?”. In this lecture, I will tackle this question, demonstrating the anatomical divergence of oxytocin neurons and their numerous central projections. In conjunction, I will describe unique composition of distinct oxytocin-sensitive neurons in different brain regions, modulating distinct forms of behaviors. Next, I will introduce new oxytocin-sensitive cell types – astrocytes, which are involved in oxytocin-mediated emotional responses to fear and pain, and describe its pathways controlling excitability of glial and neuronal cells located in brain regions relevant to socio-emotional behaviors. At the end, I will emphasize advantages and great potencies of oxytocin – in comparison to other neuropeptides – for its use for treatment of human mental disorders.

L IV Shaping of brain microcircuits – the role of endocannabinoids

Prof. Olivier Manzoni[1]

[1] Inmed Inserm, Marseille, France

Wiring & rewiring of neural circuits depend on orchestrated changes in synaptic strengths and plasticity that consequently determine behavioral adaptations to an ever-changing environment. To understand the mechanisms by which the environment and genes interact to cause long-term changes in brain & behavior it is cardinal to understand 1/ how do synaptic rules and behavior change during brain development in health and neurodevelopment diseases? 2/ How does sex influence these rules? How do events during sensitive periods program the course of development, with the result that adult characteristics are significantly and often permanently modified? In our laboratory we address these questions by studying how meso-corticolimbic microcircuits are shaped to give rise to harmonious emotional behaviors and cognitive functions in adulthood. Based on the disambiguation of complex phenotypes into new developmental endophenotypes and the design of innovative therapeutic strategies, our work reveals that the endogenous cannabinoid system, a multifaceted modulatory system quasi-ubiquitous in the CNS, is a critical determinant of how environmental and genetic insults transform the architecture and the functionality of meso-corticolimbic synapses and ultimately change the behavioral working range.

Symposia

Symposium I

Gut feelings: the interaction between the brain and the gastrointestinal system during stress

Chair: Prof. Dóra Zelena (Institute of Experimental Medicine, Budapest, Hungary; University of Pécs, Pécs, Hungary)

The first brilliant demonstration of the brain-gut interactions was the cephalic phase of gastric and pancreatic secretion discovered by Ivan Pavlov. The present director of the Pavlov Institute, Ludmila Filaretova continued his work and focused on the role of the components of the stress regulatory system in the maintenance of the gastric and intestinal mucosal integrity. Indeed, during the next presentation, Dániel Kuti from Krisztina Kovács's laboratory we will tell about structural, molecular and microbial changes in the gastrointestinal tract due to chronic stressors. However, this is a bidirectional link and the state of the intestinal environment can have profound effects on the activity of the central nervous system. Tamás Kozich will present data on multi-system metabolic reprogramming as a candidate driver for increased vulnerability to psychopathologies in male mice. In the final talk you will hear about the brain-gut connections of this disorder. Namely, how trauma affects metabolism and how changes in the metabolism might influence the development of the symptoms.

Take into consideration the fact that the number of the bacteria in our body exceed the number of our own cells and waste majority of these biologically active cells are in our gastrointestinal system we cannot neglect this organ and its effect on our brain activity.

S I.1 The realization of the brain-gut interactions with corticotropin-releasing factor and glucocorticoids

Ludmila Filaretova[1]

[1] Pavlov Institute of Physiology Russian Academy of Sciences, St. Petersburg, Russian Federation

The brain and the gut interact bi-directionally through the brain-gut axis. The first brilliant demonstration of the brain-gut interactions was the cephalic phase of gastric and pancreatic secretion discovered by Ivan Pavlov, the first physiologist who was awarded the Nobel Prize for Physiology or Medicine in 1904. The interactions occur through neuronal and hormonal pathways, components of the immune system and the microbiota. The brain-gut axis plays an important role in health and disease through a regulation of the gastrointestinal function and by this way through regulation of many other functions of the body. Stress may alter the brain-gut interactions leading to the development of gastrointestinal disorders including peptic ulcer and inflammatory bowel disease. Gastric ulcer disease remains a serious problem in clinic and stressful lifestyle make significant contributions to this disease. The hypothalamic-pituitary-adrenocortical (HPA) system is a key hormonal branch of the brain-gut axis in stress and corticotropin-releasing factor (CRF) is a main stimulator of the HPA system. The primary receptors that mediate CRF-induced increase in ACTH levels belong to type 1 CRF receptors on pituitary corticotrophs. ACTH stimulates the adrenal gland to release glucocorticoids that in turn provide life-saving processes of the body. We will discuss how an endocrinological approach to gastroenterological field can advance an understanding of the HPA axis role in regulation of gastric mucosal integrity. According to our findings activation of the HPA system is gastroprotective component of the brain-gut axis in stress but not ulcerogenic one as it was generally accepted. Corticotropin-releasing factor (CRF) and glucocorticoids are important natural players provided gastroprotection. The results obtained in our experimental studies suggest that an initial action of endogenous glucocorticoids, including stress- and CRF-produced ones, as well as exogenous glucocorticoids, even used at pharmacological doses, is physiological gastroprotective. Prolongation of the hormonal action may lead to the transformation of gastroprotective hormonal effect to proulcerogenic one. Our findings demonstrate that corticotropin-releasing factor and glucocorticoids contribute to the realization of the brain-gut interactions and that activation of the HPA system is gastroprotective component of this interaction in stress.

The study was supported by grant of Russian Science Foundation (RSF) N 19-15-00430

S I.2 Chronic stress-induced changes in colon microbiome and its effect on behavior

Dániel Kuti[1], *Krisztina Kovács*[1]

[1] Institute of Experimental Medicine, Molecular neuroendocrinology, Budapest, Hungary

Generally, chronic stress could induce a bacterial shift in the colon microbiome, in which the level of pathogenic bacteria increase. These changes contribute and aggravate the negative effects of chronic stress.

In our experiments, we hypothesized that the function of the gut barrier is reduced during chronic stress. Thus, gut permeability increases and could result endotoxemia, which exaggerates the chronic stress induced psychogenic symptoms like anxiety and depression. To reveal the relationship between microbiome composition, stress-induced gastrointestinal functions and behavior, we treated chronically stressed mice with non-absorbable antibiotic rifaximin. Newborn mice were separated from their mother (maternal separation MS) for 3 hours daily through 12days and later were exposed to chronic variable stress (CVS) for 4 weeks in adulthood. Composition of the gut microbiome was analyzed in colon content. Results showed an increased amount of Bacteroidetes and Proteobacteria at phylum level and Clostridium at family level. In MS+CVS treated mice, reduced mucosa thickness, elevated plasma LPS level and macrophage infiltration into the lamina propria were seen. After CVS, behavior tests were performed and the chronically stressed mice displayed behavioral signs of anxiety and anhedonia. Rifaximin treatment normalized Clostridium level, gut permeability and LPS plasma concentration in the stressed mice. The mRNA levels of tight junction proteins (TJP1,TJP2) and occludin were also increased to rifaximin treatment. However, these beneficial effects of rifaximin was not accompanied with positive effect on behavior in the stressed mice.

Our data highlight that the treatment of gut microbiome by non-absorbable antibiotic could positively affect the adverse effects of chronic stress in the colon, however it could not change stress-induced behavior.

S I.3 Multi-system metabolic reprogramming as a candidate driver for increased vulnerability to psychopathologies in male mice

Tamás Kozich[1,2], Anouk Tengeler[2], Graeme Preston[1], Emmerzaal Tim[1,2], Eva Morava[1]

[1] Mayo Clinic, Department of Clinical Genomics, Rochester, United States
[2] Radboud University Medical Center, Department of Medical Imaging, Anatomy, Nijmegen, Netherlands

Mitochondrial metabolism is increasingly implicated in psychopathologies. I will first discuss that mice presenting with post-traumatic stress-like phenotype display metabolic reprogramming in both cerebellum and plasma consistent with increased energetic demand, mitochondrial metabolic reprogramming, and increased oxidative stress. Specifically, multilevel analyses have identified metabolic pathways with significant metabolic pathway shifts associated with trauma exposure, including amino acids and tricarboxylic acid (TCA) cycle metabolites in the cerebellum and plasma. Notably, several transformed metabolic pathways observed in the cerebellum were also reflected in plasma, connecting central and peripheral biosignatures of PTSD-like behavior. Besides stress, exposure to antibiotic treatment has also been associated with increased vulnerability to various psychopathologies. However, a research gap exists in understanding how adolescent antibiotic therapy affects behavior and cognition. Many antibiotics that target bacterial translation may also affect mitochondrial translation resulting in impaired mitochondrial function. I will discuss that mice exposed to chloramphenicol showed increased repetitive and compulsive-like behavior in the marble burying test, an accurate and sensitive assay of anxiety, concomitant with decreased mitochondrial activity. These findings could direct further studies and offer insights into potential metabolic interventions, either pharmacological or dietary, to improve stress resilience.

S I.4 Posttraumatic stress disorder and metabolic dysfunction

Dóra Zelena[1], Krisztina Bánrévi[2], Csilla Lea Fazekas[2], Eszter Sipos[2], Bibiána Török[2]

[1] University of Pécs, Szentágothai Research Centre, Medical School, Centre for Neuroscience, Institute of Physiology, Pécs, Hungary
[2] Institute of Experimental Medicine, Laboratory of Behavioural and Stress Studies, Budapest, Hungary

Posttraumatic stress disorder (PTSD) is a highly prevalent, devastating psychiatric disorder. However, only vulnerable subject develop symptoms after a traumatic experience. Identifying markers of resilience maybe helpful for prevention. Our goal was to explore the possible link between the gut microbiota and PTSD in a rat model with the hope to identify a suitable probiotic cocktail, which will increase resiliency.

Male Long Evans rats were traumatized using electric footshock and - based on z-score calculated from the behavioral parameters (social interest, freezing in trauma context, extinction of freezing etc.) – vulnerable and resilient subgroups were determined. After sacrifice ileal morphology and caecal microbiota content were studied.

The results of the histological examinations confirmed that the thickness of the intestinal villi was significantly smaller in the vulnerable subjects compared to both resilient and control animals. The most prominent difference in the intestinal microbiota composition determined by next-generation sequencing was in the relative abundance of the bacterium *A. muciniphila*, which was nearly ten times higher in the susceptible group compared to the control and resistance groups. This group had elevated Muc2 mRNA level in their intestine wall measured by rtPCR.

Our findings show that trauma may contribute to the development of PTSD symptoms in susceptible individuals by altering microbial composition and intestinal anatomy.

Symposium II

New pharmacological targets to inhibit neuroinflammation

Chairs: Prof. Maria Deli (Biological Research Centre, Szeged, Hungary) and Prof. Zsuzsanna Helyes (University of Pécs, Pécs, Hungary)

The symposium focuses on neuroinflammation, which plays a central role in many CNS diseases. The four topics cover important, but often overlooked aspects of this field. The role of the blood-brain barrier, which not only acts as an interface between the systemic circulation and the CNS, but participates in the pathology of several neurological diseases with neuroinflammatory components, will be discussed in two talks. Novel, unpublished data will be presented on the role of macrophage colony-stimulating factor 1 receptor (CSF1R) in cerebrovascular pathology and also on how protection of the blood-brain barrier is recognized as a pharmacological target in systemic and neuroinflammation. Another important point addressed by this symposium is the translational aspect of research related to neuroinflammation. A new, translational passive transfer-trauma model will be shown for the complex regional pain syndrome (CRPS). In this model the effect of IL-1 receptor antagonist anakinra, the soluble TNF-alpha receptor etanercept and the JAK-STAT inhibitor tofacitinib provide evidence for the contribution of neuroinflammation in CRPS-related severe, persistent pain and pointing out their therapeutic potentials in this indication. The role of glial subtypes, especially microglia, will be discussed in CRPS but further elaborated in a separate talk on how microglia-mediated processes in neurological diseases can be modulated. Both established professors and group leaders as well as young researchers are represented. Prof. Helyes, Dr. Dénes, and Prof. Deli are well-known experts in their respective fields. Dr. Campbell, an ESR grant awardee, is a rising star of the blood-brain barrier community. Dr. Walter has received last year a Junior Prima prize in Science category.

S II.1 Attenuated CSF-1R signalling drives cerebrovascular pathology

Matthew Campbell[1]

[1] Trinity College Dublin, Genetics, Dublin, Ireland

Recently, we have identified novel mutations in colony stimulating factor-1 receptor (*CSF1R*) in an autosomal dominant condition termed Adult-Onset Leukoencephalopathy with Axonal Spheroids and Pigmented Glia (ALSP). Cerebrovascular pathologies such as CAA and perivascular astrogliosis were some of the primary neuropathological features of this condition. We have identified two families with different dominant acting variants located in the kinase region of the *CSF1R* gene, which confer a lack of kinase activity and signalling. We show that depletion in CSF1R signalling induces BBB disruption and decreases the phagocytic capacity of peripheral macrophages but not microglia. CSF1R signalling appears to be critical for macrophage and microglial activation, and macrophage localisation to amyloid appears reduced following the induction of *CSF1R* heterozygosity in either endothelial cells or macrophages themselves.

Finally, using microglia and endothelial cells isolated from mice with *Csf1r* heterozygosity, we show that endothelial/microglial cross-talk and concomitant CSF1R signalling causes re-modelling of BBB associated tight junctions and suggest that regulating BBB integrity and systemic macrophage recruitment to the brain may be therapeutically relevant in ALSP and other Alzheimer's-like associate dementias.

Our data suggest that CSF1R signaling and activity in ALSP patients may be affecting not only the microglia but also the peripheral macrophages. Reduced monocyte maturation may result in decreased infiltration of peripheral macrophages into the brain parenchyma, this compounded with a reduced phagocytic capacity may contribute to the cerebrovascular amyloid pathology seen in the disease.

S II.2 Role of neuroinflammation and cytokine signaling in a translational mouse model of Complex Regional Pain Syndrome

Zsuzsanna Helyes[1,2], Valéria Tekus[1], Nikolett Szentes[1], Krisztina Pohoczky[3,1], József Kun[1,4], Péter Urbán[4], Attila Gyenesei[5], Kata Bölcskei[1], Éva Szőke[1], Tímea Aczél[1], Serena Sensi[6], Ádám Dénes[7], Andreas Goebel[6]

[1] University of Pecs, Szentagothai Research Centre & Medical School, Department of Pharmacology and Pharmacotherapy, Pecs, Hungary
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[5] University of Pecs, Szentagothai Research Centre, Genomics and Bioinformatics Research Group, Pecs, Hungary
[6] University of Liverpool & The Walton Centre National Health Service Foundation Trust, Department of Translational Medicine, Liverpool, United Kingdom
[7] Institute of Experimental Medicine, Laboratory of Neuroimmunology, Budapest, Hungary

Complex Regional Pain Syndrome (CRPS) represents a severe primary chronic pain condition with hypersensitivity and inflammatory symptoms. Autoimmunity, sensory-immune-vascular interactions and neuroinflammation contribute to its pathophysiological mechanisms. We demonstrated earlier that glia activation in pain-related central nervous system regions, but not peripheral inflammation plays a predominant role these processes. Its therapy is unsatisfactory, therefore, there is a great need to explore the main mediators and identify novel drug targets.

Since pathophysiological changes at the level of the primary sensory neurons are unknown, unbiased transcriptomic analysis of the L3-5 dorsal root ganglia (DRG) was performed in our novel passive transfer-trauma mouse model to identify key signaling pathways. Female C57Bl/6 mice were treated daily with purified serum-IgG from CRPS patients or healthy volunteers following plantar skin-muscle incision. The mechanonociceptive threshold of the paw was measured by dynamic plantar aesthesiometry and RNA was isolated from the DRGs on day 5. RNA sequencing was performed with Illumina NextSeq 550, differentially expressed genes and networks were determined by bioinformatic analysis. The discovered potential pathophysiological pathways were confirmed by pharmacological tools using functional measurements and neuroinflammatory markers in the central nervous system.

In the unilateral DRG samples 125 genes were differentially expressed in 5 days after plantar incision and IgG injection of CRPS patients. These are predominantly related to inflammatory and immune responses, inflammatory cytokines, chemokines and neuropeptides. Pathway analysis revealed the involvement of the IL-1, TNF and JAK-STAT signaling. These were confirmed by abolished or significantly reduced CRPS IgG-induced increased hyperalgesia by daily treatments with the IL-1 receptor antagonist anakinra, the soluble TNF alpha receptor etanercept and the JAK-STAT inhibitor tofacitinib. They significantly reduced microglia and astrocyte markers in the spinal dorsal horn and pain-associated brain regions such as the periaqueductal grey and the somatosensory cortex.

This is the first evidence for inflammatory cytokine signaling in the DRGs related to the pathophysiological mechanism of CRPS. This suggests therapeutic potentials of anakinra, etanercept and tofacitinib, that have long been used in autoimmune diseases such as arthritis.

National Brain Research Program-2 20017-1.2.1-NKP -2017-00002 (NAP-2; Chronic Pain Research Group), GYTK-KA-2020-01, UNKP-20-4-II-PTE-465 and 2019-1-HU01-KA203-061251 "Educating Experts of the Future: Developing Bioinformatics and Biostatistics competencies of European Biomedical Students - BECOMING".

S II.3 Microglia modulate neuronal and vascular responses via dynamic, compartment-specific mechanisms

Ádám Dénes[1]

[1] Institute of Experimental Medicine, Laboratory of Neuroimmunology, Budapest, Hungary

Microglia are key regulators of inflammatory processes in the CNS. Microglial activity is altered in common brain diseases and changes in microglial function have major impact on outcome in experimental models of neurological disorders. However, the mechanisms through which microglia contribute to the maintenance of normal brain function and impact on common CNS disorders are not well understood. We have recently identified a novel form of microglia-neuron interaction, which is present in the majority of neurons in mouse and human brain. Somatic microglia-neuron junctions possess specialized nanoarchitecture optimized for purinergic signaling. We show that activity of neuronal mitochondria is linked with microglial junction formation, which is induced rapidly in response to neuronal activation and blocked by inhibition of P2Y12 receptors. Brain injury-induced changes at somatic junctions trigger P2Y12-dependent microglial neuroprotection, regulating neuronal calcium load and functional connectivity. Microglia also shape cerebrovascular responses via compartment-specific actions in the neurovascular unit. Our results suggest that motile microglial processes exert fine-tuned actions to influence the functioning of neurons and the vasculature in the healthy and the injured brain. Understanding the mechanisms of microglia-neuron-vascular interactions is likely to help the identification of novel therapeutic targets in common neurological disorders.

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S II.4 Blood-brain barrier protection as a pharmacological target in systemic and neuroinflammation

Fruzsina R. Walter[1], Szilvia Veszelka[1], Ana Raquel Santa-Maria[1], Lilla Barna[1], András Harazin[1], Attila Hunyadi[2], Yoichi Morofuji[3], Mária A. Deli[1]

[1] Biological Research Centre, ELKH, Institute of Biophysics, Biological Barriers Research Group, Szeged, Hungary
[2] University of Szeged, Institute of Pharmacognosy, Szeged, Hungary
[3] Graduate School of Biomedical Sciences, Nagasaki University, Department of Neurosurgery, Nagasaki, Japan

The blood-brain barrier (BBB), as part of the neurovascular unit, plays a fundamental role in the maintenance of the homeostasis and protection of the central nervous system (CNS). Neurovascular dysfunction emerges as the result of CNS diseases and contributes to neuropathological changes. Although the BBB is damaged in several neurological diseases, protection of brain capillary endothelial cells and neighboring cell types is often neglected. We described that the pentraxin serum amyloid P component protected the BBB against bacterial lipopolysaccharide (LPS) induced toxicity in mice, while the polyanionic polysaccharide pentosan polysulfate reduced the damaging effects of LPS on barrier integrity, P-glycoprotein activity, and reactive oxygen species production in cultured brain endothelial cells. Pentosan and serum amyloid P component attenuated the barrier injury exerted by amyloid-β 1-42 in a BBB culture model. Several studies have been performed to counteract neuroinflammation modeled *in vitro*. We found that the neuropeptide α-melanocyte stimulating hormone exerted anti-inflammatory and BBB protective effects by blocking the NFκB pathway and that grape phenolic compounds also protected against cytokine induced BBB damage in brain endothelial cells. Since oxidative stress is an important element of neuroinflammation, it was also separately studied. Edaravone, a potent antioxidant used in stroke therapy, restored cell viability, barrier integrity and decreased reactive oxygen species production in methylglyoxal induced stress. Edaravone, as well as two other drugs used in clinical practice, simvastatine and dexamethasone, were effective against kainate induced brain endothelial injury. We revealed that fasudil, a Rho kinase inhibitor used in stroke, protected the BBB integrity and cell viability in oxygen-glucose deprivation by reducing brain endothelial injury elicited by pericytes and astrocytes via cyclooxygenase-2 and thromboxane A2 receptors. In addition, we discovered that natural plant ecdysteroids show basal impedance elevating effect on cultured brain endothelial cells and protection against oxidative stress induced damage. These results corroborate that the BBB can be considered as a pharmacological target in systemic and neuroinflammation. Several clinically used drugs and natural compounds that show beneficial effects on brain endothelial cells could have therapeutic potential to protect the BBB and prevent neuronal damage.

The fasudil study was supported by the bilateral grant of the Hungarian Academy of Sciences and JSPS, the ecdysteroid study was supported by grants from Gedeon Richter Plc. Centenarial Foundation, H1103 Budapest, Gyömrői út 19-21 and NKFIH (K134704).

Symposium III

Cellular and transcriptomic investigations of schizophrenia and autism spectum disorder

Chair: Dr István Adorján (Semmelweis University, Budapest, Hungary)

The current symposium proposal endeavours to interrogate the contribution of neuronal subtypes to disease pathology in schizophrenia and autism spectrum disorder. Schizophrenia (SCH) and autism spectrum disorder (ASD) are chronic and serious mental illnesses which put an enormous burden on the individual, families and society. According to careful estimates there are approximately 100 million people worldwide and 170.000 in Hungary affected by SCH or ASD. The conditions have multiple genetic risk factors, possibly interplaying with several environmental risk factors. However, the neuropathology of SCH/ASD is still unclear and much remains to be discovered about the neuroanatomical correlates and causes of these conditions.

The recent development of emerging high throughput techniques such as whole genome sequencing and single nucleus RNA sequencing allowed to start to unravel the molecular mechanisms of neuropsychiatric disorders at unprecedented level. However, when applied on their own, these approaches often lose topographic information, or fail to grasp the exact architecture of neuronal circuits in which the cells of interest operate. Therefore, it is compelling to associate these with other techniques such as immunohistochemistry and in situ hybridization which enable to recognize altered gene expression in a morphological and topographical context. Also, when all these approaches combined and complemented with in vitro assays a synergistic potential emerges which allows to achieve a comprehensive view on the pathology of SCH and ASD.

The symposium will encompass lectures which are parts of an initiative already in progress aiming to increase our understanding of the neuropathology in SCH and ASD. Coordinated efforts have been made to organize a multidisciplinary approach between research groups in order to maximize the outcome of post-mortem and in vitro neuropathological investigations. The proposed lectures range from post-mortem human investigations at cellular, proteomic and transcriptomic levels, through in vitro induced pluripotent stem cell approaches to experiments on non-human primate models in order to investigate these conditions in evolutionary context. Also, our framework has a direct translational potential as the identified cellular and molecular alterations will represent targets for future drug screening/pharmaceutical experiments.

S III.1 Cellular biomarkers of autism spectrum disorder and schizophrenia

István Adorján[1]

[1] Semmelweis University, Anatomy, Histology and Embryology, Neuropsychiatry Research Group, Budapest, Hungary

Schizophrenia (SCH) and autism spectrum disorder (ASD) are chronic and serious mental illnesses which put an enormous burden on the individual, families, and society. According to careful estimates, there are approximately 100 million people worldwide and 170.000 in Hungary affected by SCH or ASD. The conditions have multiple genetic risk factors, possibly interplaying with several environmental risk factors. However, the neuropathology of SCH/ASD is still unclear and much remains to be discovered about the neuroanatomical correlates and causes of these conditions.

In my lecture, I plan to outline the logistical, methodological, and conceptual challenges while unraveling the complex neurohistological background of these conditions. The involvement of different cell types such as neurons, astroglia, and microglia will be discussed. Also, the Primate Brain Collection Initiative will be presented which is an essential step to put human data and disease aetiology in an evolutionary context.

This project was supported by the Semmelweis Departmental Start-up Grant 2017-2020, the Institutional Excellence in Higher Education Grant (FIKP 2018-2020), the Thematic Excellence Programme (TKP, 2020-2021), the Semmelweis Fund for Science and Innovation (STIA_2018), the Science and Technology Fund 2019-2021 (TÉT) and the ÚNKP-21 Bolyai/Bolyai+ Grants.

S III.2 Novel bioinformatics approaches and transcriptomic investigations of schizophrenia at single nucleus resolution

Konstantin Khodosevich[1]

[1] University of Copenhagen, Copenhagen, Denmark

Schizophrenia is a highly complex disorder with largely unknown etiology. Emerging single cell and spatial omics technologies are potentially powerful tools that could unravel the complexity of a developing brain and identify which cell types contribute to disease etiology and how they contribute to pathogenic mechanisms. However, single cell and spatial data is often noisy and contain large number of potential confounders. In my talk, I will show how single cell and spatial omics could be implemented to study development of schizophrenia and brain impairment in adult brain using human tissue and animal models.

S III.3 Diversity in origin and migration of interneurons and their contribution to disease pathology

Zdravko Petanjek[1], *Ana Hladnik*[1]

[1] School of Medicine University of Zagreb, Department of Anatomy and Clinical Anatomy & Croatian Institute for Brain Research, Zagreb, Croatia

Evolutionary increase in complexity of cortical GABAergic network leads to significant variability in places of origin and routes of migration for GABAergic neurons. Here we present data from postmortem human and monkey fetal brain tissue processed for classical histological and immunohistochemical methods, with aim to identify their places of origin and their migratory routes.

Our data confirmed that the ganglionic and septal eminence are the most important sources of GABAergic neurons. From these sources, they tangentially migrate through the subventricular and intermediate zone of the dorsal telencephalon (pallium). During the middle fetal period, significant production of GABAergic neurons also occurs in the pallial proliferative zones. The vast majority of the progenitors as well as migratory neurons from aforementioned sources strongly expressed GAD65 but not GAD67. Through early and middle fetal period numerous tangentially migrating cells within ganglionic eminence and lateral migratory stream were calretinin expressing cells.

Intensive GAD67 expression was observed in a population of migratory cells that accumulates in the basal telencephalon and migrate to the pallium through the marginal zone and the layer under the cortical plate. These cells originate from, until now undescribed specific parts of the proliferative zones in the rostro-dorsal and caudal part of the medial telencephalic wall, proliferative zones between medial and lateral ganglionic eminence, hypothalamus and reticular thalamic nucleus. During middle fetal period they also originated from the proliferative zone at the top of the temporal lobe from where they enter into the marginal zone. Somatostatin positive migratory cells were densely packed in part of the zones where strong GAD67 reactive cells are found.

Around mid-gestation, GAD65 positive small migratory like cells increased in number through marginal zone, forming 5-10 cells thick densely packed sublayer. By the end of middle trimester, lateral migratory stream was still massive and ganglionic eminence maintained its size. These data strongly support the view that important fraction of cortical GABAergic neurons is produced in the last trimester of gestation.

Extended production, new sources and migratory routes of cortical GABA-ergic neurons participate to increased diversity of this cell population in the human cerebral cortex, but also makes them more prone to pathological alterations during development.

Supported by the Ministry of science Croatian – Hungarian bilateral project (Investigation of the neuropathology of autism spectrum disorder and schizophrenia), by the Croatian Science Foundation Grants 5943 (Microcircuitry of higher cognitive functions) and 3182 (Brain extracellular matrix in development and in perinatal hypoxia); co-financed by the Scientific Centre of Excellence for Basic, Clinical, and Translational Neuroscience (GA KK01.1.1.01.0007) funded by the EU through the ERDF.

S III.4 Investigation of de novo mutations in schizophrenia by induced pluripotent stem cell based disease modeling and CRISPR genome editing

János Réthelyi[1]

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Schizophrenia (SCZ) is a severe neuropsychiatric disorder of complex, poorly understood etiology. Both genetic and environmental factors play a role in the development of SCZ. De novo mutations (DNMs) represent a recently described new source of genetic variation in the background of SCZ. While several mutations have been associated with SCZ, in most cases their biological significance remains unclear. The aim of this research is to investigate the biological mechanisms connected to DNMs in SCZ by combining induced pluripotent stem cell (IPSC) based disease modeling and CRISPR based genome editing.

To this end we selected two SCZ case-parent trios, where the affected patients carry a potentially disease causing DNMs. Based on exome sequencing studies we chose a patient harboring a zinc finger MYND domain-containing protein 11 (ZMYND11) 1495C>T nonsense DNM resulting in a R399X stop codon. ZMYND11 encodes a chromatin reader protein that specifically binds H3.3K36me3, co-localizes with highly expressed genes and promotes intron retention. The other SCZ patient was a carrier of 3 non-synonymous DNMs in genes LRRC7, KHSRP, and KIR2DL1. The K-homology type splicing regulatory protein (KHSRP) is a RNA-binding protein that modulates RNA life and gene expression at different levels, including mRNA decay, miRNA biogenesis, and interactions with lncRNAs.

In both SCZ cases we used RNA sequencing, morphological, and functional assays to test for transcriptomic and functional differences between patient-derived and healthy control cell lines at the neuronal progenitor cell (NPC) and mature neuronal stages. In the CRISPR experiments we introduced monoallelic or biallelic frameshift mutations into a control wild type IPSC line using CRISPR non-homologous end joining (NHEJ). Next IPSC lines were generated from each member of the case-parent trio using Sendai virus based reprogramming. The investigated ZMYND11 mutation was corrected using CRISPR homology-directed repair (HDR) in the affected IPSC line. These isogenic IPSC lines were taken forward to neuronal differentiation experiments.

Results show distinct molecular alterations characteristic fro schizophrenia-derived NPCs and neurons. RNASeq analyses at the NPC and neuronal stage showed the massive upregulation of neuronal differentiation genes in the mutant cell lines, and downregulation of cell adhesion genes. These approaches can shed light on the molecular disease pathway underlying schizophrenia.

The study was funded by the Hungarian Brain Reserach Program, NAP 2017-1.2.1-NKP-2017-00002 grant.

Symposium IV

Circuits and computations in preclinical species: the next vista towards understanding the human brain

Chair: Dr. Dániel Hillier (Research Centre for Natural Sciences, Budapest, Hungary)

For almost two decades, the ever-growing versatility and precision of genetic tools propelled mice into an attractive model for neuroscience. To bridge the gap between rodent and human brain, preclinical species (cats, dogs and primates) are poised to gain importance due to the recent development of new viral tools and transgenic primate lines. To treat disorders of the human brain, the link between behavior and underlying computations implemented in neuronal circuits in preclinical species needs to be better understood. The symposium focuses on computations and behavior relying on the sensory modality that is most precious to humans: vision.

S IV.1 Driving adult cortical plasticity and perceptual learning in primates

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Compelling correlation-based evidence in monkeys has shown that dopaminergic neurons in the ventral midbrain signal the discrepancy between an obtained reward and its prediction, which is also known as a reward prediction error. These dopaminergic signals are suggested to play a critical role in many forms of learning, and in adult cortical plasticity. Rodent studies have targeted the ventral midbrain using microstimulation and optogenetics and confirmed its causal role in cortical plasticity. For example, strong dopaminergic-dependent plasticity was observed in rat auditory cortex when a tone was repeatedly paired with electrical stimulation of the ventral tegmental area (VTA). Such causal evidence, however, is lacking in primates, in which the mesocortical and mesolimbic systems expanded substantially compared to rodents. In my presentation, I will present functional magnetic resonance and electrophysiology data showing that repeated microstimulation of the VTA can lead to changes in visual representations in monkey cortex. Furthermore, I will also show that pairing of a visual feature with VTA stimulation leads to perceptual improvements for these features, such as also observed during reward-driven (task-irrelevant) perceptual learning experiments. Importantly, I will show that both the functional and perceptual changes occur in the absence of selective attention to these features. These data indicate that the VTA plays a critical role in driving adult cortical plasticity and perceptual learning-like phenomena in primates.

S IV.2 Mesoscopic deep-brain mapping of multimodal stimulus selectivities in cats

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Linking specific brain functions to their neuronal implementation is a central avenue of neuroscience. In transparent, small organisms the whole nervous system can be studied at single cell level by light microscopy. Brain-wide access to neuronal activity has remained challenging in mammals, especially in large-animal preclinical species. Putting single cell function into the context of the whole brain remains difficult as the dominant whole-brain imaging technique, fMRI, precludes simultaneous multielectrode recordings or fluorescence imaging. Functional ultrasound imaging has emerged recently as a mesoscale imaging technique that can be applied to image whole-brain dynamics in head-fixed mice. We present our preliminary data on mesoscale multimodal imaging performed in awake cats without head fixation. Functional ultrasound imaging enables us to record from unprecedentedly large brain volumes in awake, behaving animals or during anesthesia with high spatio-temporal resolution. We use this tool to investigate the mapping of visual feature spaces onto subcortical and cortical visual areas during behavior. Our results demonstrate the great potential of functional ultrasound imaging for linking neuronal function to mesoscopic brain activity in awake, behaving animals.

This work was supported by icGEB grant CRP/HUN20-01, by the Lendület ("Momentum") Programme of the Hungarian Academy of Sciences to DH as well as by project no.129120 that has been implemented with the support provided by the Ministry of Innovation and Technology of Hungary from the National Research, Development and Innovation Fund, financed under the FK18 funding scheme.

S IV.3 Hidden topographies of horizontal connections in the visual cortex

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Identification of shapes is essential for the rational portrayal of visual objects. The incorporation of shapes follows Gestalt principles to frame the visual representation of the element (Wertheimer, 1923). There is a wealth of experimental data to support the view that form incorporation processes must use communication between receptive fields of various locations in the visual field. For example, it is commonly accepted that receptive fields representing similar orientation can produce line segments which in turn induce shape recognition (Kapadia, Ito et al. 1995, Bauer and Heinze 2002). These studies also suggest that long-range horizontal connections in V1 are the neuronal substrate for contour integration (Kisvárday 2016). Our research aims to reveal the underpinnings in the topography of long-range horizontal connections and, through this, understand their putative role in visual contour integration.

To this end, intrinsic signal optical imaging was used for determining orientation and retinotopic maps in V1. Neurons (11 excitatory pyramidal and spiny stellate cells) were labelled intracellularly and histologically processed, then reconstructed in 3D using the Neurolucida reconstruction system. The visual plane representation of the labeled boutons was used for a quantitative assessment of the connections. All reconstructed cells showed a mixed connection topography. For obtaining inferences of contour integration, three main types of connectivity models could be established based on the convolution of orientation preference and visual field location. Clearly, the majority of the connections preferred iso-orientation although, within this category, the so-called co-linear type was the least frequent. Other types of connection, i.e. radial and co-axial, formed a substantial proportion.

In summary, correlation of orientation preference of the boutons with visual field representation revealed a complex nature of long-range horizontal connections with the potential to serve a broad range of interactions for contour integration of connections.

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S IV.4 Comparative brain imaging reveals analogous and divergent patterns of species- and face-sensitivity in humans and dogs

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Conspecific-preference in social perception is evident for multiple sensory modalities and in many species. There is also a dedicated neural network for face processing in primates. Yet, the evolutionary origin and the relative role of neural species-sensitivity and face-sensitivity in visuo-social processing are largely unknown. In this comparative study, species- and face-sensitivity to identical visual stimuli (videos of human and dog faces and occiputs) were examined using functional magnetic resonance imaging in dogs (*n*=20; 45% female) and humans (*n*=30; 50% female). In dogs, the bilateral mid suprasylvian gyrus showed conspecific-preference, no regions exhibited face-preference, and the majority of the visually-responsive cortex showed greater conspecific- than face-preference. In humans, conspecific-preferring regions (the right amygdala/hippocampus and the posterior superior temporal sulcus) also showed face-preference, and much of the visually-responsive cortex showed greater face- than conspecific-preference. Multivariate pattern analyses identified species-sensitive regions in both species, but face-sensitive regions only in humans. Across-species representational similarity analyses revealed stronger correspondence between dog and human response patterns for distinguishing con- from heterospecific faces than other contrasts. Results unveil functional analogies in dog and human visuo-social processing of conspecificity but suggest that cortical specialization for face perception may not be ubiquitous across mammals.

S IV.5 Probing cholinergic mechanisms of alertness, temporal attention and visual short-term memory in a primate pharmacological model of cognitive decline

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Despite the growing body of evidence on neural and cognitive processes underlying alertness, temporal expectation, and visual working memory (VWM), studies that measure cholinergic effects as a function of time in non-human primates has been scarce. Here we set out to conduct two sets of experiments to probe cholinergic influence on basic measures of cognitive function with the aim of developing a preclinical model with high translational validity for drug development against pathological cognitive decline. Male rhesus macaque monkeys were involved in the study. In Experiment 1, four subjects performed a variable foreperiod (1-10s) simple reaction time (RT) task. In Experiment 2, the delayed matching to sample (DMTS) VWM task in six rhesus macaques, we studied how cholinergic neuromodulation influences VWM maintenance across a wide range of delays (1 to 72 s). In both experiments we investigated whether and to what extent the effects of muscarinic antagonist amnestic agent scopolamine were reversed by donepezil, a cholinesterase inhibitor widely used for the treatment of dementia. In Experiment 1, in the control condition, RT showed a continuous decrease as the foreperiod duration increased (1-10 s), which indicated the effect of temporal expectation on RT. Scopolamine eliminated the foreperiod effect and slowed overall RT. Donepezil improved scopolamine-induced impairments only on the average RT reflecting a general beneficial effect on alertness without any improvement in temporal expectation. In Experiment 2, scopolamine decreased VWM performance only in medium delays, where donepezil partially rescued the impairments. These results are in line with our current knowledge on the role of muscarinic acetylcholine receptors in the maintenance of working memory. Taking delay length into account can be a valuable component of basic and preclinical pharmacological research on the behavioral manifestations of temporal expectation and VWM maintenance and can deepen our current understanding of attention and short-term memory and its general age-related impairments.

Poster session - Topic 1
Stem cells and development

P1.01 Progenitor cells in the adult human retina

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The retinas of lower vertebrates have a certain degree of regenerative capacity. The main element of regeneration is the Müller cell which can also generate new glia and neurons by entering the cell cycle and transforming into a multipotent progenitor stem cell. Müller cells in the mammalian retina were thought to lose their progenitor nature and their ability to divide. However, recent studies have indicated that division and neurogenesis of Müller cells in adult rodents can be induced under specific conditions. There is insufficient data on the regenerative capacity of Müller cells in the human retina.

Eyes of adult multi-organ donors without known ocular disease were enucleated within an hour of cardiac arrest and were subjected to immunohistochemistry on oriented frozen sections and whole mount preparations after fixation.

The Ki-67 proliferation marker detected a significant amount of dividing cells. A subset of Ki-67 positive cells expressed Pax6 protein, indicating their retinal origin. A small fraction of dividing cells colocalized the Müller cell-specific Sox9 protein. The expression of Sox2, Pax6 and S100β proteins showed a significantly decreasing gradient from the periphery to the centre. The anti-S100β antibody labelled a subgroup of Müller cells. Labelling with the Müller cell-specific Sox9 protein revealed co- expression of Sox2 and Pax6 proteins in a fraction of peripheral Müller cells.

To our knowledge, we are the first to demonstrate that Müller cell division occurs in vivo in the original, intact, three-dimensional environment, without addition of growth and other stimulatory factors. Contrary to previous concepts, our studies suggest that human Müller cells are composed of several functionally distinct populations. Furthermore, a subset of peripheral Müller cells exhibits properties of retinal progenitor cells, and in the adult human retina at least a fraction of Müller cells retains the ability to divide.

Our findings suggest that selective, vector-mediated transduction of Müller cells could be used to induce their division in a targeted and controlled manner. Based on the results of experiments in rodent models, it may be feasible in the future to replace lost neurons by genetic reprogramming the cells generated.

P1.02 Dual role of P2X7 receptor in dendritic outgrowth during physiological and pathological brain development

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Introduction. P2X7 receptor is a purinergic receptor, activated with an increased concentration of extracellular ATP in pathological conditions. However, little is known about its role during development, where ATP itself is expressed in early embryonic stage during brain development, influencing cellular differentiation, proliferation and apoptosis. Dendritic branching is studied as a marker for correct brain development and correlation of abnormal dendritogenesis and human pathologies may offer some understanding to these currently untreatable diseases. Indeed, neurons in some regions of the brain affected in schizophrenia show reduced dendritic length suggesting an abnormal dendritic outgrowth in schizophrenic patients. Here we present for the first time the dual role of P2X7 receptor in dendritic outgrowth during physiological and pathological brain development.

Methods. Morphological analyses were performed with Sholl analysis from primary hippocampal neurons from P2X7R wild-type (WT) and knockdown mice (KO) obtained from E17.5–E18.5 embryos and dams from a maternal immune activation (MIA) model for schizophrenia. A series of tests were performed to study the possible behavioural deficits in the young adult offspring.

Results. Deficits in dendritic outgrowth can be observed in hippocampal neurons from P2X7R deficient mice, but also, in primary hippocampal neurons from embryos of WT but not KO dams exposed to MIA. In young adult mice, schizophrenia-like behaviours (deficits in spontaneous alternation in T maze and novel object recognition, PPI) correlated with these changes.

Conclusion. P2X7R seems to have different functions regarding the time point in the individual (developmental stages or young adulthood) and being a key element during a pathological inflammatory event (e.g., when it is activated in an immune activation model like MIA during pregnancy, driving schizophrenia-like behaviours).

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P1.03 Purinergic Receptor Agonists Activated Ca^{2+} Signalling in the Deiters' cells in the Organ of Corti during Development and in Mature state – experimental and theoretical approaches

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The supporting cells of the organ of Corti and their ATP activated Ca^{2+} signaling are studied extensively in cochlear explants of newborn mice and the fundamental role of the ATP-dependent spontaneous activity in cochlear development has been shown. However, information on purinergic Ca^{2+} signaling during cochlear development and in mature stage are much sparse.

We have investigated the ATP-evoked Ca^{2+} signals in Deiters' cells in the hemicochlea preparation in different postnatal developmental-stages from 5 days old, deaf mice to 18 days old, hearing mice.

Single-cell electroporation of Deiters' cells with Ca^{2+} sensitive dyes and their fluorescent imaging showed that the ATP and UTP-induced Ca^{2+} elevation in the phalangeal process preceded the one in the soma by several seconds. The phenomenon was present in different postnatal developmental stages and was independent from the direction of tissue perfusion. The adenosine receptor antagonist 8-(p-sulfophenyl)-theophylline did not inhibit the ATP response and perfusion of adenosine had no effect on the concentration of intracellular Ca^{2+}.

For mathematical modeling the intracellular Ca^{2+} dynamic of the Deiters' cells we set up a closed cell, minimal model, which could produce similar Ca^{2+} transients that were found in the experiments.

Our results show that adenosine receptors are not, but extracellular Ca^{2+} dependent P2X and intracellular Ca^{2+} store dependent P2Y receptors are involved in the ATP and UTP-evoked Ca^{2+} transients. The time delay between the transient in the phalangeal process and the soma of Deiters' cells might be caused by the different subcellular distribution of the ionotropic and metabotropic purinergic receptors. Subcellular differences in ATP-evoked intracellular Ca^{2+} dynamics might reflect the active role Deiters' cells play in mature hearing, i.e., participation in cochlear amplification and protection against excessive noise exposure.

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P1.04 Secretagogin-expressing cells in the developing human cortex.

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Cell migration in the developing cortex is shaped by different signaling mechanisms and intercellular actions. We recently identified secretagogin, a calcium sensor protein, to regulate neuroblast migration in the rostral migratory stream of the murine brain by enzyme externalization to digest the extracellular matrix. We now explored a large population of secretagogin-containing cells in the developing human brain which migrate in radial or tangential orientations in the cortical plate. Secretagogin-containing cells were numerous during midgestation but their density further increased at the early third semester to robustly decrease prior birth. In both frontal and temporal lobes, immunoreactive cells typically occurred in the marginal and subventricular zones. In aborti with Down syndrome, secretagogin cell density decreased in these regions with a preserved temporal profile throughout pregnancy. We found no significant changes in either the temporal or the quantitative profile of secretagogin cell distribution in the subventricular zone. When normalized to total cell number, however, we detected a relative secretagogin cell loss in the cortical plate. Bipolar secretagogin cells formed radially arranged corridors in the cortex, which were wider in Down syndrome during midgestation but not later. Of note, very few cells express secretagogin in the adult cortex. To trace cortical cells which transiently express secretagogin during development, we used secretagogin-Cre::tomato mice where all cells maintained expression throughout adulthood. In early postnatal brains, secretagogin cells labelled distinct columns in the cortex and striosomes in the striatum. Migrating cells at the border of white and grey matters were frequently identified, as well as radially moving cells in the developing cortex. Dentate gyrus cells, mossy fiber boutons and Schaffer collaterals were detectable in the hippocampal formation until the end of the first postnatal month.

P1.05 The impact of environmental exposures on the neuronal differentiation of pluripotent stem cells

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Increasing evidence demonstrates that altered conditions during the periconceptional (PC) period of gamete maturation and early embryonic development can have lasting effects on the health of progeny. Such effects can result in the onset of neurological disease and neurodevelopmental disorders ('Developmental Origins of Health and Disease (DOHaD) concept). The present study aims to link mouse models and human clinical observations with the establishment of a human induced pluripotent stem cell (hiPSC)-derived model for brain development. To assess the effect of early-life stress on neurodevelopment, the impact of oxidative stress inducing compounds will be determined experimentally using *in vitro* hiPSC-derived neuronal tissue with qualitative (Immunocytochemistry) and quantitative (RT-qPCR and Western Blotting) methods. iPSC-derived neuronal cultures have been established and validated with positive expression of neuroectodermal (SOX1, PAX6) and neuron-specific markers (MAP2, TUB3) in 2D, while the neural induction of iPSCs to neural stem cells (NSCs) has been conducted in 3D format. Preliminary toxicity testing including cell viability assays and reactive oxygen species measurements have been undertaken using oxidative stress inducing compounds such as Menadione and Paraquat. This study will establish an *in vitro* hiPSC-derived model for embryonic brain development. Our model will be utilized to investigate gene expression and phenotypic alterations, as well as epigenetic changes in brain development induced by a chronic oxidative challenge.

Conclusively, this study will promote preventative measures and potential therapeutic applications for neurological and neurodevelopmental disorders that can arise due to early-life stress, complementing animal studies and human clinical observations in the DOHaD field.

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P1.06 In vitro neurotoxicological studies using hiPSCs and SH-SY5Y cells

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An important and urgent need is to replace the expensive and ethically objectionable animal model system in the field of toxicology. Current regulatory procedures are mostly based on in vivo guideline studies, such as the Organization for Economic Cooperation and Development (OECD) test guidelines 424 (OECD 1997), 426 (OECD 2007), or 451 (OECD 2018b) on neurotoxicity, developmental neurotoxicity, or carcinogenicity, respectively. There are serious factors that are questionable to the use of state-of-the-art models, particularly in the field of developmental neurotoxicity and neurotoxicity, such as the translatability of results due to significant differences between species, 3R's conflicts, and low throughput accompanied with high costs.

SH-SY5Y cell line is a popular neuronal cell line established in 1970 by Biedler et al., which has been used in the last decades for many neurological and toxicological studies. SH-SY5Y cells can be differentiated, however there are many controversies regarding the differentiation protocols, therefore we present here a fast and easy-to-use protocol based on the differentiation protocol of Forsby et al. (DB-ALM Protocol nr. 205: Cell viability in SH-SY5Y cells by the ATP luminescence assay) which contains only 1µM retinoic acid (in contrast to the usual 10µM) and the protocol of Forster et al. In this way, there is no need for fetal bovine serum (FBS), so the protocol can be used also in GMP conform laboratories.

We also introduce an easily applicable, short-term system, where viability responses of hiPCS-derived neuronal cultures are combined with a cost-effective, high throughput transcriptomic analysis that is able to complement various classical in vitro systems by uncovering low dose responses that are not visible by traditional assays.

A transcriptomic analysis is a crucial tool to identify the primary cellular response to drug exposure, however performing a high-throughput RNA-Seq assay is still time-consuming, laborious and expensive. Therefore, wer used an alternative targeted RNA sequencing method, Templated Oligo Assay with Sequencing readout (TempO-Seq, BioSpider Inc.), which is able to overcome these limitations (Bushel et al., 2018; Yeakley et al., 2017).

in3-An integrated interdisciplinary approach to animal-free nanomaterial and chemical safety assessment-Marie Sklodowska-Curie Action-Innovative Training Network under grant no. 721975.

P1.07 Effects of depolarization patterns on neuronal development and maturation

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The phenotype of a terminally differentiated neuron is determined partly by its own history and partly by the environmental influences. Recent neurophysiological research has convincingly demonstrated that the intrinsic biophysical and physiological properties of developing neurons are strongly influenced - or tuned - by the tonic or synaptic inputs that they receive. This suggests that depolarization "training" by optogenetics using similar environmental stimulation patterns that occur in the developing nervous system may be useful to promote targeted cell differentiation.

In this work, we have systematically compared the progress of neuronal development in response to prolonged (48 h) application of different illumination patterns to immature neurons differentiated from the NE-4C mouse neuroectodermal stem cell line expressing channelrhodopsin 2 (ChR2). To achieve this, all trans retinoic acid-induced NE-4C cells were transfected with ChR2-H134R-YFP plasmid construct at the 6th day of induction, and 24 h later cultures were illuminated for 48 h with different illumination patterns. As controls, parallel cultures were kept in the dark for 48 h. For training, we used both a 1-minute repetition of a 5-second-long 2 Hz theta oscillation (which mimics the patterns that neurons are receiving in the embryonic brain) and a Poisson-distributed light flash sequence (as illumination control).

Patch clamp recordings were performed on 30-50 cells per treatment group. Our results show that the theta oscillation, repeated every minute, had a biologically active effect. It increased the percentage of neurons exhibiting strong action potential output and altered the cells' intrinsic membrane properties, too. The amplitude of the measured action potentials increased, and the active membrane properties of the cells proved a more mature neuronal phenotype compared to controls. The presence of the inward rectifying K-current also increased in response to training. When Poisson distribution pattern was used, values were similar to the dark control, indicating that the pattern of depolarization evokes the biological effect.

In conclusion, the applied theta oscillation proved to be effective in controlling the differentiation of neurons derived from mouse neural stem cells, promoting the formation of cells with more mature electrophysiological properties and selectively increasing the occurrence of a specific voltage-activated K-current.

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P1.08 Morphological and electrophysiological maturation of human neurons derived from induced pluripotent stem cells

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Human induced neurons (h-iNs) offer a valuable and reliable model to understand the physiological aspects of neuronal development and disease. To determine age-dependent neuronal characteristics of h-iNs more precisely, we aimed to analyse the timescale of neuronal maturation by following electrophysiological and morphological parameters for more than 10 weeks.

Neuronal excitability and physiological properties were analyzed using the whole-cell current clamp technique. In the early stages of neuronal differentiation, h-iNs exhibited passive behavior, manifested as simple RC-responses or as small 'spikelets' and high membrane resistance. From the 4th week of culture, cells expressed well-developed action potentials. Furthermore, membrane resistance and rheobase decreased, indicating the gradual increase of neuronal intrinsic excitability. The frequency and amplitude of excitatory postsynaptic currents (EPSCs) measured in voltage clamp showed similar behavior indicating the formation of functional neuronal network.

As patched cells were filled with biocytin, further morphological and immunocytochemical analyses were carried out on the recorded cells. Maturation of the dendritic arborization was investigated by Sholl analysis. Our results indicated a time-dependent change, represented by the appearance of long and bifurcated processes. Cells showing a high number of synaptic inputs in patch clamp measurements were found to be labelled with the postsynaptic marker Shank2 and presynaptic Synapsin I. Spontaneous synaptic activity was further proved by Fluo-3 AM Ca-imaging in 4-week-old h-iN cultures, where h-iNs were highly active and showed partially synchronized network activity.

Taken together, neuronal progenitor cells derived from human induced pluripotent stem cells differentiate into mature neurons in a reliable and reproducible manner. The uncovered progression of differentiation events validates the usability of the model system and gives us a powerful tool to plan targeted experiments in different stages of neuronal maturation.

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Poster session - Topic 2
Repair and regeneration

P2.01 Application of neuroectodermal stem cells supports functional and morphological recovery after chronic spinal cord contusion injury

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Spinal cord contusion injury leads to severe tissue loss and subsequent deficit of motor, sensory and vegetative functions below the lesion. Many of the human lesions remain untreated and a chronic injury develops. In this study we investigated whether transplantation of neuroectodermal stem cells into the injured rat spinal cord is able to induce morphological and functional improvement.

Mouse embryonic clonal neuroectodermal stem cells (NE-GFP-4C cell line, ATCC: CRL-2926) were grafted intraspinally five weeks after a thoracic (T11) spinal cord contusion injury performed in Sprague-Dawley rats. Control animals underwent contusion injury without stem cell transplantation. Functional tests (BBB, video-based locomotor pattern analysis system) and detailed morphological analysis were performed to evaluate the effects of grafted cells. Two months after the transplantation the retrograde tracer Fast Blue was applied distally to the injury to determine the extent of axonal sparing/regeneration.

Grafted animals showed significantly better functional recovery compared with control animals. Five days after transplantation the majority of grafted cells appeared to survive, formed clusters and a small proportion of the cells differentiated into neurons and astrocytes. At this time point, the NE-4C cells did not migrate away from the grafted area. Ten days after grafting the majority of the grafted cells appeared as nonviable fragments in microglia/macrophage cells. This observation suggests a fast elimination process of the transplanted stem cells. On the other hand, the grafted cells induced significant reduction of microglia/macrophage and astrocytic reactions in the treated groups compared with the control animals. Retrograde tracing studies showed a statistically significant increase in the number of Fast Blue-labelled neurons in different segments of the spinal cord, the brainstem and the sensorimotor cortex.

These data suggest that grafted neuroectodermal stem cells are able to induce morphological and functional recovery after chronic spinal cord contusion injury despite the limited survival of transplanted cells.

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P2.02 Inflammasome activation in motoneurons initiates excessive neuroinflammation and impedes regeneration after sciatic nerve injury

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Neuroinflammation is a major determinant of acute neuronal injury and neurodegeneration. Although injury-induced inflammatory changes may have both beneficial and detrimental aspects, over-activation of pro-inflammatory pathways is known to negatively influence regenerative outcome. Among these pathways, inflammasomes are one of the most potent, leading to release of active IL-1β.

Our aim was to understand how inflammasomes participate in central inflammatory reactions accompanying peripheral nerve injury. After transection of the sciatic nerve, in the first three days, elements of the NLRP3 inflammasome were markedly upregulated in the L4-L5 segments of the spinal cord, followed by assembly of the inflammasome and secretion of active IL-1β. Although glial cells are traditionally viewed as initiators of neuroinflammation, in this acute phase of inflammation, inflammasome activation was found exclusively in affected motoneurons of the ventral horn in our model. This process was significantly inhibited by MCC950, a potent NLRP3 inhibitor. Although at later time points the NLRP3 protein was upregulated in microglia too, no signs of inflammasome activation were detected in these cells. Inhibition of inflammasome activation in motoneurons in the first days after nerve injury hindered development of microgliosis in the spinal cord. Moreover, inflammasome inhibition in the acute phase significantly enhanced nerve regeneration on both the morphological and the functional levels.

Our results indicate that the central reaction initiated by sciatic nerve injury starts with inflammasome activation in motoneurons of the ventral horn, which triggers a complex inflammatory reaction and activation of microglia. Inhibition of neuronal inflammasome activation not only leads to a significant reduction of microgliosis, but has a beneficial effect on the recovery as well.

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P2.03 Exploring the mechanism of action of intravenous stem cell therapy following traumatic spinal cord injury

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Traumatic spinal cord injury (SCI) is characterized by an acute mechanical insult followed by a series of secondary lesion events including acute vascular disruption, cell death, ischemia, inflammation and demyelination. In this study we investigated the effect of delayed intravenous neuroectodermal stem cell therapy which could reduce the severity of secondary injury and enhance tissue preservation and ultimately promote functional recovery.

A moderately severe thoracic contusion injury was induced at T5 spinal level in adult female Sprague-Dawley rats, followed by an intravenous tail vein infusion of NE-GFP-4C stem cells 30 min, 1, 2 or 3 weeks after the injury. Control animals underwent contusion injury without intravenous stem cell administration. Functional tests (Basso, Beattie, Bresnahan, and kinematic analysis) and detailed morphological analysis (quantification of retrograde labelling and immunohistochemistry) were performed to evaluate the effect of grafted cells. One day after intravenous stem cell administration proteome profiler arrays were used to assess the cytokine expression in the spleen, blood serum and spinal cord.

Significantly greater functional improvement was observed in transplanted animals treated intravenously with NE-GFP-4C cells immediately or one week after injury compared with controls. Significant neuroprotection was observed in the same treatment groups and significantly more retrogradely-labelled supra- and propriospinal neurons were counted compared with control animals. Immediate and one-week-delayed stem cell administration increased levels of the tissue inhibitor metalloproteinase-1 (TIMP-1, a potent beneficial candidate for vascular integrity) in the injured spinal cord and spleen one day after cell infusion. Further, immediate cell infusion was shown to alter the expression levels of splenic cytokines, suggesting that the spleen is an important target and site of stem cell effects.

Our results suggest that immediate or one-week-delayed intravenous stem cell treatment is likely to induce nearly equal morphological and functional recovery through TIMP-1 modulation. Furthermore, our results provide a link between spinal cord injury and splenic inflammation that can help increase the effectiveness of stem cell therapy.

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P2.04 Determination of the essential number of motoneurons required to produce functionally useful limb locomotion

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An avulsion injury inflicted upon the spinal cord induces a critical loss of motoneurons followed by irreversible locomotor function impairment ranging from inadequate limb movement to complete paralysis. Recent surgical approaches tend to provide improvement in limb function, however, the question remains how many motoneurons are needed to survive and grow new axons to achieve sufficient muscle reinnervation. The aim of this study was to determine the minimum motoneuron numbers, required to reinnervate the denervated skeletal muscles of the lower limb and produce a functionally satisfactory locomotor pattern.

Sprague-Dawley rats that underwent a lumbar 4-5 (L4-5) ventral root avulsion had their L4 ventral root reimplanted and received then different doses of riluzole treatment (0.4, 0.8, 1.2 and 1.6 mg/kg every second day for two weeks postoperatively) in order to rescue incremental numbers of the damaged motoneuron pool. Control animals received no treatment. In order to determine the exact threshold of satisfactory functional reinnervation we used our very sensitive movement recording and analysing system to provide a quantifiable in-depth analysis about the motor pattern of the whole hind limb. All together nine parameters of the hind limb movement pattern were evaluated by measuring specific joint angles, footprints and gait parameters in single video frames. Four months after the operation we carried out Fast Blue retrograde tracing in order to label and count the reinnervating motoneurons.

Our results confirmed a strong relationship between functional restoration of the original movement pattern and morphological reinnervation. Surprisingly, the correlation between the dose of the riluzole and the number of rescued motoneurons was not linear, the 1.2 mg/kg dose seemed to be a border between poor and strong functional regeneration. The data of the kinematic analysis suggests that above 250 surviving and reinnervating L4 motoneurons useful movement pattern (comparable to the clinical M4 stage) could be performed by the animals, thus rescuing roughly 20% of the motor pool might be enough for a satisfactory functional performance.

P2.05 Modified brevican expression resulted by unilateral labyrinth lesion in the superior vestibular nucleus of the rat

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Damage to the inner ear labyrinth evokes static and dynamic deficits in the eye movements, posture, and autonomic functions. After a shorter and longer period, the balance function is nearly restored, perhaps via synaptic modifications in the vestibular circuit. It receives even more evidence that the plasticity of the adult CNS is associated with the temporary alteration of the extracellular matrix, primarily observed in its condensed form the perineuronal nets (PNN). This study aims to trace the changes of brevican expression in the PNNs of the superior vestibular nucleus of the rat in the postoperative 14 days following unilateral labyrinth lesion.

Our results showed that unilateral labyrinth lesion and the consequent compensatory processes are accompanied by the changing of brevican staining pattern in the PNNs of the superior vestibular nucleus. The reduction of brevican in PNNs are suggested to participate in the plastic reorganization of the synaptic machinery throughout the vestibular circuit. By suspending the non-permissive character of brevican we may suspect the morpho-functional modification of pre- and postsynaptic receptor assembly. After a transitional decrease, the brevican expression restored to the control levels, which seemed parallel with the functional repair o equilibrium. The bilateral changing of brevican expression suggest the involvement of commissural vestibular fibers in the compensatory process.

P2.06 Transcribed messenger RNA – a potential therapeutic platform for spinal cord injury

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Spinal cord injury results in irreversible tissue damage followed by limited recovery of function. Interleukin-10 (IL-10) attenuates the effects of pro-inflammatory cytokines and reduces apoptosis. In this study lipid nanoparticle (LNP)-encapsulated human IL-10-encoding nucleoside-modified mRNA (hIL-10 mRNA-LNP) and recombinant hIL-10 loaded via osmotic pump were used to induce neuroprotection and functional recovery following spinal cord contusion injury in a rat model.

A contusion injury was performed at the level of thoracic 10 (Th10) vertebra. The hIL-10 mRNA LNP or recombinant hIL-10 were administrated 7 days after injury directly into the lesion cavity. Animals in the control groups underwent the same surgical procedure and received either no treatment or lipid nanoparticle (LNP)-encapsulated green fluorescent protein (GFP)-encoding nucleoside-modified mRNA. Locomotor analysis of the animals was carried out through the use of the BBB-test and a video-based locomotor analysis system. The extent of supra- and propriospinal axonal sparing/regeneration was determined by retrograde tracing 9 weeks after the injury. After mRNA injection the level of produced hIL-10 was followed by hIL-10 enzyme-linked immunosorbent assay (ELISA) and Proteome Profiler was used to evaluate the changes of cytokine expression.

The functional analysis showed that hIL-10 in both treatment groups enhanced the coordinated movement relative to controls. Similarly, administration of hIL-10 in both treatment strategies resulted in significantly smaller lesion area at the epicentre of the injury and rescued significantly greater amount of tissue. Analysis of supra and propriospinal connections with the retrograde tracer Fast Blue indicated that hIL-10 treatment enhanced the number of connections between the segments caudal to the lesion and various cranial parts of the CNS. Astrocytes, microglial cells and neurons also expressed hIL-10 protein after hIL-10 mRNA LNP injection up to 5 days in the injured spinal cord. The mRNA treatment induced time-delayed expression of TIMP-1 and CNTF in injured spinal segment.

These results demonstrate that the delayed hIL-10 treatment is able to induce morphological and functional improvement after spinal cord contusion. The hIL-10 mRNA LNP provides a simple and controllable new therapeutic approach that is less-invasive than other treatments and does not integrate into the genome.

Poster session - Topic 3
Disorders, disease models

P3.01 Oscillatory pattern analysis in a multiple hit schizophrenia rat model (Wisket)

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Schizophrenia is a chronic, complex neuropsychiatric disorder, characterized by positive, negative and cognitive symptoms. Both human and animal electroencephalography (EEG) studies reported impairments in circadian rhythm and oscillatory activity in schizophrenia, which may be associated with the deficits in cognitive and sensory processing.

The aims of the present study were to evaluate the circadian rhythm and the state-dependent oscillatory pattern in control (Wistar) and a multiple hit schizophrenia rat model (Wisket) by chronically implanted cortical electrodes.

Male, adult control and Wisket rats (4-6 months old) were involved in the study. 21 hours-long EEG registrations were analyzed: 12 h of the light, and 9 hours of the dark phase. To filter artefacts and classify sleep-wake stages and the active and quiet awake substages, a custom-made software was developed.

The Wisket animals have clear light-dark cycle similar to controls, and their sleep-wake rhythm showed only a tendency to spend more time in NREM and less in REM stages. In spite of the weak diurnal variation in oscillation in both groups, the Wisket rats had higher power in the low frequency delta, alpha and beta bands, and lower power in the high frequency band of theta and gamma waves in most stages. Furthermore, the significant differences between the two groups were pronounced in the active waking substage.

The results showed that the impaired cognitive functions, observed in previous studies, were accompanied by altered the oscillatory pattern of this multiple hit schizophrenia rat model. These oscillatory differences may contribute to reveal relationships between behavioral, neurochemical and electrophysiological parameters which can lead to discovering novel drug targets.

P3.02 Transient Receptor Potential Ankyrin 1 cation channel-expressing cells of the Edinger-Westphal nucleus are activated in a mouse migraine model

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Background: Transient receptor potential cation channel subfamily A member 1 (TRPA1) is involved in nociception, thermoregulation and inflammatory responses. Our previous study has proved that the *Trpa1* expression is very weak throughout the mouse and human brain, except for the urocortin1 (UCN1)-immunoreactive neurons in the centrally-projecting Edinger-Westphal (EWcp) nucleus. Interestingly, our earlier studies showed that EWcp/UCN1 neurons are recruited by acute pain stress. Here we aimed at investigating the role of the TRPA1-expressing EWcp neurons in the neurobiology of migraine.

Methods: A calcitonin gene-related peptide (CGRP) injection model of migraine was applied in C57BL6 mice. Behavioral assessment was performed using light-dark box test. The neuronal activity was measured by FOS immunohistochemistry. EWcp *Trpa1* mRNA expression and UCN1 peptide contents were assessed by RNAscope *in situ* hybridization and immunofluorescence.

Results: CGRP treatment increased the time spent in the dark compartment of the light-dark box device suggesting migraine-like state-associated photophobia in mice. The increased number of EWcp/FOS positive neurons suggest the activation of gene expression. Upon CGRP administration, EWcp neurons showed higher *Trpa1* mRNA expression and stored more UCN1 peptide. This suggests that the migraine-like state was associated with the increased expression of this cation channel in UCN1 neurons that responded with elevated neuropeptide content.

Conclusion: TRPA1 in EWcp/UCN1 neurons may play role in neurobiology of migraine. In our ongoing experiments we study the underlying central mechanisms to identify new potential therapeutical targets for the therapy of migraine.

P3.03 Cognitive enhancer effects of memantine and alpha7 nicotinic acetylcholine receptor agonist PHA-543613 in a rat model of repetitive mild traumatic brain injury during acute and subchronic treatment regimes

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Mild traumatic brain injuries (mTBI) are ordinary events that do not require medical intervention. However, repetitive mTBIs (rmTBI) may lead to long-term consequences like chronic traumatic encephalitis and increased risk of neurocognitive disorders. Recently, the same drug candidates have been suggested for Alzheimer's disease and TBI. Therefore, we investigated the procognitive effects of memantine and alpha7 nicotinic acetylcholine receptor agonist PHA-543613 in a rat model of rmTBI during acute and subchronic treatments.

Rats were subjected to weigh-drop injury 5-times with 24 hours intervals. Novel object recognition test (NOR) was repeatedly performed between the 6th and 10th weeks after the injury. To study the acute effects of memantine and PHA-543613, drugs were administered in different doses 45 minutes before NOR sessions in a within-subject design. To study the long-term effects of the drugs, rats were treated in every 12 hours for 14 days. Subchronic treatment regime began on the 7th week after injury. Memory performance was measured before the beginning of the treatments, on the 3rd and last day of the subchronic treatment, and one week after the end of treatments.

Most rats that underwent rmTBI showed memory deficit in the NOR about 6 weeks after the injuries. Memantine (1.0 mg/kg) and PHA-543613 (3.0 mg/kg) exerted an acute cognitive enhancer effect, and restored object recognition memory. The rmTBI rats that were subchronically treated with memantine (0.3 mg/kg), showed improvement in the NOR as soon as on the 3rd day of treatments. Good memory performance was maintained throughout the entire treatment regime and after the end of treatments. High dose of memantine (3.0 mg/kg) did not improve cognitive performance during the treatment regime. However, rats showed good performance one week after the end of the treatments. Subchronic administration of PHA-543613 (3.0 mg/kg) also resulted in a significant procognitive effect on the 3rd day of treatments. However, the efficacy of PHA-543613 decreased during further days of drug administration, and its procognitive effect was faded away after discontinuing the treatment.

Our results suggest substantially different dynamics of cognitive enhancer effects of subchronically applied memantine and PHA-543613 in a rat model of rmTBI. In the future, we aim to test the short- and long-term effects of memantine and PHA-543613 combination treatments in the rmTBI model of cognitive decline.

P3.04 Comparison of anxiety tests presenting different amounts of novelty: The introduction of the Elevated Circular-Maze

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Anxiety disorders are causing enormous problems worldwide while their background mechanisms and risk factors are poorly understood. The best way to research such illnesses is by developing animal models. However, a critical limitation of these is that anxiety tests measure transient states of animals, but the individuals' experiences would bias a trait-focused repeated sampling. Interestingly, in tests like the most popular Elevated Plus-Maze (EPM), avoidance increases with experience, implying enhanced anxiety. Alternatively, it is possible that as novelty decreases, animals lose their motivation for exploration, meaning that repeated measures are no longer providing information of an anxious state. In the present study, we aimed to investigate these potentially overlapping hypotheses and validate measures of repeated sampling designs.

We created a novel anxiety test, the Elevated Circular-Maze (ECM), that offers a classical approach-avoidance conflict as the EPM but presents an enhanced amount of novelty, aiming to sustain explorative motivation in a repeated experimental design. The ECM is a circular platform consisting of twelve wall-separated hiding areas and an exposed interconnecting edge. We conducted a systematic comparison with the EPM through three pilot experiments in mice to investigate the short- and long-term impact of prior experiences of different frequencies and adversity.

In the ECM, mice were more explorative than in the EPM through all experiments, as indicated by several measures. However, similar to EPM, ECM exploration also decreases after more time or occasions spent in the test. Interestingly, in contrast to EPM, ECM exploration is negatively correlated with the number of previous repeats and positively correlated with the length of intervals between test occasions. In addition, animals that experienced previous adversity did not change their behaviour in response to test repetitions. Overall, first-day behaviour predicted the outcome of the following tests, regardless of experimental conditions.

In summary, we found that novelty and prior adversity are both elements of experience-based decrease of exploration in these tests. However, ECM triggers more explorative motivation in mice and is slightly less sensitive to previous repeats, implying its potential in preclinical experimenting.

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P3.05 The somatostatin 4 receptor agonist heptapeptide TT-232 inhibits pain in mouse models of arthritis and neuropathy

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Somatostatin is a regulatory neuropeptide in the central and peripheral nervous systems, influences several endocrie functions, neurotransmission and cell proliferation via its 5 G_i-coupled receptors (sst_1-sst_5). It exerts analgesic and antiinflammatory effects through the sst_4 receptor. The natural, 14 and 28 amino acid-long somatostatin is not appropriate for drug development due to its short half-life and broad range of actions. TT-232 is a stable, sst_4 agonist heptapeptide, which might be a promising analgesic and anti-inflammatory drug candidate. Here we investigated its effects in mouse models of arthritic and neuropathic pain.

Chronic arthritis was evoked by complete Freund's adjuvant (CFA), traumatic mononeuropathy by tight ligation of 1/3-2/3 of the sciatic nerve in male wildtype (WT) and sst_4 gene-deleted (KO) mice. Mechanical hyperalgesia was measured by dynamic plantar aesthesiometry, paw swelling by plethysmometry. TT-232 was administered every day in the arthritis model and once on the 7th postoperative day in the neuropathy model (100 and 200 µg/kg i.p.).

Treatment with 100 µg/kg TT-232 significantly reduced mechanical hyperalgesia by 40-60% in both models in WT mice, but it had no effect in sst_4 KO animals. The analgesic effect was further increased by the higher, 200 µg/kg dose in the neuropathy model. TT-232 did not influence paw swelling in the CFA arthritis model.

The heptapeptide TT-232 exerts potent antihyperalgesic effect in mouse models of arthritis and neuropathy mediated by the sst_4 receptor. Therefore, it is a promising analgesic candidate for the treatment of chronic inflammatory and neuropathic pain.

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P3.06 A1 adenosine receptors have a modulatory role in exogenous ketogenic supplements-evoked beneficial effect on lipopolysaccharide-generated increase in absence epileptic activity in WAG/Rij rats

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It was demonstrated previously that ketone supplemented food containing exogenous ketogenic supplement ketone ester (KE) and ketone salt (KS) decreased the lipopolysaccharide (LPS)-generated increase in SWD (spike-wave discharge) number in a well-characterized rat model of human absence epilepsy (WAG/Rij rats: Wistar Albino Glaxo/Rijswijk rats) likely through ketosis. Moreover, it has also been demonstrated that ketone supplemented food-generated ketosis may increase adenosine level thereby may alter the activity of adenosine receptors. It was suggested that adenosine is able to modulate not only neuroinflammatory processes but also LPS (neuroinflammatory processes)-evoked increase in epileptic activity through adenosine receptors such as A_1Rs and A_{2A}Rs. Thus, in order to determine whether A_1Rs and A_{2A}Rs can modify the ketone supplemented food-evoked beneficial effect on LPS-generated increase in SWD number, an A_1R antagonist DPCPX (i.p. 0.2 mg/kg) with LPS (i.p. 50 µg/kg) and an A_{2A}R antagonist SCH58261 (i.p. 0.5 mg/kg) with LPS were co-injected on the 9th day of ketone supplemented food administration. Influence of different treatments on SWD number, blood glucose and R-βHB (R-beta-hydroxybutyrate) levels, and body weight were measured. We demonstrated that DPCPX abolished the alleviating effect of ketone supplemented food (containing 10 % KE and 10 % KS in paste-like standard rodent chow) on LPS-evoked increase in SWD number whereas SCH58261 did not modify significantly the ketone supplemented food-generated beneficial effect. Our results suggest that the ketone supplemented food-evoked alleviating effect on SWD number can be modulated through A_1Rs.

P3.07 Serotonergic anxiolysis in zebrafish requires novel or previously aversive experience

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Anxiety, manifested in an abnormal form or extent, is a core symptom of many psychiatric illnesses that are a severe burden at both the individual and societal levels. Developing animal models is the most effective way to understand the neural background of such diseases. Despite their advantages, preclinical animal testing suffers from certain limitations. According to our previous results, the currently used animal models measure time-fluctuating state anxiety instead of stable traits. Consequently, the effectiveness of specific anxiolytic agents decreases or completely disappears by repeated testing, a phenomenon called one-trial tolerance (OTT). Despite extensive investigation, the neurobiological background mechanisms of OTT are poorly understood.

We use zebrafish (*Danio rerio*) to unravel the background of OTT due to the availability of high-throughput pharmacological screening and the accessibility of whole-brain imaging techniques in this model. In the present study, we aimed to i) describe OTT in larval zebrafish and ii) investigate what stimuli can exert such phenomena. To reach this goal, we submitted three weeks-old, wildtype zebrafish to different test experiences and measured their anxiety-like responses under anxiolytic treatment (buspirone) on the following day. We measured behaviour using the swimming-plus maze (SPM) and showjump (SJ) tests, both developed and validated by our Laboratory.

We demonstrated OTT in zebrafish in both tests because buspirone has not produced its anxiolytic effect after repeated testing. OTT only occurred in response to repeats but not in response to different previous tests, implying the importance of specific experience. Given these results, we pretreated zebrafish with cycloheximide, a protein synthesis inhibitor, which rescued the effect of buspirone, indicating that OTT is based on memory formation. Finally, we examined the significance of the nature of OTT-inducing memory and found that additional aversive cues in the prior experience can diminish OTT.

In summary, buspirone exerts its effect only if the animal is naive to the experience or has a previous aversive memory of it, indicating the necessity of an alarmed internal state.

P3.08 Single-cell level characterization of the chemotherapy induced cellular senescence in the neurovascular unit

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Introduction:

In the EU every year tens of thousands of people recover from malignant diseases by the help of chemotherapy. In recent years, thanks to the great improvement of the patient`s survival rates, the long-term effects of different treatment regimens have become the subject of intense research. One of the known negative side effects of chemotherapy is the chemotherapy-induced cognitive impairment, also called as chemobrain. According to previous publications the accumulation of senescence cells and the pro-inflammatory senescence associated secretory phenotype can play an important pathophysiological roll in the development of the chemo brain.

Materials and methods:

In our study the senescence reporter p16-3MR mouse had been used. A group of animals was treated with intraperitoneal injections of the chemotherapeutic agent Paclitaxel (PTX) for 10 days. Some PTX mice were injected with the senolytic agent Navitoclax (ABT) or with ganciclovir (GCV) which shows selective toxicity in p16 expressing cells in our animal model. Previously, our PTX animals was shown to have learning and memory disfunction and neurovascular uncoupling, which was reversible by the Navitoclax senolytic treatment. In our current study, mice from each experimental groups were sacrificed for spatial and single-cell transcriptomics study, using the 10X genomics platforms (Visium and Chromium). Our data was analyzed in R environment using the Seurat workflow.

Results:

According to our spatial measurements there was an accumulation of p16-expressing senescence microdomains in the PTX group relative to the aged matched control brains. The senolytic ABT treatments and the use of GCV with model-specific p16-selective toxicity decreased the area of the senescence microdomain relative to the PTX. Transcriptome annotation showed that senescent microdomains has pro-inflammatory gene expression changes. Our parallel single-cell sequencing from the neurovascular unit enriched brain samples could identify senescence signature at the cellular level. In the brain from all experimental groups there were senescence cells predominantly among the microglia and the endothelial cells. The accumulation of senescence cells and the associated pro-inflammatory changes were also observed in the PTX samples.

Conclusion:

Using our novel transcriptomic approaches, we presented critical evidence on the pathophysiological role of cellular senescence in the development of chemo brain.

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P3.09 Comparative transcriptome analysis of the dorsomedial prefrontal cortex associated with suicidal behavior

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There is a scarcity of data on the potential contribution of gene expressional changes to depression and suicidal behavior. Recent studies revealed that human brain networks, among which the resting state network (RSN) is outstanding, are affected in psychiatric disorders. However, expressional alterations related to depression have not been reported in the dorsomedial prefrontal cortex (DMPFC), a major and functionally significant component of the RSN network. We used RNA sequencing to investigate the molecular changes in suicide victims without any medication for chronic depression as compared to control subjects without identified psychiatric disorders. More than 1000 genes differed between the 2 groups using log2FC > ±1 and the p-value < 0.05 criteria, and RT-PCR validated 15 of them. In order to identify patterns in the transcriptome data, gene set enrichment analysis was used and identified functional pathways enriched in up- and down-regulated genes. The glutamatergic synapse, growth factor receptor signaling and cytokine receptor pathways were over-represented in suicide victims suggesting that these processes are involved in suicidal behavior. One of the validated differentially expressed genes were the neuronal Ca(2+) -binding protein 2 (NECAB2). Since this gene may have great and previously not fully characterized its importance in modulating neuronal function, we aimed to further characterize the NECAB2 expression by performing in situ hybridization and immunohistochemistry to describe the distribution in different layers of the DMPFC. The NECAB2 location, together with a comparison to cell type-specific gene expressional data of the Allan Brain Atlas suggest that it is located mainly in layer I-IV and VI in two different interneuron subtypes. Our results imply extensive gene expressional alterations in the DMPFC related to suicidal behavior. Some of these genes may contribute to the altered mental state and behavior of suicide victims.

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P3.10 Age-related degeneration in the motor endplates and axons of mice leaves the motoneuron soma unaffected

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Growing evidence from animal models suggests that aging affects motor end plates and thus the innervation of skeletal muscles. The cause and the time course of these degenerative processes are unknown, and they may involve less exercise associated with age and spontaneous degeneration of the end plates, or both. Here we show that there are minor degenerative changes in the motor axons and their terminals while sensory axons remain largely unaffected. Axonal degeneration is already detectable in some of the axons in both the ventral roots and peripheral nerves of 6 months old C57BL/6 mice. These changes were accompanied by an increased number of pathological motor end plates (as compared with 3 months old mice) involving various forms of degenerating end plates in the EDL and TA muscles. At later time points (12, 18 and 24 months) progressive changes are present in both the peripheral nerves and muscles. The number of motoneurons decreases slowly but axonal transport appears to be more severely affected by aging. Muscle tension recording from these muscles shows a slightly decreasing tetanic force produced by aging animals, while the number of motor units decreases more progressively. Calcium histochemistry displays differential changes in the motor end plates and in the motoneurons. These changes suggest that aging affects mainly the distal parts of the motor unit while the perikaryon remains preserved for long time.

P3.11 Terminating human epileptic seizures by closed-loop transcranial brain stimulation – a first-in-patient study

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Existing drug therapies cannot ensure seizure-free life to one-third of the patients suffering epilepsy. Surgical interventions have very limited applicability, as they require well-defined, resecable seizure foci. Vagal nerve stimulation (VNS) and deep brain stimulation (DBS) have limited therapeutic effect and generally fail to terminate emerging seizures. Conventional transcranial electric stimulation (TES) can induce only subtle changes in neuronal activity and cannot promptly abrupt robust brain network patterns.

We showed earlier that non-or minimally invasive, closed-loop TES applied with proper intensity and temporal pattern can terminate epileptic seizures in animal models. Intersectional Short-Pulse Stimulation (ISP), our patented TES method, is capable of delivering high-intensity electrical impulses aligned to the ongoing brain rhythms with millisecond precision. The immediate effect of ISP stimulation and its spatial steerability have been proven in rodents and healthy volunteers, respectively. However, its performance in seizure termination has neither been investigated in animals nor in human yet.

First, we used spatially focused ISP in a rodent model of temporal lobe epilepsy. Stimulation pattern was aligned to pathological brain activity in real time and could promptly restore healthy network patterns by oscillatory interference. By comparing to diffuse TES, we found that ISP with identical timing and intensity, but bilateral spatial foci is more effective in early seizure termination.

Second, we performed ISP stimulation of a human epilepsy patient through subgaleal electrode strips. Tolerance profile of various stimulus waveforms was mapped first to deliver high intensity stimulation without adverse peripheral effects. Then, closed-loop stimulation was applied driven by a proprietary seizure detection algorithm. We found that 25 mA ISP stimulation could instantaneously terminate the overwhelming majority of the electrographic seizures (i.e. 33 of 39) during sleep, without waking the patient up. Average seizure duration of the terminated seizures was 977±480 ms, which was attributed to the response time of the automated seizure detection algorithm.

Here we provide the first-in-patient evidence on closed-loop seizure termination using ISP stimulation. Our results suggest that time-targeted ISP stimulation is a powerful tool for intervening pathological oscillations of epilepsy and possibly other neuropsychiatric disorders in humans.

P3.12 Extracellular circulating miRNAs as potential biomarkers in multiple sclerosis and epilepsy.

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Neurological disorders, such as multiple sclerosis or epilepsy are organic brain disorders with neuropsychiatric symptoms and represent severe social and economic burden on our society. In the last decade, numerous studies focused on the molecular background of these disorders. One important aim is to investigate the potential role of micro RNAs (miRNA) in the pathophysiology of these disorders and their potential application as peripheral biomarkers to assess disease progression and therapeutic response. Results from such studies are often rather ambiguous or hard to explain, due to the wide scale of used RNA isolation-, miRNA detection methods and different starting material. For further examination of this issue, we investigated the expression levels of circulating miRNAs in peripheral blood and brain samples originating from humans and experimental mice. In the present study, we report on findings originating from patients with epilepsy (EP) and multiple sclerosis (MS). In addition to that, we used an animal for epilepsy. C57BL/6 male mice were used for induction of temporal lobe epilepsy (TLE). 52 serum samples from MS patients, 71 serum samples from EP patients and mouse brain samples were investigated. TLE mice were terminated 1, 2, 3 and 4 weeks after intra-hippocampal injection of kainic acid. Brain tissue was removed from animals, and samples containing the prefrontal cortex, hippocampus and cerebellum were collected and immediately frozen on -80°C. After blood/brain tissue collection, total RNA was isolated, and RNA was described into cDNA. For downstream workflow droplet digital PCR reaction was prepared, using specific miRNA primers which were collected based on previous literature data. ddPCR is an extremely sensitive qPCR system, that allows absolute quantification of miRNAs in case of low yield samples as well. Our preliminary findings demonstrate an association between miRNA expression levels in human serum samples and clinical examination including MRI in MS patients.

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P3.13 Effects of intracerebroventricularly injected streptozotocin treatment on the cognitive performance of aged, experienced rats

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Streptozotocin (STZ) injected intracerebroventricularly (icv) into rats produces many symptoms of Alzheimer's disease (AD) such as cognitive decline, increased phospho-tau protein, and appearance of amyloid deposits. As the model has been used in naïve albino rat strains, in our previous experiments (Gáspár et al., 2021) we tried to transfer it to naïve Long-Evans rats, and found that a higher dose of STZ was required to induce subtle AD-like symptoms. In this study we examined the effect of icv STZ treatment on aged, experienced rats.

In the study 29 male rats were used which had almost two years learning experience in the following paradigms: five choice serial reaction time task (5CSRTT), Morris water maze (MWM), pot jumping test (PJ) and pairwise discrimination (PWD) in a touchscreen apparatus. At the age of 22 months, they were bilaterally injected with 3x1.5 mg/kg STZ or vehicle into the lateral ventricles on days 1, 3 and 5. Learning and memory capabilities of the rats were then investigating in the above assays supplemented with novel object recognition (NOR), step through passive avoidance (PAL), fear conditioning (FC), open-field (OF) and elevated plus maze (EPM) tests. 15 weeks after STZ treatment animals were sacrificed and the hippocampal phospho-tau/tau protein ratio and β-amyloid level were determined by Western blot technique (WB).

We found significant impairing effects of STZ treatment in the NOR and MWM tests while in the FC test, STZ-treated animals showed significantly stronger freezing responses. In the PWD paradigm STZ-treated rats initiated a significantly higher number of trials but with similar percentage of correct responses to the controls. In the 5CSRTT, they made significantly more premature responses than the vehicle-treated group but again with similar correct% and accuracy%. We also found a significant increase in motor activity in the OF together with higher dwelling time in the central zone. In the EPM, STZ-treated animals more frequently visited the open arms. We did not find significant difference between the two groups in the PAL and PJ test, neither in the tau and β-amyloid levels.

Our findings suggest, that icv STZ treatment impaired visual recognition and spatial memory but did not affect procedural memory, fear memory and attention in aged, experienced Long-Evans rats. We interpret the overall behavioural changes induced by STZ as increased impulsivity

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P3.14 The dualistic role of the purinergic P2Y12 receptor in MPTP induced Parkinsonism in mice

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Parkinson's disease (PD) is a chronic, progressive neurodegenerative condition; characterized with the degeneration of the nigrostriatal dopaminergic pathway and neuroinflammation. During PD progression, microglia, the resident immune cells in the central nervous system (CNS) display altered activity, but their role in maintaining PD development has remained unclear to date. The purinergic $P2Y_{12}$ receptor ($P2Y_{12}R$), which is exclusively expressed on the microglia in the CNS has been shown to regulate microglial activity and responses; however, the function of the $P2Y_{12}R$ in PD is unknown. Here we show that MPTP-induced PD symptoms in mice are associated with marked neuroinflammatory changes and $P2Y_{12}R$ contribute to the activation of microglia and progression of the disease. Surprisingly, while pharmacological or genetic targeting of the $P2Y_{12}R$ augments acute mortality in MPTP-treated mice, these interventions protect against the neurodegenerative cell loss and the development of neuroinflammation in vivo. Pharmacological inhibition of receptors during disease development reverses the symptoms of PD and halts disease progression. We found that $P2Y_{12}R$ regulate ROCK and p38 MAPK activity and control cytokine production. Our principal finding is that the receptor has a dualistic role in PD: functional $P2Y_{12}R$ are essential to initiate a protective inflammatory response, since the lack of the receptor leads to reduced survival; however, at later stages of neurodegeneration, $P2Y_{12}R$ are apparently responsible for maintaining the activated state of microglia and stimulating pro-inflammatory cytokine response. Understanding protective and detrimental $P2Y_{12}R$-mediated actions in the CNS may reveal novel approaches to control neuroinflammation and modify disease progression in PD.

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P3.15 Parallel investigations on behavioural changes and ex vivo entorhinal cortical network excitability in a rat model of autism

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Autism is a neurodevelopmental disorder characterized clinically by impaired social communication and interaction, limited interest, and repetitive behavior patterns. Autism has been shown to increase the risk of developing epilepsy, so it is important to investigate the common background of both diseases. The valproic acid (VPA) rat model is commonly used to study autism-like behaviors. VPA is an antiepileptic drug whose prenatal exposure increases the risk of developing autism in offspring.

In our study, pregnant Wistar rats received an i.p. injection of VPA on gestation day 12.5 (dose 500 mg/bwkg). Pups were tested for various motor functions and behaviors from day P3 to week 6 (surface righting, negative geotaxis and air righting reflex, auditory startle, visual placing reflex, self-grooming, marble burying, social interaction test). Then, electrophysiological studies were performed in two age groups: 6 weeks and 3 months in both sexes. Horizontal entorhinal cortex slices were prepared from the offspring for field potential measurements and parallel detection of intrinsic optical signals (IOS). Excitability changes were tested with spontaneous bursts evoked by magnesium-free solution (MFR) and afterdischarges evoked by brief bursts of high frequency electrical stimulation.

Behavioral tests indicate that VPA-treated animals were able to perform surface righting reflex, negative geotaxis and visual placing reflex significantly later. The treated females spent more time self-grooming than controls. Electrophysiological results show that the pattern of electrical activity depended on the stimulation method, and the extent of the change in the IOS was also different. Valproate treatment showed an increase in seizure activity, suggesting an increased tendency to epilepsy. The optical signal showed concordance with the electrophysiological results. In MFR, sex differences have also been observed, neuronal excitability increased to a greater extent in male offspring than in females.

After prenatal VPA exposure, developmental delay and disturbed social skills can be observed. Our behavioral tests confirm the presence of autistic traits in VPA-treated animals. There are significant differences between sexes and age groups. VPA treatment may enhance these differences. As a result of VPA treatment, the excitability of the network might enhance.

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P3.16 Investigation of the glutamate transmitter receptor system in a rat model of autism

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Autism is a neurodevelopmental disorder described by several major symptoms for example impairment of sociability, impaired communicational skills, stereotypic and repetitive behaviors. According to a leading hypothesis about the background of autism, the disequilibrium of the excitation/inhibition processes within neuronal networks plays a key role. This imbalance may be caused by the hyper- or hypoactivation of the glutamatergic system or the GABAergic system. The reason behind this could be the disturbed expression of different ionotropic glutamate receptor subtypes. During my experiments, I compared the expression of different members of ionotropic receptor families (AMPA, NMDA and kainate receptors) in different brain areas related to autism: prefrontal cortex, hippocampus and entorhinal cortex.

The rodent valproate (VPA) autism model was used: pregnant Wistar rat dams were treated with VPA on the 12.5 gestation day and 3-month-old male and female offspring was used for experiments. Horizontal brain slices were stained with the immunohistoblot method. This is a reliable semi-quantitative method to examine protein expression and regional distribution of proteins without fixation of tissue samples, which would cause modification or denaturation of the proteins. Alkaline-phosphatase labelled secondary antibody was used and optical density values were compared in sections from control and treated animals. Whole-cell patch clamp recordings were also carried out on pyramidal cells in CA1 and entorhinal cortex. Standard current clamp stimulation/dynamic clamp stimulation and spontaneous EPSC analysis were performed.

Preliminary analysis of histoblots revealed differences in the expression of different kainate receptor subtypes in the entorhinal cortex, prelimbic and infralimbic cortices. VPA treatment caused a significant decrease in GluK2/3 immunoreactivity in the entorhinal cortex of female rats in all cortical layers. In addition, sex differences were significant in the cortices in relation to GluK2/3, GluK5 and GluN1 subtypes. Sex differences were also prominent in the hippocampal areas mostly in case of GluN1 subtype.

Regarding the patch clamp recordings, VPA treatment evoked significant differences in several membrane parameters of the neurons, and also EPSC frequency in the hippocampus and entorhinal cortex. Most of these changes indicated increased excitability of individual neurons and neural circuits.

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P3.17 Excitability changes in prefrontal cortical networks in a rat model of autism

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The experiments focused on brain excitability and plasticity changes in autism spectrum disorder model rats. People diagnosed with ASD exhibit impairment in social interactions and communication skills as well as repetitive behaviours. Underlying this triad of impairments, changes in neural network connectivity and excitability can be observed in several brain areas.

Experiments carried out by our group focused on the prefrontal cortex, an area, which is connected to a wide variety of functions, among others, the interpretation of others' emotions and the evaluation of social situations.

Valproate was administered to rat dams on the 12th day of pregnancy. Pups were subjected to behavioural tests at postnatal days 3-45, to observe the expected delayed development and autistic traits. Acute brain slices were prepared from 6-week-old and 3-month-old offspring of both sexes. To investigate network functions, evoked field potentials were recorded in the prefrontal cortex. Basic excitability was tested with input-output curves. To test network plasticity, long-term potentiation was induced with two different protocols (1 or 4 stimulation trains of 100Hz). Excitability of individual neurons was tested with patch clamp recordings.

Valproate treatment evoked significant delays in postnatal development and impaired social behaviour. According to preliminary electrophysiological results, the amplitude of evoked potentials' early component was lower, while late component amplitude was higher in treated 6-week-old males compared to controls, indicating an altered circuit excitability. The results also pointed out, that there is a significant sex difference between the threshold of excitation of the treated 6-week-old male and females. LTP efficacy did not differ significantly.

Intracellular recordings revealed that cells in the treated 6-week-old females were more excitable compared to their control peers, based on the finding that the membrane resistance increased and the rheobase decreased. The excitability difference was observed in the 6-week-old males due to the membrane potential becoming more positive, but the resistance of the membrane decreasing. The difference of the valproate treated and control animals diminished in the 3-month-old groups.

Further investigation is needed to increase the sample numbers in each treatment group. Testing network sensitivity by recording epileptiform activity is also planned.

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P3.18 Peptidergic neurons of the Edinger-Westphal nucleus express TRPA1 ion channel that is downregulated both upon chronic variable mild stress in mice and in human suicide victims

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Background: Transient Receptor Potential Ankyrin 1 (TRPA1) cation channel is predominantly expressed in primary sensory neurons, but its central distribution and role in mood control remains elusive. We aimed at investigating if TRPA1 is expressed in the urocortin1 (UCN1)-immunoreactive centrally-projecting Edinger-Westphal nucleus (EWcp) and hypothesized that chronic variable mild stress (CVMS) reduces its expression in mice. We anticipated that *TRPA1* mRNA is present in the human EWcp and it is downregulated in suicide victims.

Methods: *Trpa1* knockout (KO) and wildtype (WT) mice were exposed to CVMS. Behavioral tests for depression and anxiety, physical moreover endocrinological parameters of stress response were evaluated. EWcp *Trpa1* and *Ucn1* mRNA expressions and UCN1 peptide contents were assessed by RNAscope *in situ* hybridization and immunofluorescence. Human EWcp samples were tested for *TRPA1* by RT-PCR techniques.

Results: *Trpa1* mRNA co-localized with EWcp/UCN1 neurons. Non-stressed KO mice expressed higher *Ucn1* mRNA, had reduced body weight gain and greater immobility in forced swim test (FST) compared to WTs. CVMS downregulated EWcp/*Trpa1* expression and enhanced FST immobility only in WTs. *TRPA1* mRNA expression was confirmed in the human EWcp that was downregulated in suicide victims.

Limitations: Developmental compensations and the global lack of TRPA1 may have influenced our findings. Due to the lack of TRPA1-specific antibody we provide mRNA data only. Limited access to high-quality human tissues restricted sample size.

Conclusion: TRPA1 in EWcp/UCN1 neurons has physiological role in regulating depression-like behavior and stress adaptation response in mice. Human TRPA1 may potentially contribute to mood control via EWcp/UCN1 neurons.

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P3.19 Creating cholinergic neuron specific knock-out mice by combining three (CRISPR-Cas9, Cre/loxP and AAV) genome editing technologies

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Introduction: The RNA-guided Cas9 nuclease from the microbial clustered regularly interspaced short palindromic repeats (CRISPR) adaptive immune system can be used to facilitate efficient genome engineering in vivo by simply specifying a 20-nucleotide targeting gene specific sequence.

Aim: The aim of our experiment was to create a genetically modified mouse species in which we can knock-out the Estrogen Receptor alpha (ERα) (or any other receptor) specifically from cholinergic neurons, in any interested areas of the brain.

Material and methods: First, two homozygous mouse strains were crossed. One contains a cholinergic neuron-specific Cre recombinase (choline acetyltransferase; ChAT-Cre), and the other contains the Cas9 enzyme, between two loxP cleavage sites (Cas9-LoxP). All cholinergic (and not other types) neurons in double heterozygous animals, formed during crossing, will express the Cas9 enzyme. The next step was a stereotaxic injection of an adeno-associated virus vector (AAV) containing an ERα-specific guide-RNA into a specific area of the brain, in our case the nucleus basalis magnocellularis (NBM). The AAV virus was custom made in our laboratory.

Results: In these animals, Cas9, which is only present in cholinergic cells is selectively active in the NBM (not other areas of the brain), because only here the CRISPR sequence required for its function will appear. This will specifically apply only to the ERα gene (see guide-RNA), which will thus disappear from these cells.

Conclusions: The technology combining the CRISPR-Cas9, Cre/loxP and AAV virus systems is really promising to edit the genome more selectively. Creating new genetically modified animals will help the better understanding of neurological diseases and improve research tools.

EFOP 3.6.2-16-2017-00008 (The Role of Neuro-inflammation in Neurodegeneration: From Molecules to Clinics)

P3.20 Elevated serum purine levels in schizophrenia: a reverse translational study to identify novel inflammatory biomarkers

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Immunological markers and related signaling molecules in the blood are altered in schizophrenia mouse models, in acutely relapsed patients with schizophrenia, and in persons at a clinically high risk for subsequently developing psychosis, highlighting their potential as prognostic and theranostic biomarkers. Therefore, we herein aimed to identify novel potential biomarkers in the serum that are associated with purinergic signaling. To our knowledge, this is the first study to assess the correlations among the levels of human serum adenine nucleotides (ATP, ADP), adenosine, P2X7 receptor, and disease activity in patients hospitalized due to an acute relapse of schizophrenia (n=53) and healthy controls (n=47). In addition, to validate these findings using a reverse translational approach, we examined the same parameters in an acute phencyclidine (PCP)-induced schizophrenia mouse model. We found consistently elevated levels of ATP, ADP, IL-6, and IL-10 in both schizophrenia groups compared to the controls. The levels of adenosine, IL-1β, IL-12, and C-reactive protein (CRP) were also increased in the human patient samples. Moreover, ATP and ADP were significantly positively correlated with the Positive and Negative Symptom Scale (PANSS) item 'lack of judgment and insight'; IL-1β, IL-12 and TNF-α were significantly positively correlated with 'tension' and 'depression'; and 'disorientation' and 'poor attention' were correlated significantly with IL-6 and IL-8. Our study suggests the promising potential of blood purines and inflammatory markers as future prognostic tools.

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P3.21 Reinstating olfactory bulb derived limbic gamma oscillations alleviates depression

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Although the etiology of major depression has remained largely unknown, its strong mutual link with olfactory function suggests neuronal dynamics of the broadly distributed limbic olfactory network and their downstream oscillatory outputs can contribute to the pathophysiology of this disorder. The olfactory bulb (OB) is a major source of brain wide gamma oscillations and decreased gamma power was recently identified as a potential biomarker for major depression. Here we demonstrate, that olfactory bulbectomy, specific chemogenetic suppression of OB neuronal activity or temporally suppressing the OB to PirC synaptic transmission can result in impaired gamma oscillations in multiple brain areas and the appearance depressive-like behaviors. These results suggest that intact brain-wide gamma oscillations driven by OB are necessary for maintaining a healthy mood. In line with this, a temporally precise closed loop neuromodulation-based enhancement of OB derived gamma oscillations in the PirC can alleviate depressive-like behaviors in rodent models without side effects providing promising therapeutic value.

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P3.22 Role of the phosphodiesterase GDE1 in an ER-mediated pathway preventing anxiety

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Due to the increasing average human lifespan, neurodegenerative diseases (NDs) are on the rise and present an ever more significant economical and social burden. While some of these conditions have well-described causes (eg. Huntington's disease or familial Alzheimer's disease), most of them are complex diseases with multiple genetic and enviromental factors involved. The extensive neuron-loss in NDs are the result of various forms of cell death, including but not limited to apoptosis, necrosis and autophagy. Paraptosis is a special form of programmed cell death accompanied by excessive enlargement of the endoplasmatic reticulum due to ER-stress and the unfolded protein response (UPR). Paraptosis has been mainly implicated in cancer-related cell death processes so far, but recently, it has been also been demonstrated to occur in neurons of Huntington's disease mouse models. Here, we demonstrate that loss of the glycerophosphodiester phosphodiesterase GDE1 evokes a progressive and degenerative vacuolization in the hippocampus, locus coeruleus and other areas of the CNS. We also provide evidence that these vacuoles are initially produced intracellularly and accompanied by progressive degradation of intracellular organelles. Interestingly, Gde1 knockout animals also demonstrate increased anxiety and elevated context-dependent recall during fear conditioning. GDE1 has been previously proposed by several labs to be involved in an alternative synthesis pathway of the endocannabinoid anandamide downstream of ABHD4, a PLA2-type serine hydrolase. Here, we present direct and indirect evidence to demonstrate that GDE1 does not play a role in this pathway, which correlates well with recent lipidomics data demonstrating that glycero-phophoserine, glycero-phosphatidylinositol and glycero-phosphoglycerate, but not glycero-phosphoetanolamine serve as Gde1 substrates. Our current research focuses on describing the complete spatio-temporal pattern of the vacuolization process within the CNS of Gde1 knockout animals and on uncovering the intracellular localization of Gde1 in order to identify the cellular function of this molecule.

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P3.23 Peripherally induced acute neuroinflammation leads to functional changes in the prefrontal cortex at the molecular, cellular, and network levels

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Neuroinflammatory conditions (e.g., infection, neurodegeneration, and sepsis-associated encephalopathy) lead to various neuropsychiatric symptoms both in humans and laboratory animals. One of the best known physiological changes related to neuroinflammation is the manifestation of sickness behavior that resembles some features of clinical depression. However, in addition to depression-like behavior, there are other symptoms of neuroinflammatory conditions that can be associated with the deterioration of prefrontal cortex (PFC)-regulated cognitive functions. Thus, we investigated the electrophysiological and molecular alterations of the PFC using the lipopolysaccharide (LPS) mouse model of acute peripheral infection. We analyzed the fronto-occipital functional connectivity in freely moving animals by electroencephalographic (EEG) recordings, as the alterations of fronto-occipital coupling are associated with the cognitive processes impaired by neuroinflammation. Significant changes were found in the EEG correlation and in the EEG coherence of the delta and high-gamma frequency bands. The cellular background of these LPS-induced effects was investigated by patch clamp recordings in acute brain slices. We treated PFC neurons with interleukin-1 beta (IL-1β) and found a concentration-dependent enhancement of pyramidal cell excitability that could be abolished by interleukin-1 receptor (IL-1R1) antagonist. However, inhibitory interneurons showed no electrophysiological changes during IL-1β treatment, suggesting that the imbalance between excitatory and inhibitory neurotransmission may contribute to the pathophysiology of neuroinflammation. The inflammation-induced alterations of the prefrontal IL-1 system (IL-1β, IL-1R1) were analyzed by immunoassays at different time points after LPS-treatment. Furthermore, we investigated the synaptic proteome changes of the PFC using 2-D differential gel electrophoresis and found more than 90 protein spots showing significant alteration due to LPS-treatment. Thus, our results indicate remarkable electrophysiological and molecular changes in the PFC during acute peripheral infection that may explain some of the behavioral and physiological symptoms observed under neuroinflammatory conditions.

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P3.24 Deep plasma proteomics reveal age-related molecular pathways modulated by GRF6019 treatment in Alzheimer's disease patients

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Objectives

Blood has been widely investigated to discover biomarkers and gain insights into the biology of aging and age-related diseases. Its protein composition provides information about complex biological processes, as proteins are often direct regulators of cellular pathways. Using recent methodological developments allowing the measurement of thousands of proteins with very high sensitivity and specificity, we sought to understand comprehensive proteomic changes in two AD clinical trials.

Methods

Phase 2 clinical trials (GRF6019-201 n=40 and GRF6019-202 n=26) testing the safety, tolerability, and feasibility of repeated infusions of the plasma fraction GRF6019 in Alzheimer's disease (AD) were used as the source to measure more than 7000 proteins in plasma using the SOMAscan and Olink assays. To evaluate the relevance of the proteomics changes induced by GRF6019, we compared these changes to those observed in a healthy aging cohort (~5000 proteins measured in 370 subjects).

Results

Standard statistical analysis at the protein levels lacked power due to the small sample size in phase 2 clinical trials. By analyzing trajectories of groups of proteins, clinical proteomics revealed multiple clusters of proteins responding to GRF6019. Remarkably, several pathways modulated by GRF6019 were particularly relevant for the biology of aging and AD – including the complement/coagulation cascades and neuronal pathways ($q<0.05$).

Conclusion

Altogether, our results suggest that the treatment of AD patients with a complex plasma fraction modulates biological pathways that are relevant to aging and AD. Our results establish deep proteomics as a powerful tool to study human response to treatment in clinical trials.

P3.25 TDP-43 pathology includes inflammatory changes around NMJs in a mouse model of ALS

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Amyotrophic lateral sclerosis (ALS) is characterized by the progressive loss of motoneurons and motor functions. Although mutations in TDP-43 are only present in 2-3% of ALS patients, TDP-43 pathology is described in 95% of the cases, suggesting a prominent role in the pathogenesis of the disease. TDP-43 mislocalization is known to induce pro-inflammatory response in the spinal cord, around the motoneuronal cell somas, however, the degenerative alterations are also present at the level of the neuromuscular junctions (NMJs). In our experiment we aimed to characterize the inflammatory changes around NMJs and their possible role in the propagation of TDP-43 pathology.

For our experiments we used a mouse line overexpressing human wild-type TDP-43 under control of Thy1 promoter, resulting in a neuron-specific TDP-43 pathology. Animals were sacrificed at postnatal day 20, representing a late-stage disease pathology. Age-matched hTDP-43 -/- animals were used as controls. The tibialis anterior (TA) and gastrocnemius (GC) muscles were then used for immunostaining and quantification of CD45+ leukocytes, CD3+ T-cells and CD68+ macrophages. Cell counting was carried out both in the innervation zone (IZ) and outside of the innervation zone (OIZ). Furthermore, changes in cytokine and chemokine levels were assessed with proteome profiler antibody array.

In both the TA and GC muscles the number of CD45+ and CD3+ cells was elevated compared to control samples. This increase was significantly greater in the IZ compared to OIZ. Furthermore, these changes were more prominent in the GC, than in the TA muscle. The CD68+ cell count was only elevated in the IZ of both muscles. In the control samples cell counts showed no significant difference amongst the IZ and OIZ. Proteome profiler analysis revealed an increase of pro-inflammatory and chemotactic factors in both the TA and GC.

Our results show extensive immunreaction in the muscle samples of hTDP-43 +/+ animals. While muscle atrophy might contribute to the increased leukocyte number, we observed enhanced immunactivity around the degenerating NMJs, suggesting the presence of NMJ-specific processes. Interestingly, the higher leukocyte density and chemotactic activity in the GC muscle is inversily proprotional to the rate of NMJ degeneration, since denervation is known to be more prominant in the TA, than in the GC muscle. These results imply that immune cell activation might amplify denervation through NMJ-targeted mechanisms.

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P3.26 Cannabinoid receptor type 1 expression in the fetal cortex and its alterations in Down syndrome

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We traced the expression of cannabinoid receptor type 1 (CB_1R) in axons and axonal endings/boutons in fetal brains and its changes during pregnancy. Between days 98-136, a wealth of fibers appeared at the border of the subventricular and intermediate zones in both temporal and frontal cortices. This expression was less intense in Down syndrome and a similar phenomenon appeared in the fibers surrounding the ganglionic eminence. Phylogenetic age did not affect CB_1R expression change: in the archicortical hippocampus and in the fornix CB_1R expression was weaker in Down syndrome feti by the end of the fourth gestational month. Between days 138-157, we observed an opposite phenomenon: developing cortices of Down syndrome subjects showed higher CB_1R expression. In cerebellum and cingulate gyrus, immunoreactive fibers appeared during the second trimester in Down syndrome with a different expression pattern in control subjects. By late gestation, except some minor variations, no consistent differences in CB_1R expression could be observed between control and Down syndrome subjects: the fibers of the subcortical white matter, corpus callosum, fornix, and internal capsule were devoid of CB_1Rs, but the hippocampal formation and parts of the neocortex were enriched in CB_1R expressing terminals. Conclusively, CB_1R-expressing axons and boutons appear later in Down syndrome during brain development which is equalized during the third trimester.

P3.27 A mouse model of comorbid anxiety and depression

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Nearly half of patients who are diagnosed with major depressive disorder also have a comorbid anxiety disorder diagnosis. Co-occurrence of these conditions results in more severe symptoms and less effective pharmacotherapy compared to single diagnoses. Nevertheless, the underlying neurobiological factors of comorbid anxiety and depression are not well understood, and the lack of established animal models of comorbidity have greatly hindered such studies so far. Therefore, our aim was to establish a simple, reliable rodent model of comorbidity with high clinical translational validity.

We have previously designed a behavioral sampling protocol to measure trait anxiety, rather than commonly measured anxious states. This involves repeated sampling of behavior using the most popular anxiety tests, the Elevated Plus-Maze, Light-Dark and Open Field tests, all measuring the avoidance of species-specific aversive stimuli. Through this project, we aimed to extend this method to preclinical tests measuring passive coping (a key attribute of depressive disorders), namely the Forced Swim, Tail Suspension and Back tests. Consequently, we examined the association of anxiety- and depression-related traits of individuals and characterised a subpopulation that consistently displayed high avoidance and strong passive coping. Correlation between measures of trait anxiety and coping can be increased by averaging results from repeated tests, thereby strengthening the detection of comorbidity between these traits.

Subsequently, we performed the Learned Helplessness (LH) depression model, which simultaneously measures coping and induces a depression-like behavioural phenotype in vulnerable individuals. According to our results, animals that show strong avoidance and passive coping in tests previous to the LH are significantly more likely to develop a strong level of learned helplessness subsequently. Using a semi-supervised machine learning algorithm we were able to simplify the testing protocol into a reduced number of tests. These tests allowed us to predict with high accuracy the level of helplessness an animal develops in the subsequent depression model, and also its long-term consequences on behavior.

In conclusion, we developed a simple and reliable preclinical model of comorbid anxiety and depression, which enables us to uncover the underlying neurobiological factors, thereby leading to the characterisation of novel pharmacological targets.

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P3.28 Examination of the PAC1 receptor colocalization with Ca^{2+}-binding proteins and cochlea-efferent markers in the auditory pathway of pituitary adenylate cyclase-activating polypeptide - knock out (PACAP KO) and wild type (WT) mice

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The neuroprotective and cytoprotective effects of PACAP are well known. In PACAP KO mice, we showed elevated hearing tresholds along with higher apoptosis rate and increased synthesis of Ca^{2+}-binding proteins (parvalbumin, calretinin) of hair cells in the organ of Corti.

In this present study, we examined the role of PACAP in the auditory pathway of 1.5, 4, and 8-month-old mice. The synthesis of Ca^{2+}-binding proteins and of PAC1 receptor were visualized with calretinin-parvalbumin-PAC1 receptor immunostaining in the cochlear nuclei of PACAP KO and WT mice. Choline acetyltransferase (ChAT)-tyrosine hydroxylase (TH)-PAC1 receptor triple immunostaining was performed in the nuclei of the superior olivary complex participating in cochlear efferentation.

PAC1 receptor showed colocalization with parvalbumin and calretinin positive cells in the ventral cochear nucleus. The number of parvalbumin positive cells significantly increased with the age in both genotype, however, the number of PAC1 receptor containing parvalbumin positive cell had a less pronounced increase. In the dorsal cochlear nucleus we also found a similar, but less pronounced elevation in the KO animals. In young animals, PAC1 receptor was colocalized more with parvalbumin positive cells than with calretinin positive cells in the dorsal cochlear nucleus in both genotypes. In the superior olivary complex, PAC1 receptor was detected in the third of ChAT and TH positive cells. We did not find significant differences between the age groups and the genotypes.

The age-related increase of parvalbumin in the auditory pathway is known. Based on our experiment this elevation is less marked in the cells of the ventral cochlear nucleus which also synthesize PAC1 receptor. Higher PAC1 receptor association with parvalbumin cells in the dorsal cochlear nucleus could show that PACAP does not affect all cells similarly in this nucleus. PAC1 receptor colocalize with ChAT and TH positive neurons - which take part in the efferent innervation of cochlea - in both genotype. Our experiments prove that PACAP plays a role in the auditory system not only in the cochlea, but also in other parts of the auditory pathway.

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P3.29 Protection against vincristine-induced peripheral neuropathy in WLDS and SARM1$^{-/-}$ mice

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Chemotherapy-induced peripheral neuropathy (CIPN) is a common and potentially dose-limiting side effect of many cancer chemotherapy drug treatments. CIPN is thought to begin with degeneration of sensory axons, or their terminal regions. One of the leading hypotheses is that axons undergo 'dying back' pathology because axonal transport is impaired by several chemotherapy drugs. A protein we identified, the slow Wallerian degeneration protein (WLD^S) and the reported genetic deletion of SARM1 (sterile alpha and TIR motif containing protein 1) strongly protect axons from degeneration when axons are injured, or axonal transport is blocked. Here we tested if SARM1 deletion and WLD^S protects against vincristine-induced peripheral neuropathy in a mouse model. Vincristine was used in a low (0.5mg/kg) and high dose (5mg/kg) to induce peripheral neuropathy in wild type (WT*), WLD^S and SARM1$^{-/-}$* mice. Both doses caused a pronounced mechanical hypersensitivity only in the WT mice but not in the *WLD^S or SARM1$^{-/-}$* mice. A significant increase in heat sensitivity was found in WT mice but not *WLD^S or SARM1$^{-/-}$* mice following the treatment with the high dose of vincristine. In addition, WLD^S and SARM1$^{-/-}$ blocked vincristine induced degeneration of intraepidermal nerve fibers (IENFs) in the footpads of mice.

Our findings suggest that targeting SARM1 could be a viable therapeutic approach to prevent vincristine-induced peripheral neuropathy. WLD^S and SARM1$^{-/-}$ prevent neuropathic pain likely by blocking axon degeneration. Our study also reports for the first time a protective effect of WLD^S against vincristine-induced peripheral neuropathy.

P3.30 Infrared thermal modulation of optogenetically induced epileptic activity

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Around 50 million people worldwide suffer from different kinds of epilepsy, making it one of the most common neurological diseases globally. Since almost 30% of patients have drug-resistant forms of epilepsy, novel treatments are required. One possible approach is the local heating of brain tissue, as it has been shown to influence the general network excitability, and influence the frequency of neural oscillations. The aim of the present project is to reveal the influence of local temperature increase on generalized tonic-clonic epileptic seizures.

We used a recently developed optogenetic epilepsy model, in which we can reliably induce tonic-clonic seizures by rhythmic stimulation of layer 6 corticothalamic cells. Experiments were made on NTSR1 double transgenic mice, expressing channelrhodopsin in their layer 6 corticothalamic cells. Generalized tonic-clonic seizures were provoked by 447nm blue light stimulation of corticothalamic pathway, under freely moving conditions. The local field potential of epileptiform activity was recorded from motor, somatosensory, auditory, and visual cortices, as well as from the hippocampal formation. Local rise of temperature was induced by 1550 nm infrared stimulation applied at the site of epileptogenesis. Each day only one seizure was evoked from each animal, alternating the heating and non-heating epochs.

Local infralight heating of the site of epileptogenesis was unable to inhibit the development of the epileptiform activity, however, it was able to change the pattern of seizure events. A significant decrease in the duration and so far a non-significant decrease in the prevalence of seizures were found in the heated sessions of seizure induction. The power of spectral components also showed a prominent rearrangement due to the rise of local temperature, but there was no change in spreading patterns or latencies of synchronized activity among distant cortical regions.

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P3.31 Inoculation with blood sera from ALS patients with identified mutations eventuates elevated calcium levels and loss of lumbar motor neurons in mice

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Increasing evidence support that neuroinflammation has a key role in the pathobiological processes of amyotrophic lateral sclerosis (ALS) both in animal models and human patients. Immunoglobulins from ALS patients showed anti-motoneuronal properties and their presence resulted in increased proinflammatory factors in the spinal cord of mice after intraperitoneal injection. Furthermore, these autoantibodies can bind and modify the function of calcium channels, which leads to disturbance in the calcium homeostasis and eventually neuronal loss. Utilizing these degenerative effects, blood sera from ALS patients without known genetic alterations or with identified mutations in the SOD1, C9ORF72, SQSTM1, CCNF, NEK1, TBK1, and UBQLN2 genes were injected into Balb/c mice to create an immune-based experimental motoneuron degeneration model. Previously we demonstrated increased calcium content in the axon terminals of spinal MNs after passive transfer of ALS sera but the effect of such treatment in the perikaryon reminded unknown. Similarly to the axon terminals, a significant increase in the calcium level of the cell bodies of lumbar motoneurons was observed in the ALS serum treated group compared to the untreated and the healthy serum treated groups. Correlation in the mutual increase in the distal and central parts of the lumbar motor neurons was corroborated by the analysis of the fitted linear model of these data. As a consequence of the elevation of the calcium content, a remarkable loss of motor neurons was observed in the lumbar spinal cord of mice after passive transfer of ALS sera. Interestingly, we found that mice treated with ALS sera from patients with C9ORF72 hexanucleotide repeat expansion showed the most prominent elevation in the calcium level which was accompanied by the most robust motoneuronal loss. Expectation-Maximization cluster analysis based on the correlation of calcium elevation and motoneuronal loss is capable of separating controls and ALS patients, furthermore, C9ORF72 mutation sorted into a more progressive subgroup. Since this mutation has a significant role in numerous immune-mediated processes, in the future, we would like to examine these events which might lead to novel therapeutic targets for ALS.

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P3.32 Maternal P2X7 receptor inhibition prevents autism-like phenotype in male mouse offspring through the NLRP3-IL-1β pathway

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Autism spectrum disorder (ASD) is a complex neurodevelopmental condition caused by interactions of environmental and genetic factors. Recently we showed that activation of the purinergic P2X7 receptors is necessary and sufficient to convert maternal immune activation (MIA) to ASD-like features in male offspring mice. Our aim was to further substantiate these findings and identify downstream signaling pathways coupled to P2X7 upon MIA. Maternal treatment with the NLRP3 antagonist MCC950 and a neutralizing IL-1β antibody during pregnancy counteracted the development of autistic characteristics in offspring mice. We also explored time-dependent changes of a widespread cytokine and chemokine profile in maternal blood and fetal brain samples of poly(I:C)/saline-treated dams. MIA-induced increases in plasma IL-1β, RANTES, MCP-1, and fetal brain IL-1β, IL-2, IL-6, MCP-1 concentrations have been shown to be the effect of P2X7/NLRP3 regulation. Offspring treatment with the selective P2X7 receptor antagonist JNJ47965567 was effective in the prevention of autism-like behavior in mice using a repeated dosing protocol. Our results highlight that in addition to P2X7, NLRP3, as well as inflammatory cytokines, may also be potential biomarkers and therapeutic targets of repetitive behavioral and social deficits observed in autism spectrum disorder.

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P3.33 Perisomatic inhibition and its relation to epilepsy and to synchrony generation in the human neocortex

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Inhibitory neurons innervating the perisomatic region of cortical excitatory principal cells are known to control the emergence of several physiological and pathological synchronous events, including epileptic interictal spikes. In humans, little is known about their role in synchrony generation, although their changes in epilepsy have been thoroughly investigated. Now, we describe how parvalbumin (PV)- and type 1 cannabinoid receptor (CB1R)-positive perisomatic interneurons innervate pyramidal cell bodies, together with their role in synchronous population events spontaneously emerging in the human epileptic and non-epileptic neocortex, in vitro. Quantitative electron microscopy showed that the overall, PV+ and CB1R+ somatic inhibitory inputs remained unchanged in epilepsy. However, the size of PV-stained synapses increased, and their number decreased in epileptic samples, in synchrony generating regions. Pharmacology demonstrated – in conjunction with the electron microscopy – that although both perisomatic cell types participate, PV+ cells have stronger influence on the generation of population activity in epileptic samples. The somatic inhibitory input of neocortical pyramidal cells remained almost intact in epilepsy, but the larger and consequently more efficient somatic synapses might account for a higher synchrony in this neuron population. This, together with the epileptic hyperexcitability might make a cortical region predisposed to generate or participate in hypersynchronous events.

P3.34 The role of spreading depolarization in the insufficiency of reperfusion after cerebrovascular occlusion

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Cerebrovascular anatomical variations determine the efficiency of recanalization therapies the ischemic stroke care. In fact, incomplete collateral circulation might provoke secondary pathological events, such as spreading depolarizations (SDs) that cause perfusion deficit and lesion progression. Here, we aimed to prove that (i) incomplete collateral anastomoses predict SD evolution and (ii) the pharmacological blockade of SDs improves reperfusion in mice.

Male 8-12 weeks old C57BL/6 mice (n=15) were anesthetized with isoflurane (0.6-0.9%). A baseline of 10 min was followed by a transient (45 min) bilateral common carotid artery occlusion (2VO) and a subsequent 60 min reperfusion. Cerebral blood flow (CBF) variations were captured using green light reflectance and laser speckle contrast imaging. The irreversible NMDA receptor antagonist MK801 was applied intraperitoneally (0.3 mg/kg) (n=8). Post stroke neurological deficit was classified after 24 hours on a Composite Garcia Neuroscore scale (0-21). Anatomical features of the Willis circle were examined after carbon black ink perfusion.

Low CBF early under ischemia favored SD evolution (SD vs. no SD; <25% vs. >35%). SDs occurred in both hemispheres (bilateral) in 33%, in one hemisphere (unilateral) in 54% and in neither hemisphere (no-SD) in 13% of mice. Also, insufficient reperfusion was measured in 33% of animals, partial reperfusion in 54%, and complete reperfusion in 13% (28.74±9.89 vs. 55.12±13 vs. 96.88±5.33% CBF, bilateral vs. unilateral vs. no SD). The hemispheric SD incidence and insufficient reperfusion were associated with the ipsilateral lack of the posterior communicating artery (PcomA). MK801 treatment reduced SD incidence (6/6 vs. 10/6 SD/animal; MK801 vs. Control) and the size of the cortical area invaded by SD (67.12±11.3 vs. 85.32±14.32%; MK801 vs. Control). In concert, increased reperfusion magnitude (92.1± 12.37 vs. 54.5±33.2%; MK801 vs. Control) and higher neuroscore values (18.33±1.08 vs. 16.33±0.57; MK801 vs. Control) were measured in the MK801 group.

Insufficient reperfusion is usually attributed to out-of-time intervention or reduced collateral circulation. Our data substantiate the association of SDs with subsequent insufficient reperfusion in ischemic stroke. Based on our findings, pharmacological inhibition of SDs may be beneficial to improve cerebral perfusion after recanalization.

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P3.35 Neurodegeneration in the centrally-projecting Edinger-Westphal nucleus contributes to the non-motor symptoms of Parkinson's disease in the rat

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Besides the classical motor deficit of the Parkinson's disease (PD) both depression and anxiety are important in this neurodegenerative disorder, but their neuropathological background is not exactly understood. It is known that the Edinger-Westphal nucleus is also affected in PD, but its importance in PD associated mood disorders has not been clarified. Our research group has been investigating the morphological changes in the centrally-projecting Edinger-Westphal (EWcp) nucleus with highlighted attention on its urocortin 1 containing (UCN1) cells.

Our main hypothesis was that the loss and/or damage of EWcp/UCN1 neurons directly contributes to anxiety and depression as non-motor symptoms of PD.

To test our hypothesis, we applied the systemic chronic rotenone treatment model as well as selective ablation of the UCN1 neurons. Rotarod, open field and sucrose preference tests were performed to assess motor performance and mood status. Biochemical and histopathological tools - like multiple immunofluorescence labelling combined with RNAscope – were used to disclose the morphological changes. Two-sample Student's t test, Spearman's rank correlation analysis and Mann-Whitney U tests were used for statistics

As result the rotenone treated rats showed not only motor deficit but anxious and depression-like phenotype as well. Significant neuron loss, alpha-synuclein accumulation, astro- and microglial activation were found both in substantia nigra and EWcp. Other mood status-related brainstem nuclei did not show notable histopathological changes. Selective EWcp/UCN1 neuron ablation provoke similar mood status without motor symptoms.

Our findings collectively suggest that neurodegeneration of urocortinergic EWcp contributes to the mood-related non-motor symptoms in toxic models of Parkinson's disease, in the rat.

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P3.36 The role of astrocytic insulin-like growth factor 1 receptor in the development of vascular cognitive impairment

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Introduction:

The population of the western world is rapidly ageing which significantly increased the prevalence of cognitive impalement and dementia. Vascular cognitive impairment is the second most common cause of dementia among the elderly. The available data in the literature showed that the levels of circulating insulin-like growth factor 1 (Igf1) decreases with ageing which can contribute to the development of the neurovascular disfunctions by time. Despite all of the previous investigations the mechanism of Igf1 signalization at the level of the neurovascular unit is not well understood and the exact pathological role of Ifg1 in in the vascular cognitive impairment is ambiguous.

Methods:

Our mouse model carried a astrocyte specific inducible Igf1 receptor (Igf1r) knockout system (GFAP-Cre^{ERT2}/Igf1r$^{f/f}$). The animals either received intraperitoneal injections of tamoxifen dissolved in corn oil or sham for 5 days at 3 months old. After a transcardial PBS perfusion the cerebral cortexes were quickly harvested. RNA was isolated, the quantity and quality of the RNA was checked and cDNA libraries were created using commercially available kits. The sequencing was performed on an Illumina NovaSeq 6000 platform. The sequenced reads were aligned to the mouse genome version GRCm38 using Kallisto. The downstream analysis was performed in the R environment using multiple R and Bioconductor packages.

Results:

The observed gene expression changes between the Igf1r knockout and the control animals were considered statistically significant if the logarithm of the RPKM normalized fold change was at higher than 1.5 and the adjusted p-value calculated with the DESeq2 packages was under 0.05. In the Igf1r knockout group in comparison to the control we successfully identified 75 differentially expressed genes. Furthermore, the principal component analysis and the hierarchical clustering of the gene expression values showed that the two experimental groups were well separated. During the functional annotation of the differentially expressed genes, we found multiple significantly enriched Gene Ontology terms associated with inflammation and extracellular matrix remodeling.

Conclusion:

Gene expression changes associated with the disruption of the astrocytic insulin-like growth factor 1 receptor show potential pathophysiological role of Igf1 signaling at the level of the neurovascular unit in the development of vascular cognitive imperilment.

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P3.37 Attempt to transfer a pharmacological neurovascular uncoupling model from mice to rats

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The objective of the present study was to establish a pharmacologically induced neurovascular uncoupling (NVU) method in rats as a translationally valid animal model of human cognitive decline.

Diminished neurovascular coupling (NVC) has been shown during aging and in various brain disorders. Pharmacologically induced NVU with subsequent neurological and cognitive defects was described in mice (Tarantini, 2015), however, no similar procedure has been reported so far in rats.

In this study, we used 32 male Hannover Wistar rats. NVU was induced by intraperitoneal administration of a pharmacological "cocktail" consisting of N-(methylsulfonyl)-2-(2-propynyloxy)-benzenehexanamide (MSPPOH, a specific inhibitor of epoxyeicosatrienoic acid)-producing epoxidases, 5 mg/kg), L-NG-nitroarginine methyl ester (L-NAME, a nitric oxide synthase inhibitor, 10 mg/kg) and indomethacin (a nonselective inhibitor of cyclooxygenases, 1 mg/kg) and injected twice daily for 8 consecutive days.

Animals were tested in Morris water-maze and fear-conditioning assays on days 5-7 and 4 and 8 of the treatment period, respectively. Blood pressure of the animals was monitored on days -1, 2, 5 and 7. NVC was measured in the barrel cortex in a non-recovery operation. A laser Doppler probe was used to detect changes in cerebral blood-flow (CBF), while the contralateral whisker pad was stimulated. Brain and small intestine tissue samples were collected *post mortem* and processed for prostaglandin E_2 (PGE2) level measurements.

When contrasted with the control group, the animals treated with the "cocktail" showed no impairment in their performance in any of the cognitive tasks. However, we observed an overall higher blood pressure in these rats. They also showed a greater than 50 % decrease in CBF, while their barrel cortex was under stimulation. Intestinal bleeding and ulcers were found in some of the treated animals and ELISA assays of the tissue samples revealed significantly decreased levels of PGE2 both in the brain and small intestine.

Although we could evoke NVU by the applied mixture of pharmacons, it also induced adverse side effects such as hypertension and intestinal alterations. Furthermore, the treatment did not cause cognitive impairment. Thus, further refinements are still required for the development of an applicable model, mostly with regard to finding the appropriate dosages and learning assays.

P3.38 A novel approach to measure trait-dependent behaviour reveals a plasticity-focused genetic profile of anxiety

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According to the WHO, anxiety-related illnesses are the most prevalent psychiatric disorders and may become the utmost disease burden of society by 2030. Developing animal models is our primary approach to discovering neural mechanisms and novel therapies of anxiety, but even these often fail to reach clinical relevance. These models seem to be poorly associated with each other and weakly predict the subjects' future behaviour or physiological states, suggesting the lack of a solid, shared basis of measures. We hypothesize that this is because conventional behaviour readouts are more of a consequence of the animals' fluctuating internal states than their stable traits.

Here, we suggest a novel sampling approach that offers trait anxiety markers by benefiting but rethinking classical measures of rodent models. We created summary measures (SuMs) from semi-randomly repeated sampling of the three most popular anxiety tests and compared those with conventional single measurements (SiMs).

In contrast to SiMs, SuMs i) reveal or enhance correlations between anxiety models and can predict ii) anxiety in a future noxious situation or iii) foretell the level of fear generalization after a life-threatening experience. Given these stable trait-like markers, we conducted a whole-transcriptome RNA-seq analysis on prefrontal cortex samples of 27 Wistar rats. We found that SuMs define the outcome of our analysis quantitatively as well as qualitatively. First, SuMs five-fold enhance the number of anxiety-associated gene discoveries in a false-discovery-rate corrected analysis. Second, according to our functional analysis, while SiMs mostly reveal acute stress-related transcripts (38%), the genetic profile of SuMs is dominated by genes of neuronal plasticity (45%). Finally, our literature analysis showed that by using SuMs, we revealed five and three times more genes that were previously mentioned in preclinical and clinical studies, respectively, in the context of mental illness.

In summary, a significant enhancement in the depth and resolution of sampling enables us to trace out stable traits of the animals. Best to our knowledge, by using the approach above, we were able to identify the highest number of gene targets and present the most detailed transcriptomic profile of natural anxiety in rodents so far.

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P3.39 Effect of aging on the antidepressant role of extracellular zinc and P2X7 deficiency in mice

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Major depression is the most frequent psychiatric disorder in the world. Zinc is the second most important essential trace element in our body, and within the brain, it is found in the highest concentration in mossy fibres innervating CA3 pyramidal cells of the hippocampus.

Zinc is an endogenous modulator of ionotropic receptors, such as P2X purinergic receptors. For P2X7 receptor (P2X7R), zinc strongly inhibits its functioning. Using a high dose of zinc during animal experiments has an antidepressant like effect, while it's deprivation produces the opposite reaction. It is already proven, that the presence of P2X7R also influences the state of depression, so we hypothesized that the antidepressant effect of zinc is mediated via the inhibition of P2X7Rs.

During our experiments C57/BL6 P2X7 wild-type (P2xr7 WT) and knockout (P2xr7 KO), male animals were used, with varying age groups, both young (2 to 3 months old), and elderly (43 to 46 months old). They were subjected to different behavioural tests, such as the Forced Swim Test (FST), the Tail Suspension Test (TST), and the LPS induced anhedonia measured by the sucrose preference test (SPT). At the end of these tests, monoamine concentrations of different brain areas were analysed by HPLC. We also examined the change in the BDNF levels from the hippocampus, and the concentration of serum zinc in the blood.

$ZnCl_2$ (1 mg/kg i.p.) decreased immobility in FST and TST using P2xr7 WT mice. P2X7R deficiency by itself exhibited an antidepressant effect, but the effect of zinc was still detectable in these mice. Zinc deprivation increased immobility in both FST and TST and the effect in the TST is attenuated in P2xr7 deficient mice. In the case of elderly animals, the pro-depressant effect of zinc deprivation is also lost in the absence of P2X7Rs.

In conclusion, experiments in the presented study show that the amount of zinc intake correlates with mood disorders and age changing. Using mice, the antidepressant-like effect of zinc in the TST and FST is independent from P2rx7s, whilst the effect of zinc deprivation is partly mediated by P2rx7.

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P3.40 Age-related changes in the activity of basal forebrain cholinergic neurons during Pavlovian conditioning

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Acetylcholine is a neuromodulator that has a crucial role in mediating cognitive functions like arousal, attention, sensory processing, reinforcement expectation, reward and addiction. The basal forebrain cholinergic neurons' widespread projections to the cortical mantle play a key role in these modulatory processes. Age-related loss of cholinergic function has been observed across species, characterized by the degeneration of dendrites, synapsis, and axons. However, the link between cholinergic activity during learning and the normal or pathological age-related neurodegeneration is still missing.

In order to better understand the age-related changes in the activity of basal forebrain cholinergic neurons, we combined fluorescent in vivo techniques and optogenetic manipulations in headfixed mice during an auditory cued Pavlovian conditioning task. On one hand, we imaged the acetylcholine release in the basolateral nucleus of the amygdala (BLA) using fiber photometry techniques and the recently developed acetylcholine sensor. On the other hand, we optogenetically manipulated learning acquisition by specifically inhibiting the horizontal diagonal band of Broca (HDB) cholinergic neurons during tone presentation (conditioned stimuli, CS).

Our results suggest that cholinergic cells respond with an increase in activity and acetylcholine release after US (both punishment and reward) and reward-predicting CS. Our data suggest that acetylcholine release in the BLA occurs during reward-predicting but not punishment-predicting sensory stimuli. Optogenetic inhibition of HDB cholinergic neurons during the auditory CS presentation impaired the learning process of the animals compared to mice injected with control virus lacking the optogenetic actuator. This suggests that acetylcholine release from basal forebrain neurons is required during the acquisition of the CS-US association during Pavlovian conditioning.

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P3.41 Alzheimer's disease modelling by hiPSC-derived neurons and microglia like cells

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Microglia cells represent 0.15-16% of the total cell population in the brain, being the resident macrophages of the tissue. They play an essential role in neural network maintenance and act as first responders to inflammation. Their major responsibility is removing the damaged neurons, cell debris, and infectious agents. Alzheimer's disease (AD) is an incurable neurodegenerative disorder and the most prevalent cause of dementia. Microglia plays an important role in neurodegenerative disease development and runoff. The cells phagocytic activity has a crucial function in AD prevention by abolishing the accumulation of the toxic β-amyloid peptides. In our study, we established a 3D differentiation protocol to generate induced pluripotent stem cell (iPSC)-derived microglial cells. The weekly harvested cells expressed high levels of microglial markers (Iba1, CD11c, TMEM119) but low CX3CR1 and P2YR12, suggesting an early developmental stage of the cells, in need of a further maturation step. We established a complex 2D and 3D system where we included the iPSC generated microglia-like cells in a culture with neuronal and astroglia cells, in order to mimic better the in vivo brain architecture. The data obtained by this in vitro model demonstrates that the environment supports the microglia-like cells maturation process and it is suitable for further studying the microglia characteristics, and its functionality, besides the cell-cell interactions. The differentiated microglia-like cells respond to activation with LPS, resulting in the production of multiple pro-inflammatory cytokines and an increase in phagocytic activity, which indicates the cells immune direction. Our findings demonstrate that iPSC based in vitro cellular model can recapitulate AD pathology and provide a platform for drug screening and development. In the future, we aim to further investigate the role of microglia on the development and progression of AD.

P3.42 Effects of dorsal root avulsion injury on the spinal ganglia and spinal cord

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High impact vehicle accidents and sport injuries often result in avulsion of the dorsal and ventral roots of the spinal cord. The changes in the ventral horn after ventral root injury are well-known, however, there are only few studies investigating the effect of dorsal root avulsion (DRA). Here we examined the avulsion-induced changes in the affected cell populations of the dorsal root ganglia and spinal cord.

The lumbar 4 and 5 (L4-5) dorsal roots were avulsed in deep ketamine-xylazin anaesthesia. Animals were perfused 3, 8, 21 and 90 days after the surgery (n=4each). The injured and contralateral dorsal root ganglia along with the L4-5 spinal segments were removed. The expression of TrpV1 receptor, CGRP, NF-200kDa was detected immunohistochemical analysis, while lectin histochemistry was used to visualize the GSA B4 isolectin. A self-established semi-quantitative fluorescent analysis was applied to detect the changes of the protein expressions.

Three days following DRA a membrane-bound ring-like TrpV-1 expression could be observed in the large neurons of affected ganglia. Later the ring-like expression could not be detected anymore and the overall expression of TrpV-1 increased until day 90. The CGRP expression showed a maximum in the injured ganglia at day 8 while GSA-B4 staining intensity reached its maximum 21 days after DRA in injured neurons. The expression of TrpV-1 and CGRP also showed minor changes in the contralateral ganglia.

Significant decrease of NF-200 kDa expression could be found in the affected ganglia and in the ipsilateral gracile tract of the spinal cord 21 days after the injury. The decreased density of CGRP-positive fibers was already significant in the affected dorsal horn as early as 3 days after the injury. In contrast, GSA-B4-positive fibers remained well preserved for at least 3 weeks after the injury.

Our data suggest that DRA induces unique expression pattern changes of the investigated markers not only in the injured dorsal root ganglia, but in the contralateral ones, too. Relevant changes appeared in the ipsilateral spinal cord, too.

P3.43 Ectopic neurons in the dentate gyrus in human temporal lobe epilepsy

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Temporal lobe epilepsy is the most frequent form of drug-resistant epilepsies. In the background, hippocampal sclerosis (HS), malformation of cortical development (MCD) and intracranial tumors are the most common causes. During cortical development, neuronal migration can be disrupted, which results in the presence of ectopic cells in various positions. The aim of our study was to examine ectopic neurons in the human dentate gyrus.

Ectopic cells were detected by immunohistochemistry based on the presence of calcium binding proteins: calretinin (CR), parvalbumin (PV) and calbindin (CB) in neurosurgically removed sections of the hippocampal formation of patients with therapy-resistant temporal lobe epilepsy. Patients' groups were formed based on preoperative MRI: 1) HS, 2) HS+MCD, 3) cortical developmental disorder, 3) non-detectable cortical alteration by MRI (MR-negative). The aim of our work was to shed light on the possible association of the presence of ectopic neurons that belong to different neuronal groups based on the expression of calcium-binding proteins, which may suggest a common mechanism in the appearance of ectopic neurons.

Patients in the HS group had the largest density of ectopic CR-immunoreactive (IR) and PV-IR neurons. Significantly larger number of ectopic CR-IR cells were in those patients who had dispersion of granule cells. Density of ectopic PV-IR neurons in the dentate molecular layer was significantly higher in those patients whose right hippocampi were removed. No significant correlation was found between density of ectopic CR- and PV-IR cells numbers, as well as between density of ectopic PV-IR neurons and the morphology of the granule cell layer.

We can conclude that in HS, large number of ectopic neurons are present in the dentate gyrus. The lack of correlation between the appearance and density of different ectopic neuronal groups suggests that the appearance of ectopic PV-, CR-, and CB-IR cells occurs by different mechanisms.

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P3.44 Effect of Urocortin 2 on the maturation of parvalbumin-immunoreactive neurons in organotypic hippocampal slice culture

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Urocortins, members of the corticotropin-releasing factor (CRF) peptide family, are small, 38-40 amino acid-long peptides. Urocortins are expressed in various regions of the central nervous system and exert several effects in the nervous system, including neuroprotection as well as stimulation of learning and memory processes. Previously we have shown that the development and maturation of parvalbumin (PV)-immunoreactive hippocampal neurons is a long-lasting event both *in vivo* and *in vitro* hippocampal organotypic slice cultures. In this study we examined the effect of urocortin 2 on the maturation of PV-immunoreactive neurons in hippocampal slice culture. Hippocampal slice cultures were dissected from postnatal 9 days (P9) old Long-Evans rats. Explants were treated with 10 nM of urocortin 2 on the 5th, 7th, 9th, 11th and 13th days, then slices were fixed on the 15th day. PV immunohistochemistry was performed on cryostate sections and neuronal density was determined using iTEM program. PV-immunoreactive neurons, yet immature, were visible in Ammon's horn at P9, and P10-12 in the dentate gyrus hippocampal slices. After 15 days of culturing, both in control and urocortin-treated slices, mature PV-immunoreactive cells with long, arborized dendrites, as well as with PV-immunoreactive synaptic terminals were observed along the principal cell layers of Ammon's horn and the dentate gyrus. Compared to controls, PV-immunoreactive neuronal density increased dose-dependently and parallel with the number of urocortin treatments. Our results indicate that urocortin stimulates the expression of PV in hippocampal slice culture, however, the elucidation of the exact mechanism needs further investigation.

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P3.45 Long-term effects of regular exercise training on the muscle-brain axis in healthy and hyperlipidemic mice

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In modern society, there is a strong association between cognitive decline and obesity. Although little is known about the exact molecular mechanisms underlying communication between muscle and brain, physical activity is one of the most effective strategies for reducing the incidence of obesity-related neurological disorders. For therapeutic use, exercise should be personalized, therefore we need to better understand the production of muscle-derived factors and their possible effects on the brain in healthy and obese individuals, as well as in both sexes.

To model obesity and regular exercise training, normal-diet fed wild-type (healthy) and high-fat-fed (HFD) ApoB100-overexpressing transgenic (hyperlipidemic) mice performed a medium-intensity treadmill running five times a week for 7 months. We used 12 female and 12 male mice per group. Based on our Open-field test results, females had higher locomotor activity than males, while the Barnes-maze test showed that daily treadmill running significantly improved the spatial learning abilities of females, but this effect could not be observed in males. The activation of glial cells was studied using immunohistochemistry, which showed that the level of the astrocyte marker GFAP decreased upon exercise training and HFD as well. The gene expressional analysis revealed that in the brain *leptin*, the *leptin receptor*, and the lactate receptor *Hcar1* changed sex-dependently in response to HFD or exercise, which factors have previously been shown to affect neuronal functions. Examining the musculus quadriceps femoris (QF), we found that its weight increased significantly in response to exercise training and HFD in males, the latter indicating a possible fat accumulation in the muscle tissue. Moreover, the gene expression of several factors having a role in the regulation of muscle contraction, hypertrophy, metabolism, and energy expenditure showed remarkable differences between the sexes: these alterations suggest that this long-term medium-intensity training is more suitable for females.

In conclusion, our results showed that long-term moderate-intensity exercise training improved the learning abilities of female animals. This sex-dependent memory improvement can be associated with the altered regulation of metabolic and hormonal factors in the brain. The more favorable skeletal muscle response in females can also contribute to the positive effects of exercise on cognitive function.

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P3.46 Role of PACAP in age-related systemic amyloidosis

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Introduction: PACAP (Pituitary Adenylate Cyclase Activating Polypeptide) is a multifunctional neuropeptide, which can be found in many tissues and organs of the body. Its general cytoprotective, anti-inflammatory, and anti-apoptotic effects have been proven; however, there are just a few data available of its role in aging. The aim of our experiment was to compare the tissues of wild-type (WT) and PACAP (KO) deficient mice of different age groups to explore the role endogenous PACAP in aging.

Materials and Methods: Samples were taken from more than 20 organs of two age groups of WT and PACAP KO mice (n=30). We divided the following age groups: 3-12-months, 13-24-months-old animals. 3-µm-thick sections of the samples were stained with hematoxylin-eosin, Congo-red staining and anti-ß-amyloid immunohistochemistry, after we have found signs of amyloid deposits. A semi-quantitative scoring to grade Congo-positive deposits from 0-3 was performed according to pathological criteria. Complete blood count, serum analysis from the animals' blood and cytokine array examinations from kidney samples were performed.

Results: Histopathological analysis showed that in the PACAP KO mice the lesion in all organs seemed more severe and was present at a younger age. Among the WT and PACAP KO mice, significant difference occurred in the esophagus, kidney, liver, spleen, thyroid, and skin. Complete blood count, serum analysis and cytokine array examinations (BLC, IL-1ra, RANTES) have shown differences, due to the lack of PACAP.

Conclusion: Using young and aging PACAP KO mice, here we demonstrated that in mice lacking endogenous PACAP senile amyloidosis appeared accelerated, more generalized, more severe and affected more individuals. In summary, here we describe accelerated systemic senile amyloidosis in PACAP KO mice, which might indicate an early aging phenomenon in this mouse strain. Thus, PACAP KO mice could serve also as a model of accelerated aging, with human relevance.

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P3.47 Generation and characterization of neural progenitor cell lines and neural cultures from monozygotic twins with type 2 diabetes

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Background: Type 2 diabetes (DM2) is characterized by insulin resistance, which may lead to decreased insulin secretion. Long-term consequences of the disease include retinopathy nephropathy and neuropathy However, recently an increased comorbidity between type 2 diabetes and certain psychiatric disorders has been shown. The association is primarily observed in Alzheimer's, Parkinson's disease, autism and OCD. The main objective of our work is to generate induced pluripotent stem cell lines from patients diagnosed with diabetes (monozygotic twins), and then to generate neuronal progenitors and neurons from these IPSC lines. Our model system may help to investigate DM2 related cellular phenotypes and possible neuronal co-morbidities for a better understanding of disease pathomechanisms.

Methods: IPS cells were generated via Sendai virus transduction of the four Yamanaka factors. The pluripotency of the stabilized lines was validated by immunocytochemical staining (Oct4, Nanog), qPCR and spontaneous differentiation, among others. The genetic integrity of the cell lines was checked by karyotyping and their origin by STR analysis. Neuronal progenitor cells were generated from the resulting lines (1-1 line from each twin). The resulting lines were validated by immunocytochemical staining for several neuronal progenitor markers (Sox1, Sox2, Pax6, Nestin). We also confirmed the elevated expression of Nestin and Sox2 compared to IPSC lines by qPCR. From the stabilized NPC lines, we started neuronal differentiation. After the 4th week of the differentiation, we were able to detect spontaneous and drug-induced calcium transients by Ca-imaging. Glutamatergic neuronal cultures were generated based on cell responses to kainate, AP5 and CNQX. In the 6th week immunocytochemical staining was performed, and the obtained neurons showed MAP2 and Synapotophysin positivity.

Results: From the diabetic twin pair we successfully established iPSC lines. We successfully established NPC lines from both twins. The generated cell cultures show neuronal morphology, spontaneous Ca^{2+}- transients, responsivnes to kainate, AP5, CNQX, and glutamate sensitivity.

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Poster session - Topic 4
Cellular neuroscience

P4.01 Morphological and neurochemical characterization of glycinergic neurons in laminae I to IV of the mouse spinal dorsal horn

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It is becoming increasingly evident that glycinergic neurotransmission in the spinal dorsal horn plays an important role in spinal pain processing, especially in the development of mechanical allodynia. However, how glycinergic neurons contribute to the formation of neural circuits underlying spinal pain processing is still waiting for exploration. The lack of this essential knowledge makes the interpretation of the role of glycinergic neurons in spinal pain processing vague. Therefore, we investigated the morphological and neurochemical properties of glycinergic neurons in laminae I-IV of the spinal dorsal horn using a GlyT2::CreERT2-tdTomato transgenic mouse line. By using immunohistochemical and in situ hybridization methods, first in the literature, we provided experimental evidence that there are glycinergic neurons in laminae I-II that do not express GABA and can thus be referred to as glycine-only neurons. We have reconstructed the dendritic morphologies of tdTomato labeled glycinergic neurons from 100 µm thick sagittal sections. According to dendritic morphologies and the shape of cell bodies, we divided the labeled glycinergic neurons into three morphological categories in laminae I-II and classified them into six groups in laminae III-IV. Investigating the co-localization of tdTomato labeling with neuronal markers, which were identified earlier as markers of inhibitory neurons in the spinal dorsal horn, we demonstrated that proportions of the labelled glycinergic neurons co-express neuronal nitric oxide synthase, parvalbumin, the receptor tyrosine kinase RET and the retinoic acid-related orphan nuclear receptor β (RORβ), but they do not express galanin, calretinin, and neuropeptide Y. The present findings may advance our knowledge about glycinergic neurons in the spinal dorsal horn, and thus may contribute to a better understanding of spinal pain processing.

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P4.02 Nanoscale distibution of Munc13-1 and Cav2.1 in identified hippocampal synapses.

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Synaptic strength and plasticity show great heterogeneity in neuronal circuits. We have previously demonstrated that different nanoscale spatial arrangements of voltage-gated calcium channels (VGCC) and synaptic vesicles can underlie some functional diversity at different types of cerebellar synapses. However, it is unknown whether similar structural organization explain variable strengths at synapses established by a homogeneous population of presynaptic neurons. Axons of hippocampal CA1 pyramidal cells (PCs) excite postsynaptic cells with different presynaptic release probability and short-term plasticity depending on the identity of the target cell. They form strong synapses on Kv3.1b expressing fast spiking GABAergic interneurons (INs) and weak synapses on mGluR1α positive INs. To test whether different nanoscale topographies of VGCCs and synaptic release sites underlie differences in the synaptic strength at these hippocampal synapses, we studied the subsynaptic distribution of Cav2.1 subunit of VGCCs and Munc13-1, a molecular marker of release sites, at excitatory synapses on Kv3.1b and mGluR1α positive INs in CA1 area using SDS-digested freeze fracture replica labeling. Gold particles labeling Cav2.1 and Munc13-1 were both enriched in the active zone (AZ) of hippocampal excitatory synapses. Although Munc13-1 formed small nanoclusters, the sub-AZ distribution and density of Munc13-1 gold particles and Munc13-1 clusters were not different in the two AZs. The density of Cav2.1 was 20% higher in CA1 PC – mGluR1α synapses, but in both synapses Cav2.1 gold particles distributed in a non-random, non-clustered manner. Bivariate Ripley analysis revealed a repellent interaction between Cav2.1 gold particles and Munc13-1 clusters. The distances between the Munc13-1 clusters and the nearest Cav2.1 gold particles were comparable in both synapse populations. Distribution of Cav2.1 around Munc13-1 clusters was consistent with the exclusion zone model, with similar exclusion zone radii in both synapses. Our results reveal that the different synaptic strength of these hippocampal excitatory synapses cannot be explained by distinct nanotopography or different coupling distances between VGCCs and the release sites.

P4.03 Target cell type-dependent enrichment of Munc13-2 in presynaptic active zones of hippocampal pyramidal cells

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Postsynaptic target cell type-dependent differences in synaptic release probability (P_v) and short-term plasticity of excitatory synapses have profound impacts on cortical network dynamics. A candidate mechanism to bestow such differences is the selective distribution of proteins that regulate synaptic vesicle release in presynaptic active zones (AZs). Two molecules – metabotropic glutamate receptor (mGluR) 7 and 8 have been identified so far to be selectively enriched in hippocampal pyramidal cell (PC) AZs that innervate somatostatin and mGluR1α expressing interneurons (mGluR1α+ INs). These INs receive small, facilitating unitary EPSCs (uEPSCs) from PCs suggesting low P_v from their glutamatergic input synapses. The mGluR1α+ INs express Elfn1 – a postsynaptic density protein – that trans-synaptically recruits mGluR7 into the presynaptic AZ of PC axons and renders the receptor constitutively active. This activity has been shown to contribute to the characteristically low Pv of this synapse. Here we show that Elfn1 also has a role in the selective recruitment of another presynaptic protein – Munc13-2 – to PC AZs. In its absence the amount of both mGluR7 and Munc13-2 are dramatically reduced in the PC – mGluR1α+ IN synapses. Munc13 proteins are essential regulators of synaptic vesicle docking and priming. Hippocampal glutamatergic neurons express another isoform, Munc13-1 as well that is ubiquitously present at every presynaptic AZ. In Elfn1 knock-out mice, uEPSCs in mGluR1α+ INs have 3-fold larger amplitudes with less pronounced short-term facilitation, which might be the consequence of the loss of mGluR7 or Munc13-2 or both. Conditional genetic deletion of Munc13-2 from CA1 PCs results in the loss of Munc13-2, but mGluR7 and Elfn1 expression and distribution remain unaltered. Removal of the Munc13-2 has no effect on the amplitude of uEPSCs and leaves the characteristic short-term facilitation intact in PC – mGluR1α+ IN connections. Our results demonstrate that Munc13-2 does not contribute to the initial low P_v at PC – mGluR1α+ IN synapses and Munc13-1 alone is capable of imposing high and low P_v at PC output synapses.

P4.04 P2X7Rs Modulate Excitatory Neurotransmission in Mouse Dentate Gyrus

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P2X7 receptors (P2X7Rs) the ligand gated ion channels, regulate a diverse array of normal and pathological brain function, including learning and memory formation, mood and behavior. However, the cellular mechanisms underlying these functions are far from fully understood yet. Here, we investigated the involvement of P2X7Rs in excitatory neurotransmission in dentate gyrus granule cells (DG-GC) and the potential role of P2X7Rs in animal model of schizophrenia. To address these questions, we utilized in vitro patch clamping to record AMPA-mediated and NMDA-mediated sEPSC and mEPSC in both wild-type and P2rx7-/- mice. We found that genetic ablation and blockage of P2X7Rs decreased AMPA-mediated and NMDA-mediated EPSC frequency but amplitude kept unchanged. Paired pulse ratio experiments further indicated that it was Perforant-Granule cell pathway (PP-GC) (especially LPP-GC) but not Mossy cell-Granule cell pathway (MC-GC) participated in P2X7-regulated neurotransmission process. Meanwhile, the possible involvement of postsynaptic site was ruled out by analysing dendritic morphology and spine number of DG-GC. Importantly, two photon microscopy calcium imaging showed that activation of P2X7Rs by agonist BzATP could partially increase axonal bouton-related calcium influx in LPP-GC pathway which was infected by injecting pAAV1-hsynapsin1-axon-GCaMP6s virus in to lateral entorhinal cortex (LEC). Lastly, in postnatal phencyclidine (PCP)-induced schizophrenia model, we found that the deficiency of P2X7Rs significantly reversed PCP-induced locomotor hyperactivity and prepulse inhibition. To summarize, P2X7Rs participated in excitatory neurotransmission via presynaptic mechanism, partially by regulating of axonal bouton calcium influx. Deletion or blockage of P2X7Rs might be a potential therapeutic strategy to alleviate postnatal PCP-induced schizophrenic-like symptoms.

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P4.05 Investigation of astroglial heterogeneity in the human cortex and caudate nucleus

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Astroglia and neurons populate the human cerebral gray matter in a 1:1 ratio. While there is much information available on the diversity of neuronal populations, relatively little is known about that of the astroglia. We aimed to quantitatively investigate different astroglial populations present in the human cortex and caudate nucleus with morphometric and topographic analyses. We focused on the dorsolateral prefrontal cortex whose involvement in neuropsychiatric disorders is already demonstrated. Human brain tissue was provided by the Netherlands Brain Bank and Oxford Brain Bank.

Our results showed that GFAP+ and ALDH1L1+ astroglial populations were distributed in a partially overlapping pattern in the dorsolateral prefrontal cortex. The GFAP+ population was preferentially located in L1 and L6, whereas the ALDH1L1+ population was predominantly found in L2-L5. Furthermore, two times more ALDH1L1+ than GFAP+ astroglia was found in both the cerebral cortex and caudate nucleus.

Our study indicates diverse astroglial populations distributed in the human cerebral cortex and caudate nucleus in a complementary fashion. Furthermore, our results suggest that the use of GFAP in routine pathological investigations only informs about approximately one-third of the cortical astroglia. Regional distribution of diverse astroglial populations was mapped quantitatively in the human grey matter which will allow future investigations of potential astroglial alterations in conditions such as autism spectrum disorder and schizophrenia.

P4.06 Optical recording of unitary synaptic connections using Voltron

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A longstanding goal in neuroscience is to understand synaptic connectivity that underlies function-specific neuronal activities. Patch-clamp recording enables precise resolution to map the synaptic properties between individual neurons. However, the number of connections that can be tested is limited. Our goal is to replace patch-clamp recordings of individual postsynaptic neurons with optical imaging that enables simultaneous monitoring of several potential postsynaptic cells. To measure small voltage fluctuations at high spatio-temporal resolution we sparsely expressed a new genetically encoded voltage indicator (GEVI), the Voltron in hippocampal neurons using rAAV vectors. 4-7 weeks after the injections we prepared acute slices that were incubated with a fluorescent dye (Janelia Fluor 549) that bounds to the expressed Voltron protein and enables simultaneous monitoring of membrane voltage in many neurons using epifluorescent illumination and a fast CMOS camera at high speed (0.67-1 kHz) and large field of view (375x235 µm).

First, we tested the applicability of Voltron imaging in acute slices. For this, we determined the scattering of fluorescent signals in simultaneously recorded neurons, and tested various illumination equipments and developed analysis algorithms. Our results showed that the Voltron is capable to report small membrane potential changes with high fidelity. We also observed a quasi-linear correlation between voltage changes and fluorescent emission changes, within experiments. Next, we tested whether unitary synaptic connections can be resolved. For this, we stimulated individual dentate gyrus granule cells using patch-clamp recording and imaged putative postsynaptic target neurons in the hilus. The results showed that Voltron signal can resolve individual postsynaptic responses in individual cells and it also captures the cell-to-cell variability of the responses. Subthreshold responses can be readily distinguished from postsynaptic firing due to the sufficient temporal resolution of the imaging system. Furthermore, the persistence of Voltron signal after conventional fixation allowed us to map the identity of imaged neurons (both responding and silent) using posthoc immunolabelling.

Together, these results indicate that Voltron-imaging is a powerful tool for detecting synaptic responses in a large pool of neurons and it is suitable for detailed and precise mapping of synaptic connectivity.

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P4.07 Relations between the kynurenergic and GABAergic systems in the mouse brain - A neuroanatomical study

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Kynurenic acid (KYNA) plays an important role in neuroprotection and neuromodulation due to its broad-spectrum receptor modulatory effects. In many neurodegenerative and psychiatric disorders, abnormal levels of KYNA have been observed. KYNA production is catalyzed by enzymes called kynurenine aminotransferases (KATs). So far, four KAT isoforms (KAT-1,-2,-3,-4) have been identified from which KAT-2 is described as the major biosynthetic enzyme of KYNA both in the murine and the human brain. The treatment of diseases affected by abnormal KYNA levels requires the manipulation of the kynurenergic system. Since KAT-2 has the ability to regulate KYNA levels, it could be advantageous to study the tissue- and cell-type-specific localization of the enzyme. Previous studies found that in the rat brain KAT-2 is localized not in neurons but in astrocytes. In the mouse brain, however, KAT-2 expression was observed in neurons too.

This study aimed to further investigate the cell-type specific localization of KAT-2 in the mouse brain using fluorescent immunohistochemistry. We observed a broad KAT-2 distribution in the whole mouse brain. The results show that the most prominent KAT-2^+ cells in the mouse brain (along with astrocytes) are GABAergic neurons. Most of the KAT-2^+ cells showed complete overlap with many GABAergic neuronal markers while using double immunolabeling. The present study provides data about the GABAergic neuronal localization of KAT-2 in the mouse brain. These anatomical results are supporting future pharmacological and kynurenergic manipulation studies in mice, and highlight the fact that there might be relations between the kynurenergic and GABAergic systems.

P4.08 Inflammasome-dependent communication between cerebral endothelial cells and pericytes

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As integral elements of the neurovascular unit, cerebral endothelial cells and pericytes actively contribute to the neuroinflammation via a number of molecular mechanisms including upregulation of cell adhesion molecules and secretion of pro-inflammatory cytokines. As previously shown, both vascular cell types can activate inflammasomes, notably cerebral endothelial cells (CECs) through the canonical pathway, whereas pericytes only through the noncanonical pathway. Using complex *in vitro* models, we demonstrate that the noncanonical inflammasome pathway can also be induced in CECs, leading to a more robust secretion of active interleukin-1β (IL-1β) than what is observed in response to activation of the canonical pathway. Along with it, a more pronounced disruption of tight junctions (TJs) takes place. Our findings also show that CECs respond to inflammatory stimuli coming from both the apical/blood and the basolateral/brain directions. As a result, CECs can detect factors secreted by pericytes in which the noncanonical inflammasome pathway is activated and respond with inflammatory activation and impairment of the barrier properties. In addition, upon sensing inflammatory signals, CECs release inflammatory factors toward both the blood and the brain sides. Consequently, CECs activate pericytes by upregulating their expression of NLRP3 (NOD-, LRR-, and pyrin domain-containing protein 3), an inflammasome-forming pattern recognition receptor. In conclusion, cerebral pericytes and endothelial cells mutually activate each other in inflammation.

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P4.09 Ill-priming of docked vesicles contributes to low release probability at hippocampal glutamatergic synapses

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One of the most intriguing examples of the functional diversity of synapses is the postsynaptic target cell type-dependent differences in release probability (Pv) in cortical networks. Here we show – with whole-cell patch-clamp paired recordings – that adult mouse hippocampal CA1 pyramidal cell (PC) to fast spiking interneuron (FSIN) connections have 15-fold larger unitary EPSC (uEPSC) amplitudes than those made by the same PCs on oriens lacunosum-moleculare (O-LM) interneurons. Two-photon imaging shows that action potential-evoked [Ca^{2+}] transient peak amplitudes are only 40% higher in PC boutons targeting parvalbumin immunopositive (FSIN) dendrites than in those synapsing on mGluR1α immunopositive (O-LM) ones. This difference cannot account for the 15-fold difference in the uEPSC amplitudes, because when the [Ca^{2+}] at PC – O-LM boutons was increased with the K^+ channel blocker 4-AP (5 µM) to the level measured in the PC – FSIN boutons, it caused only a 2.7-fold increase in the uEPSC amplitudes, leaving an additional ~5-fold difference between the 2 connections unexplainable. However, the application of a phorbol ester analog (PDBU) that activates the synaptic vesicles docking/priming molecule Munc13 increased the uEPSC amplitudes by 4.5-fold at PC – O-LM and only 77% at PC – FSIN synapses, suggesting that vesicle docking/priming is incomplete at PC – O-LM synapses. Serial section electron microscopy (EM) and EM tomography show that there is no difference in the density of docked vesicles at these synapses ruling out low release sites occupancy at the PC – O-LM synapses and suggesting that ill-primed vesicles limit the output of these synapses.

P4.10 Prefrontal calretinin interneurons are impaired in schizophrenia

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Excitatory/inhibitory imbalance plays a major role in neuropsychiatric disorders such as schizophrenia (SCH). In the last decades several independent workgroups investigated the post mortem prefrontal cortex of schizophrenic patients and observed both decreased and unchanged densities of parvalbumin and calbindin neurons, however, calretinin-expressing cell density was largely found unchanged. To this day only one manuscript reported decreased density of calretinin (CR+) neurons in the Brodmann area 9 in SCH. It is highly important to take into account the individual variance and the challenging heterogeneity of schizophrenia; the inconsistent results might foreshadow the existence of a cellular/ molecular schizophrenia spectrum. We propose the following question: could we broaden the horizons of schizophrenia research by not necessarily thinking of heterogeneity as a limitation, but as a potential opportunity to gain more information?

The current study is part of a project aiming to give a comprehensive view of all major interneuron types of the dorsolateral prefrontal cortex (DLPFC) and their possible impairment is SCH.

Formalin-fixed tissue from 15 cases with SCH and 15 age- and gender matched control (CTR) cases was obtained from the Netherlands, Newcastle, Kings' College and Oxford Brain Banks and was immunohistochemically stained. We quantified CR+ and PV+ neurons in cortical columns after delineating cortical layers based on Nissl staining. Linear mixed models were applied for statistical evaluation of layerwise density values.

CR+ interneuron density was significantly lower in the DLPFC of the SCH group. Interestingly the samples could be subdivided into a 'control-like' group with no density reduction and an 'affected' group with considerably lower density values, not confounded by age, gender or port mortem interval. This pattern can also be viewed as a spectrum.

As a recent transcriptomic work also found potential subgroups in the SCH cohort, it is most important to further study within-group differences, which could aid the better understanding of schizophrenia. Cell types identified as impaired can be subjects of targeted experiments such as iPSC assays and animal models, which are crucial to gain insight into the network-level disturbance present in schizophrenia.

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P4.11 A new pathway from basal forebrain somatostatin neurons to cortical areas

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The mammalian basal forebrain (BF) modulates cortical activation and the sleep/wake cycles, and it has important roles in motivation, learning and memory. BF cholinergic, glutamatergic and GABAergic parvalbumin neurons target different cortical regions and play a crucial role in cortical rhythmic activity. Here we discovered a previously unrecognised, long-range GABAergic, somatostatin-expressing cell population in the BF, which innervates interneurons in the dentate gyrus of the hippocampus and in the retrosplenial cortex (RSC). The RSC is thought to play an important role in spatial navigation, contextual memory encoding and retrieval in tandem with the hippocampus, providing similar coding, thus making these systems more robust. Using viral tract tracing in SOM-Cre/vGAT-Flp double transgenic mice, immunohistochemistry and confocal laser scanning microscopy, we found that BF SOM cells establish multiple putative inhibitory synaptic contacts on the somata and dendrites of parvalbumin-, somatostatin- and calretinin-expressing GABAergic interneurons in the RSC. Our results suggest that BF SOM cells may disinhibit selected subpopulations of RSC principal neurons, which may have a crucial role in modulating the involvement of these principal cells in RSC related coding.

P4.12 Spreading depolarization-induced astrocytic Ca^{2+} waves and subsequent non-synchronized Ca^{2+} oscillations coincide with arteriole diameter changes in the mouse cerebral cortex

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Spreading depolarization (SD) is a principle of secondary cell death in acute brain injury, that induces marked Ca^{2+} waves in neurons and astrocytes. SD is coupled with profound changes in the local blood flow, which may further compromise cerebral perfusion after ischemia. Since astrocytes mediate neurovascular responses, it is yet to be explored how the passage of SD alters glial activity that may be implicated in the regulation of the SD-coupled CBF response. In the present study we describe enhanced post-SD astrocyte Ca^{2+} oscillations and investigate the concurrent vascular diameter changes in the mouse cortex.

Astrocytes were selectively labeled by the astrocyte marker SR101, and their intracellular Ca^{2+} events were indicated by Fluo-4 AM fluorescence intensity changes. Concomitant, SD-related arteriolar diameter alterations were visualized simultaneously using multiphoton microscopy in anesthetized mice.

Simultaneous with SD evolution, astrocytes displayed a Ca^{2+} wave engaging all astroglia identified in the field of view. High frequency, unsynchronized Ca^{2+} oscillations evolved minutes behind the astrocytic Ca^{2+} wave front of SD and engaged fewer astrocytes at a given time point. The fluorescence intensity changes indicative of Ca^{2+} oscillations were of smaller amplitude in comparison to Ca^{2+} waves. Furthermore, our data confirmed a coincidence b arteriolar constriction and Ca^{2+} waves, while post-SD Ca^{2+} oscillations occurred during the peak of the SD-related vasodilation.

The present study is the first to describe the high frequency post-SD astrocyte Ca^{2+} oscillations *in vivo*. Our results provide novel insight into the spatio-temporal correlation between glial reactivity and cerebral arteriolar diameter changes upon and behind the SD wavefront and provide a novel model for further investigations on astrocyte oscillations.

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P4.13 Examination of the role of nesfatin-1 in the supraoptic nucleus of rats

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Nesfatin-1 is an anorexigenic neuropeptide that also inhibits water intake. It is coexpressed with oxytocin (OT) and vasopressin (AVP) in the osmosensitive magnocellular cells of the hypothalamic paraventricular and supraoptic nucleus (SON). In order to investigate the role of nesfatin-1 in the magnocellular cells, we silenced the expression of nesfatin-1 in the SON by *AAV*-delivered *shRNA in rats*. A scrambled shRNA (scr)-AAV was used as control, delivered into the SON of a second group of rat. After three weeks, the rats were divided into two additional groups, and received 2% NaCl solution, or tap water to drink for a week. The bodyweight as well as the daily water and food intakes of rats were measured. After 7 days, the animals were perfusion-fixed. Serial coronal sections containing the SON were immunostained for nesfatin-1, OT and AVP, and the cell nuclei were labelled with DAPI. Pictures were taken by a confocal microscope and analyzed using the ImageJ software. Our findings show that the nesfatin-1-shRNA fully inhibited the expression of nesfatin-1 in the infected neurons. Salt loaded animals lost weight during the experiment, which effect was amplified by the lack of nesfatin-1 in the SON. Salt loaded animals consumed higher amount of fluid and their food intake was reduced compared to the tap water group. In the SON, both salt loading and nesfatin-1 deficiency increased the density of AVP immunoreactive fibers, and the lack of nesfatin-1 potentiated this effect. Salt loading reduced the intensity of OT immunostaining, but the absence of nesfatin-1, reduced this effect. Additionally, salt loading increased the size of the SON and the absence of nesfatin-1 in the nucleus enhanced this effect. Based on our results, we suggest that nesfatin-1 plays a role in the development of dehydration-induced anorexia.

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P4.14 Dendritic synaptome of GABAergic interneurons in the mouse visual cortex

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In spite of the vast amount of literature regarding the light microscopic morphology of GABAergic interneurons (INs) their synaptic inputs impinging on the dendrites are almost completely missing.

Our central aim was to fill this cavity by providing a morpho-functional database called, dendritic synaptome, for dendrites of calcium-binding protein containing (parvalbumin (PV), calretinin (CR), calbindin-D_{28K} (CB)) as well as somatostatin (SOM)- and vasoactive intestinal polypeptide (VIP)-immunopositive GABAergic INs.

High-resolution serial-section electron microscopy remains the only unbiased and adequate method for resolving the different structural components of synapses and identifying the presynaptic partners. In order to preserve high fidelity of ultrastructure and reveal the subtype of GABAergic cortical neurons we developed an immunohistochemistry-correlated electron microscopy method using the “mirror technique”.

Using the “mirror technique” CB+, CR+, PV+, SOM+ and VIP+ IN cell bodies were identified in 60-80 µm thick vibratome sections. Next, the same neurons were re-sectioned (50 nm) for serial section transmission electron microscopy analysis within the entire thickness of the vibratome section. So far, seven dendrites belonging to CB, CR, PV and VIP-containing INs and their presynaptic boutons were traced (photographed) and reconstructed in 3D. The following synaptic parameters were determined for the dendrites: spatial distribution of synapses; surface area and volume of presynaptic boutons and area of the active zones.

Significant differences in the morphometric parameters of the presynaptic boutons were detected between CB+ and CR+ IN dendrites. On the contrary, PV+ IN dendrites were less different from those of the other IN types on the basis of the same metrics. Regarding the spatial distribution of synapses along dendrites, only the VIP+ dendrite showed clustering. For the latter subtype, more than half of the synapses were inhibitory (symmetric) in contrast to 10-30% of the synapses which encountered for CB+, CR+ and PV+ INs.

Our findings provide essential structural information to establish realistic neuronal network models for studying the function of neocortical microcircuits.

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P4.15 Organization of extracellular matrix in the hindbrain of mouse embryo

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Normal brain development requires communication between developing neurons and their cellular environment. During the last decades, remarkable efforts were made on establishment the roles of different extracellular matrix molecules (ECM) in regulation of different aspects of neural development. These studies showed that ECM forms a physical barrier which restricts movements of the cells, it binds and sequesters growth factors and concentrate them close to their receptors on the surface of developing neurons and glial cells, and directly activates intracellular signalling pathways through interaction with cell surface receptors.

The aim of our study was to give a qualitative and quantitative analysis of expression of ECM components in the hindbrain during embryonic development of the mouse. We investigated the distribution of hyaluronic acid and lecticans including aggrecan, neurocan and versican (V0,V1), as well as tenascin-R and HAPLN1 link protein. We gained embryos from the uterus at certain developmental stages. After paraffin embedding and sectioning, the ECM molecules were detected with the help of histochemical and immunohistochemical methods. The staining intensities of different extracellular molecules were measured with the help of ImageJ software.

Our results showed that hyaluronan and neurocan molecules first appeared in the interstitium at E13.5 and they showed intensive labelling until the time of birth. The amount of hyaluronan did not change significantly between different embryonic stages whereas the level of neurocan was higher than the hyaluronan at E13.5 but appeared significantly lower levels by P0. The versican and tenascin-R molecules were detected in lower concentration in the interstitum during the whole embryonic period. At the time of birth the hyaluronan was detected in highest intensity, the neurocan and tenascin appeared to be about the same level which was significantly lower comparing to HA whereas versican reaches the lowest concentration.

These findings suggest the importance of hyaluronan and neurocan in embryonic development of the mouse hindbrain. Due to its polyanionic character the HA maintains the physical properties of the extracellular environment and also acts via its cell surface receptors to regulate several cellular functions. It was proved that neurocan controls migration of developing neurons and aggregation of neurons into nuclei during embryonic development.

P4.16 Sema3 is essential in the differentiation of neuroprogenitor cells and the regulation of cell cycle in chicken embryo's spinal cord

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Semaphorins are secreted or membrane-bound proteins, which play an essential role in the differentiation of neurons and regulation of axonal growth. The semaphorin signalling pathway also plays a role in the guidance of spinal cord neurons, mainly responsible for the organization of the ascending and descending tracts formed by crossing axons within the white matter. Recently it has been found that semaphorins are also involved in the regulation of cell cycle during early neuronal development. Here we describe a detailed expression pattern of Sema3s and their receptors in the immature spinal cord of chicken embryos for finding the candidate molecules important for progenitor cell differentiation and migration of postmitotic neurons into the spinal dorsal horn. Immunohistochemical study showed that both neuropilin 2 was expressed together with plexin A2 and preferentially with Sema 3F and 3A while neuropilin 1 pattern was similar to that of Sema 3C and Sema 3B. We used a plasmid-based dominant negative (DN) technology by in ovo electroporation into the spinal cord for examination of the effect of inhibited neuropilin 1 and 2 receptors' signal transduction. The growth of the basal processes of the neural-progenitor cells and then the leading processes of the postmitotic neurons were impaired in cells expressing DN-neuropilin 2 while the processes of the DN-neuropilin 1 expressing cells were de-fasciculated. The transfected cells are typically stuck in the ventricular zone. After BrdU staining, we observed that DN-neuropilin 2 expressing cells are postmitotic, while in the case of DN-neuropilin 1 we found double labelled cells consequently, these treated cells don't leave the mitotic cycle. Our investigations have shown that the semaphorin signalling pathway has an essential role in the cell cycle of neural-progenitor cells in the spinal dorsal horn just as during interkinetic and radial migration.

P4.17 Unique properties of dendritic Ca^{2+} spikes in hippocampal CA3 pyramidal neurons

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The hippocampus has an essential role in spatial representations and contextual memory. Its subfields are thought to form a sequential information processing network with special input systems and different roles. The CA3 region, a circuit formed by recurrently connected pyramidal cells (CA3PCs), is often proposed to be involved in pattern separation and completion; however, the cellular and subcellular mechanisms underlying these functions are not well understood.

Dendrites of PCs can produce regenerative responses that fundamentally influence input-output transformation. As a prominent dendritic spike type, Ca^{2+} spikes may have an important role in dendritic integration. Ca^{2+} spikes are generally considered to produce a slow afterdepolarization (ADP) following action potentials (APs) that evokes complex spike bursts (CSB) at the soma. CA3PCs have high propensity to produce CSBs, but there is pronounced heterogeneity of this property among individual neurons. Putative dendritic Ca^{2+} spikes have been observed in CA3PCs, but remained poorly characterized, and their role in CSB generation remained incompletely understood.

We combined somatic and dendritic patch-clamp recordings with two-photon microscopy in acute adult rat brain slices to elucidate the generation and biophysical properties of dendritic Ca^{2+} spikes and understand the relationship of these properties with the unusually high propensity and variability of CSBs in CA3PCs. We found a large cell-to-cell variability in the kinetic properties of dendritic Ca^{2+} spikes, which strongly depended on the proximo-distal position of the cells. Using direct dendritic recordings, we discovered distinct types of dendritic Ca^{2+} spikes: 1) ADP-type global Ca^{2+} spikes that promote bursts, and 2) a novel fast Ca^{2+} spike form that is initiated without backpropagating APs, is compartmentalized to the activated dendritic subtree and promotes strictly single APs at the soma. Our results point to unique properties of Ca^{2+} spikes in CA3PCs compared to other PC types and suggest a potentially important role for these spikes in input-output transformation of CA3PCs during navigation and associative memory functions of the CA3 network.

P4.18 Comparison of popular fluorescent actin markers to measure actin dynamics in dendritic spines

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Excitatory synapses in the central nervous system are mainly localized on dendritic spines, highlighting the importance of these protrusions in learning and memory. Changes in synaptic activity induces rapid remodelling in the actin cytoskeleton leading to morphological changes of the dendritic spines. FRAP (fluorescent recovery after photobleaching) is widely used to study the dynamics of actin cytoskeleton, based on photobleaching fluorescently labelled actin-bound signals within a small area, followed by measuring the return of fluorescent signal intensity within the bleached regions. This technique provides tools to calculate the kinetics of the actin remodelling and determine the proportion of stable and rapidly rearranging microfilaments within certain cellular areas.

Within the last decades, numerous actin labelling fluorescent markers have been developed. To select the most suitable for FRAP experiments, murine embryonic hippocampal cell cultures were transfected with three different actin labelling fluorescent markers. The EGFP-Actin fusion protein is covalently labelled with EGFP and incorporates into the F-actin network. Actin-Chromobody-GFP is a monomeric camelid antibody, while LifeAct-GFP has an actin binding domain which can bind to filamentous and monomeric actin. Neurons expressing the freely diffusible EGFP protein only were used as controls.

Actin-FRAP experiments were performed in dendritic protrusions under control conditions and after F-actin stabilization by Jasplakinolide. In addition, we compared how the different labelling methods affected the motility of dendritic protrusions and general neuronal morphology. There was no significant difference between the motility of the filopodia expressing Actin-Chromobody-GFP, LifeAct GFP and EGFP, while EGFP-actin expression reduced motility. Sholl analysis of dendritic arborisation revealed that cells expressing Actin-Chromobody-GFP have shorter dendrites and lower number of branches. Fluorescence recovery of the covalently labelled EGFP-Actin was completely blocked by F-actin stabilization. On the other hand, both indirect actin labelling constructs recovered almost completely after bleaching, indicating that free, diffusible fusion proteins mask the detection of actual actin dynamics. Thus, only the covalently labelled EGFP-actin method is suitable for FRAP experiments.

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P4.19 Investigating the function of septin-3 in cortical neurons

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Alzheimer's disease can be characterized with the enhancement of synaptic decline and synapse loss. Synapses are eliminated by the microglia in complement mediated synaptic pruning, where the complement protein C1q binds to dysfunctional synapses. Using proteomics techniques, we found an elevation of the apoptotic cleaved caspase-3 and an increase in the levels of mitochondrial stress factors in the C1q-tagged synapses of wild-type and APP/PS1 mice. Interestingly, we also found that several cytoskeletal-like septin proteins of the P-loop GTPases were enriched. Septins can form homo- and hetero-oligomeric filaments and can associate to many cellular compartment membranes. To validate the positive correlation between C1q deposition and septin enrichment, we investigated septins-3 and 5 with co-localization analysis based on signal intensity separation. To unravel the cause of septin enrichment in synapses we investigated septin-3 further using structural bioinformatics. Based on our results, new possible interactions were tested using human recombinant proteins by microscale thermophoresis, fluorescence shift and fluorescence polarization. To inspect the localization of septin-3 and binding partners, we used immunochemistry of primary neuronal cells and cortical slices from mice with confocal- and electron microscopy. Here, we introduce new interactions and suggest possible roles of septin-3, regarding synaptic decline.

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P4.20 Fast astrocytic calcium signals are revealed by high-frequency imaging during epileptiform activity

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Astrocytes are considered to be slow followers of neuronal activity. However – using high-speed two-photon microscopy on astrocyte soma – we previously observed that astrocytes display oscillatory activity during slow-wave sleep in vivo at frequencies that match the pattern of neuronal rhythms. Moreover, we demonstrated that this astrocytic activity is a prerequisite for the emergence of neuronal slow wave activity. Thus, we argue here that the widely unchallenged view of slow astrocytic signalling is the result of the commonly applied low time-resolution fluorescent calcium imaging technique; and the spectral width of Ca2+ imaging of network-embedded astrocytes is remarkably broad.

We evaluated fast speed calcium-imaging experiments from astrocytes of acute hippocampal rat brain slices and simultaneously recorded neurons electrophysiologically. Astrocytes were bulk loaded with the astrocyte-specific morphological tracer SR101 and the calcium dye Oregon Green 488 BAPTA-1 AM. During low magnesium-induced epileptiform activity we observed oscillatory activity in SR101+ stratum radiatum astrocytes in the frequency of 1-10 Hz on the soma that were not present at low acquisition rate (1 Hz). However, we observed frequency components in electrophysiological recordings that matched calcium oscillation frequencies - implying a causal relationship.

We conclude that at low acquisition rate, glial responses are critically undersampled, which basically results in undersampling in the astroglial fast response frequency range. Offline downsampling of calcium traces recorded at high acquisition rate also reinforced the view that lowering acquisition rate not only reduces the information content of the detected astrocytic signals, but introduces artificial lower frequency components. Overall, the observed mechanism endows astrocytes with fast ‘parallel computing’ capabilities in the network.

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P4.21 Cell-type specific features of serotonergic modulation in the anterior piriform cortex

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Originating from the brainstem raphe nuclei, serotonin is an important neuromodulator involved in a variety of physiological and pathological functions including sensory coding and depression. Specific optogenetic stimulation of serotonergic neurons results in the divisive suppression of spontaneous, but not sensory evoked activity in the majority of neurons in the primary olfactory cortex and an increase in firing in a minority of neurons. To reveal the mechanisms involved in this dual serotonergic control of cortical activity we used a combination of in vitro electrophysiological recordings from identified neurons in the primary olfactory cortex, optogenetics and pharmacology and found that serotonin suppressed the activity of principal neurons, but excited local interneurons. The results have important implications in sensory information processing and other functions of the olfactory cortex and related brain areas.

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P4.22 Cortical and subcortical neuronal dynamics during absence seizures in awake animals

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Absence seizures (ASs) are sudden, transient lapses of consciousness associated with lack of voluntary movements and generalized 2.5–4 Hz spike-wave discharges (SWDs) in the EEG. In addition to the primary somatosensory (S1) cortico-thalamo-cortical system, where these pathological oscillations are generated, multiple neuronal circuits have been involved in their modulation and associated comorbidities. Most previous studies exploring the ictal neuronal dynamics in various brain regions have been performed under anesthesia, a regime known to drastically alter neuronal activity. Here we performed intracellular and extracellular single unit recordings from freely moving and immobilized animals to explore the neuronal dynamics in various brain areas. We found that neuronal activity in the cortical initiation network is more heterogeneous than previously thought with only a fraction of neurons being phase locked to SWDs. A prominent rhythmic activity characterizes S1 FS neurons which also undergo ictal hyperpolarization. In higher order thalamic nuclei the activity of individual TC neurons is characterized by ictal rhythmic burst firing. In the lateral hypothalamus neuronal activity is coupled to SWDs exclusively on a long timescale and not to the individual cycles of the SWDs. These results provide novel insights into the neuronal activity in various brain areas and highlight the importance of using drug free preparations when revealing the neuronal activity during (patho)physiological functions.

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P4.23 Cellular and molecular mechanisms of age-related changes in a defined neuronal network encoding associative memory

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Due to the complexity of central nervous system (CNS), the study of aging processes in vertebrates is not an easy task at the level of neural circuits and individually identified neurons. As a result, aging research heavily relies on invertebrate model organisms. One such invertebrate model is the great pond snail (*Lymnaea stagnalis*), which has been used extensively for decades to study the functioning of the nervous system with a characteristically integrative top-down approach (behaviour-to-circuits and molecules). Its use as a versatile aging model showcased this species as a contemporary choice for modelling the behavioural, circuit, cellular, and molecular mechanisms of aging and age-related memory impairment.

We have identified several evolutionarily conserved homolog sequences, such as *klotho*, *huntingtin*, *presenilin*, and *RbAp48*, in the whole neuronal transcriptome of *L. stagnalis* to genes involved in aging, age-related memory impairment, and neurodegenerative diseases (e.g., Parkinson's disease) of vertebrates including humans. We hypostatize that the proteins encoded by these sequences are involved in age-related impairments of learning mechanisms in *L. stagnalis* by targeting the identified components (e.g., NMDA receptor) of the signalling pathways of long-term memory (LTM) formation. Furthermore, we propose that their effects may be exerted at the level of transcriptional regulation of some of the key molecules involved in LTM formation and that some of the newly described sequences (e.g., *RbAp48*) could also be transcriptionally regulated in an age-dependent manner. Using 4-month-old and 16-month-old snails, we investigated the molecular footprint of aging in the whole CNS. From the total 72462 transcripts expressed, 960 showed significant changes during aging. Highlighting, the expression of several key molecules of learning, such as NMDA receptor and CREB-binding protein, showed an age-related decline.

The identified gene expression changes at the system level may play a role in aging and age-related memory impairment. Developing the CRISPR/Cas9-mediated gene modification method for *L. stagnalis* will open revenues for the investigation of molecular processes underlying age-related memory decline in more detail leading to the discovery of novel mechanisms operating not just in molluscs but also in higher organisms.

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P4.24 The age-dependence and roles of the astrocyte-dependent, NMDA-receptor mediated cortical slow inward currents in human and mouse neocortical samples

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The slow inward currents (SIC) are known as excitatory events of neurons caused by astrocytic glutamate release and activation of neuronal extrasynaptic NMDA receptors. The SICs have significantly slower kinetics than the spontaneous or miniature excitatory postsynaptic currents (sEPSCs).

We investigated the role of SICs in the synaptic plasticity and its (patho)physiological significance. For these experiments, we used mouse neocortical preparations for slice electrophysiology. An astrocyte-dependent phasic depolarization (related to SICs) was either injected as voltage commands with various temporal relationships with the evoked EPSCs or SICs were elicited by selective chemogenetic activation of astrocytes.

We found that SICs of mice have similar age dependence as in humans: in older (1-year-old) mice the charge transfer by SICs is declined. With application of artificial SICs (a recorded astrocyte-dependent phasic depolarization as a voltage command) at different time shifts with evoked EPSCs In the present project, in temporal cortical samples - during we used artificial SICs - we recorded EPSCs which were elicited by electrical stimulation. We found that a single SIC as electrical signal altered the EPSC amplitude in a time dependent way: if SICs preceded EPSCs or appeared at the same time, EPSC amplitude was facilitated in almost half of the cases. If SIC was in delay compared to EPSC, the EPSC amplitude was depressed in half of the cases. Next, SICs were elicited by chemogenetic activation of astrocytes using hM3 DREADD (Designer Receptor Exclusively Activated by Designer Drug) and clozapine-N-oxyde (CNO). SICs elicited by chemogenetic astrocyte activation increased the amplitude of evoked EPSCs.

Spontaneously occuring SICs also caused increase in spontaneous EPSC amplitude and frequency.

In summary, one might conclude that SICs contribute to astrocyte-dependent homeostatic synaptic plasticity mostly as electrical signals and not simply "hallmarks" of astrocytic activation. Decline of this signaling can be seen both in humans and mice with age.

P4.25 Chemogenetic investigation of brainstem neuromodulatory actions on locomotor regulation

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Pontine and mesencephalic areas influence locomotion in a complex way. The pontine caudal nucleus is the main structure for startle reflex which is under control of other sensory and regulatory brainstem nuclei. Brainstem nuclei involved in startle regulation seem to be the target of cholinergic neuromodulation via ion channels composed of KCNQ4 subunits responsible for M-current. The pedunculopontine nucleus (PPN) is part of the mesencephalic locomotor center which displays a complex role in locomotor regulation ranging from muscle atonia to explorative movements. This nucleus is also involved in circadian regulation as a member of the reticular activating system. We previously found that astrocytic activation of PPN inhibits a certain subpopulation of the nucleus, whereas another subpopulation is stimulated.

In the present project we aimed to activate brainstem networks for startle or astrocytes of the pedunculopontine nucleus using DREADD technology. Behavioral changes were tested with eliciting acoustic startle reflex, and by activity wheel test.

In vivo chemogenetic activation of hM3 in brainstem neuronal network responsible for startle increased the amplitude of acoustic startle reflex; which resembled to the increase of startle reflex observed in KCNQ4 knockout mice. Acute in vivo chemogenetic activation of PPN astrocytes did not alter activity cycles significantly but increased locomotion. After chronic activation of astrocytes, the time spent with locomotor activity was significantly increased.

Although these results are preliminary at the moment, one might conclude that the in vitro neuromodulatory actions by M-current or by astrocytic activation previously described by us might be able to modulate locomotor behavior in an in vivo animal model.

P4.26 Astaxanthin exerts anabolic actions via pleiotropic modulation of the excitable tissue

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Astaxanthin is a lipid-soluble carotenoid which exerts actions on lipid metabolism, body weight and insulin sensitivity. In the present paper, a systematic analysis of acute and chronic actions of astaxanthin on different organs is provided. Actions of chronic astaxanthin feeding were analyzed on general metabolism, expression of regulatory proteins in the skeletal muscle, as well as changes of excitation and synaptic activity in the hypothalamic arcuate nucleus of mice. Acute actions were also tested on cardiac muscle and different neuronal populations of the hypothalamic arcuate nucleus. Dietary astaxanthin was found to significantly increase food intake. It increased protein levels affecting glucose metabolism and fatty acid biosynthesis in skeletal muscle. Inhibitory inputs innervating neurons of the arcuate nucleus regulating metabolism and food intake were strengthened by both acute and chronic astaxanthin treatment. Moderate changes of cardiac action potentials, including shortening of duration, depression of plateau, and reduction of the maximal rate of depolarization were also seen. Based on its complex actions on metabolism and food intake, our data supports the previous findings that astaxanthin is suitable for supplementing the diet of patients with disturbances in energy homeostasis.

P4.27 Photobleaching alters morphometric parameters of different cell types during immunofluorescent imaging of spinal cord

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The sensitivity of immunofluorescence to the illumination time and intensity is well known; however, its effects on morphological parameters are poorly understood. Morphometric changes in different cell types can indicate the underlying molecular events in the region of interest; for example, the thickness and the number of the fine processes of microglia can imply their activation state. Our aim was to determine whether different illumination times and fluorophores are capable to alter the morphometric parameters of cells in the nervous system.

Immunofluorescent staining of microglia and neurons was performed on mouse spinal cord using different fluorophores, and immunohistochemistry with photostable diaminobenzidine staining was served as non-bleaching standard. Time series using 0.5, 1, 2, 5, 10 and 15 minutes of illumination after the initial image were acquired from each spinal cord section using epifluorescence microscopy without any changes in the acquisition parameters. On the native images, changes in the intensity were measured and numerous morphological parameters were extracted. Dynamic and relative total areas of microglial coverage were measured with an Image Pro-Plus macro developed in our lab used routinely for quantifying glial activation. Fractal geometrical parameters (fractal dimension, lacunarity, density, span ratio, perimeter, area, circularity) were measured using a specialized Fiji plugin, FracLac.

Mean color intensity measurements imply that photobleaching strongly depends on the type of fluorophores. Dynamic and relative total area density comparisons showed that microglia can fade to the point of becoming non-detectable even after 2 minutes of illumination using Alexa Fluor 546 or Alexa Fluor 594 while fine processes of the microglia remained detectable after immunostaining with Alexa Fluor 488. In lacunarity, density, area, and span ratio, at least 20% alterations can be observed regardless of the fluorophore.

Our results are in accordance with the literature which suggest that immunofluorescent staining is an excellent method for localizing different cells relative to each other or mapping the cellular and subcellular distribution of molecular markers, however morphological measurements require photostable staining regardless of the fluorophores. Our observations indicate that using immunofluorescent staining to examine the changes in the morphometric parameters of cells may alter the results of the experiment.

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P4.28 Structure-based short peptides designed to disrupt the STEP-GluA2 complex enhance cognitive performance in rats

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STriatal-Enriched protein tyrosine Phosphatase (STEP) is a brain-enriched tyrosine phosphatase playing an important role in modulating synaptic plasticity. Dysregulation of STEP has been reported in various neurological disorders. Among its identified substrates are the GluA1/GluA2 subunits of AMPA-receptors, through which it can exert a negative effect on synaptic plasticity by the dephosphorylation of certain tyrosine residues, resulting in reduced synaptic transmission and subsequently the internalization of the receptor. Thus, inhibition of STEP is predicted to perform memory enhancement, but the active site inhibitors are unspecific to STEP. Here we report the structure-based design of peptides capable of disrupting the formation of the STEP-GluA2 complex, hence obstructing the clathrin-mediated endocytosis of AMPA-receptors. From virtual peptide libraries, GluA2-CT analogue short peptides were determined, tested and selected in further in vitro binding experiments using surface plasmon resonance and flourescence polarization. Electrophysiological experiments were carried out to evaluate in vivo efficacy by measuring population-spikes in the hippocampus after the direct administration of peptides to the ventricles in rats in two different doses. We revealed a marked increase in excitability, indicated by the significant elevation of population-spikes' area and slope 1 hour after delivery and amplitude 3 hours after delivery. To assess their effect on cognition, memory tests were performed on rats using a scopolamine-based cognitive impairment model, in which the memory deficit was rescued successfully by the short peptid analogues. In conclusion, the structure-based interference peptides introduced in these experiments showed promising results against memory impairment and may give rise to new cognitive enhancers in the future.

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P4.29 Modelling of Neuronal Responses to Rotating Extracellular Electric Field Gradients

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Modern biophysical simulation environments can model the dynamics of different cell types as well as the responses to extracellular fields in details. Numerous articles characterized the activity of various neuron models in stationary electric fields eliciting subthreshold and suprathreshold responses.

In our work, we used the NEURON simulation environment to simulate the effects of extracellular fields of different orientations on geometrically precise models of various neuron types. Our aim was to study the underlying dynamics of the integrative nature of cell membranes exposed to repetitive subthreshold and suprathreshold stimuli. We also tracked the course of charge-accumulation during exposure to high-frequency rotational field leading to the emerge of an action-potentials.

The software NEURON was developed to model responses to simple stationary stimuli and thus, it is less suitable to handle stimuli with complex temporospatial layout. In order to model the instantaneous effects of the sequential pulses of high-freqeuncy rotational stimulation, we developed a Python wrapper around the original C-based environment of NEURON.

Neuron cell models were taken from the database of Aberra et al. (2018). These models include five types of morphologies and channel dynamics, from five cortical layers: layer 1 neuroglia cells (L1-NGC), layer 2/3 pyramidal cells (L2/3-PC), layer 4 large basket cells (L4-LBC), layer 5 tick-tufted pyramidal cells (L5-TTPC) and layer 6 tufted pyramidal cells (L6-TPC).

One stimulation period was defined by a set of consecutive rotations of the electric field, where the dendroaxonic axis of the modelled neuron and the normal of the field vector was moving from parallel to orthogonal in seven steps. Rotation was advanced at four frequencies (200, 500, 1000, 2000 Hz) and 12 amplitudes (from 0 V/m to 80 V/m in 5 V/m steps) were tested.

In conclusion, higher field intensities were needed to generate the first spike when the field was rotated faster around the modelled neurons. Therefore, we conclude that charge accumulation plays an important role in action potential generation. Angular variability showed that in general, directions perpendicular to the axons required higher intensities to generate spikes, but the neuronal morphology results in complex spatiotemporal response profiles, which play a more important role in neuronal responsivity than the structural components.

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P4.30 Altered H-current in cortical interneurons of drug-resistant epileptic patients

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Approximately 30 % of the epileptic patients are drug resistant. Most of this drug resistant group has a well-defined, focal onset region. For these focal-onset, drug resistant epilepsy patients the surgical resection might be the best therapeutic option for achieving seizure freedom. In many cases the pathological high-frequency oscillations (pHFO) are continuously present in the epileptogenic zone (EZ), however, the pHFOs do not always propagate to neighboring areas. The mechanism which blocks this continuously ongoing pHFOs from generating a generalized epilepsy is not entirely known. In the present study we compared the basic electrophysiological properties of GABAergic interneurons of surgically removed EZ and control (non-epileptic, subcortical tumor patient) cortical interneurons. Most GABAergic interneurons in the control cortical preparations showed prominent sag-potentials caused by H-current. In contrast, sag-potentials were less prominent or completely missing in epileptic interneurons. H-current in fast-spiker interneurons have been shown previously to be involved in the maintenance of repeated, high-frequency bursting of interneurons. We hypothesize that the epileptic interneurons are unable to maintain high-frequency bursting activity for longer period, which contributes to the propagation and generalization of pHFOs in epilepsy.

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P4.31 Feedback inhibition in the entorhinal cortex mediated by neurogliaform cells

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The role of local GABAergic inhibitory neurons in generating the entorhinal specific cell activities is still not entirely known. Several studies focused on the function of parvalbumin+ fast spiker interneurons, and only limited data has been published on the connectivity-matrix of many other GABAergic cell types. Many interneurons are localized in the layerI, where apical dendrites of layerII-V pyramidal and stellate cells are located. The majority of these critically positioned interneurons are neurogliaform cells. Neurogliaform cells have been shown to elicit elongated GABAA and GABAB receptor mediated inhibition in the neocortex and hippocampus in virtually all cell types which are located within the range of the rich axonal clouds of the neurogliaform cells. They are generally supposed to perform feed-forward inhibition: in the somatosensory cortex thalamic input; in the dentate gyrus entorhinal input; in the CA1 entorhinal and CA3 inputs give excitatory synapses on neurogliaform cells. The feedback inhibition, however, has not been linked with neurogliaform cells.

In the present work we aimed to shed light on the involvement of layerI GABAergic interneurons in the local microcircuits. Specifically, we investigated whether these cells receive excitatory inputs from the layerII pyramidal and stellate cells and whether neurogliaform cells show correlation to the island-like “patchy” structures which is a hallmark of MEC superficial layers. Our results showed strong, monosynaptic excitatory connection between layerII pyramidal cells and neurogliaform cells. Therefore, we hypothesize that neurogliaform cells are involved in effective feedback inhibition of the entorhinal cortex microcircuits. Moreover, we found that the neurgliaform cells are evenly distributed in layerI, therefore, they can elicit inhibition in all cell types sending dendrites to layerI.

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P4.32 Comparison of CCK+ perisomatic inhibition throughout multiple layers of the entorhinal cortex

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Perisomatic inhibition is considered as one of the most effective regulator in neuronal circuits. Two types of basket cells target the perisomatic regions of principal cells: fast-spiker parvalbumin expressing and regular spiking CCK positive interneurons. In the entorhinal cortex parvalbumin positive fast spiker basket cells have been shown to play a major role in forming grid-like firing of layerII principal cells. Little is known, however, about the CCK positive basket cells in this brain region. This lack of knowledge is mostly due to the heterogenous expression of CCK throughout different neuronal types including pyramidal cells. This expression pattern made the transgenic approaches for specific cell-type labeling extremely difficult. Here, with the help of a VGAT-IRES-Cre/BAC-CCK-eGFP-coIN trangenic mice we show the overall distribution of CCK+ interneurons and their specific targets in the medial entorhinal cortex. We found that CCK basket cells target mainly layerII and layerV pyramidal cells and avoid layerII stellate and layerIII pyramidal cells. Moreover, we found a population of CCK+ cells in the layerI. These GABAergic interneurons show distinct morphological and electrophysiological features, enabeling them to control several surrounding elements of the circuitry.

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P4.33 Down-top inhibition of neurogliaform cells by somatostatin positive interneurons

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The majority of GABAergic interneurons in the entorhinal cortical layerI are neurogliaform cells. This cell population is critically positioned by receiving thalamic and cortical excitatory inputs and convey feed-forward inhibition on virtually all cell types which have dendrites in the axonal cloud of the neurogliaform cells. However, the inhibitory inputs of neurogliaform cells are not entirely known. In the present study we utilised cell-type specific optogenetic approach to investigate the potential inhibitory inputs of neurogliaform cells. We found that somatostatin expressing putative Martinotti cells inhibit monosynaptically the neugliaform cells located in layerI, however, this inhibition is significantly smaller than the inhibition on neighbouring principal cells.

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P4.34 Comparison of action potentials in small and large mossy fiber axons using direct patch-clamp recording and voltage imaging

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Axonal action potentials (APs) are fundamental digital signals that determine neuronal output. However, it is not clear whether and how AP shape is maintained within individual axons as there are substantial morphological variabilities along individual axons that influence local voltage generation due to slower membrane time constants of smaller structures. The variable AP shape would compromise their digital output function. To explore the influence of the size-dependent filtering on AP signaling within variable axon morphology, we compared small and large axonal structures of hippocampal mossy fiber axons (MFs) using direct patch-clamp and voltage imaging. MFs are ideal subject for this general question because they form boutons in a wide diameter range, from typical small axons to exceptionally large boutons. These recordings required innovative methods that correct distortions in voltage signals caused by the small size of the structures.

We found that passive membrane properties depended on the axonal size, and as expected, passive voltage generation was slower in small axons. However, the amplitudes and shapes of the active APs were surprisingly similar regardless of the axonal thickness. Calculations based on recorded signals and their morphological substrate predicted that similar AP shapes can be maintained by different Na^+ and K^+ current ratio in MFs with different size. Direct measurements of Na^+ and K^+ currents in membrane patches from MFs confirmed this prediction.

Thus, our results suggest that uniform digital AP signal is achieved by size-dependent contribution of Na^+ and K^+ currents along individual axons that possess boutons with variable caliber.

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P4.35 Modulation of firing activity of CA1 hippocampal pyramidal neurons by systemically applied alpha7 nicotinic acetylcholine receptor selective compounds and memantine in the anesthetized rat, in vivo

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Promising new strategies for the treatment of cognitive decline (especially in Alzheimer's disease, AD) target either the cholinergic system by the activation of acetylcholine receptors (AChR) or modulate the glutamatergic N-methyl-D-aspartate receptors (NMDAR). There is also an increasing interest in low-dose combination therapies co-targeting these neurotransmitter systems to reach greater efficacy over the monotreatments.

To investigate direct cellular correlates of cognitive enhancer treatments in the hippocampus, a brain region relevant to learning and memory, our present aim was to measure the individual and combined modulatory effects of the α7nAChR selective agonist PHA543613 (PHA), and a proprietary α7nAChR PAM compound (CompoundX) and the NMDAR antagonist memantine on the firing activity of CA1 pyramidal neurons in three different conditions: spontaneous firing, NMDA-evoked firing and ACh-evoked firing. Extracellular firing activity of neurons was recorded in anesthetized rats. The application of pharmacological agents was performed by local microiontophoretic drug delivery. Cognitive enhancer agents were applied systemically with s.c. injections in behaviourally relevant doses. In addition to monotreatments, four different combined treatments were applied (memantine + PHA and memantine + CompoundX, both combo in low and high doses).

We found that high-doses of memantine decreased NMDA-evoked firing activity having no effect on the spontaneous firing activity of the neurons, however, none of the doses had any remarkable effect on ACh-evoked firing activity. In contrast, systemically applied PHA had a robust potentiating effect on ACh-evoked firing activity with having no effect on NMDA-evoked activity. In addition, CompoundX increased both NMDA-evoked firing activity and ACh-evoked firing activity, with having no effects on spontaneous activity. The combined simultaneous systemic administration low doses of memantine and PHA increased firing activity in all conditions and a similar effect was observed using low doses of memantine and CompoundX.

The present results demonstrate that the applied α7nAChR agents, especially the PAM compound successfully potentiate glutamatergic activation in the hippocampus and their combined application with AD medication memantine in low therapeutic doses may exceed the monotherapeutic efficacy of memantine, suggesting new experimental avenues of preclinical research in cognitive enhancement.

P4.36 Ecdysteroids protect the viability and barrier integrity of cultured human brain endothelial cells under oxidative stress

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Ecdysteroids have been examined since decades. They were discovered as molting hormones in arthropods, but these polyhydroxylated sterols are abundantly present in plants as well. The health benefits of ecdysteroids were discovered in mammals including humans due to their anabolic, antidiabetic and antioxidant effects, while their acute toxicity is negligible. Plants' ecdysteroid composition is dominated by the 20-hydroxyecdysone (20E), which is the most studied member of the family. Recently a derivate of the 20E, calonysterone was synthesized and prepared for biological testing.

In central nervous system-related diseases blood-brain barrier (BBB) dysfunction is often identified, still during the therapy of these pathological conditions the protection of the BBB is often neglected. Therefore, the search for new agents which exert beneficial effects on barrier functions of brain capillary endothelial cells in health and disease could not be more important. The aim of the present study was to test the effect of 20E and its derivative, calonysterone on human brain capillary endothelial cell functions in healthy conditions and under oxidative stress. During our experiments we used the hCMEC/D3 human brain endothelial cell line. To induce oxidative stress cells were treated with tert-butyl hydroperoxide. Edaravone, a known antioxidant and drug used in clinical practice served as a control. We performed barrier integrity measurements, fluorescent staining for interendothelial junctional molecules and measured the production of reactive oxygen species.

We found, that both 20E and calonysterone elevated the basal impedance of brain endothelial cells in low treatment concentrations, which reflects to a barrier tightening effect in normal culture conditions. Both agents protected brain endothelial cell barrier integrity and restored junctional morphology under oxidative stress. 20E and calonysterone could not reverse the excess reactive oxygen species and nitric oxide production after tBHP treatment, but both agents decreased the basal nitric oxide production. Our results show that ecdysteroids have a beneficial effect on the barrier properties of cultured human brain endothelial cells. We hypothesize, that these compounds could have therapeutic potential to protect the BBB in neurovascular dysfunctions.

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P4.37 Information flow between the dentate gyrus and CA3 regions during sharp wave-ripple complexes in rat hippocampal slices

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The hippocampal formation is yet in the focus of attention among the most widely examined parts of the nervous system. It is presumed to have a crucial physiological role in cognitive functions, such as learning, memory formation, and spatial orientation. Nonetheless, our knowledge about how diverse memories are coded and stored has gaps. Sharp wave-ripple complexes (SPW-Rs) are considered to be the neuronal correlates of memory trace formation and transmission. These synchronous population discharges are observed in the mammalian hippocampus on EEG during slow-wave sleep and immobility.

An in vitro SPW-R model was investigated on rat hippocampal slices using a 24-channel linear electrode. Instead of assuming a classical trisynaptic circle of information flow from the DG to the CA3 (Type I), our studies showed that SPW-Rs could also be propagated in the opposite direction (Type II), or generated simultaneously in both areas (Type III). Based on these results, it can be stated that bidirectional information flow occurs between the DG and the CA3 region of the rat hippocampus. The role of reverse information flow might be to intensify the information packages transmitted by the SPW-R complexes by adding another network to the trisynaptic circle for more efficient memory consolidation.

In the second part of the project, the role of different cell types was investigated in the generation of distinct SPW-Rs. More cells were activated at the initiation site of the SPW-R complexes than at other regions. Pyramidal cells of the CA3 fired more and showed denser connectivity than granule cells of the DG. A metabotropic glutamate receptor agonist, DCG-IV was used to investigate the role of the mossy fibres in the generation of SPW-Rs. In DCG-IV bath, the recurrence frequency of Type I SPW-Rs increased, while that of Type II SPW-Rs showed a slight drop. Furthermore, the propagation of SPW-Rs decelerated, while the LFPg deflections and the superimposed multiunit activities were reduced. Our results emphasise the prominent role of CA3 region on the one hand and the influential but not essential role of mossy fibres, on the other hand, in the generation of SPW-R complexes.

P4.38 Postnatal developmental change in the expression of ChAT, NKCC1, and KCC2 mRNAs in the mouse basal forebrain

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Basal forebrain cholinergic (BFC) neurons provide projections directly engaged in cognitive processing in mammals. They receive input from GABAergic/glycinergic neurons including those derived from the brain stem reticular formation. These transmitters, depending on the target cells' intracellular chloride concentration and receptor(s) (glycine and/or NMDA) involved in signaling, can exert both excitation and inhibition and consequently contribute to activation or silencing of BF neuronal circuits. A developmental shift in the expression of chloride transporters and receptor subunits, therefore, is a critical determinant of the temporal role played by the target cells in the circuit activity.

The current study aimed to investigate (1) the postnatal expression pattern of the neuronal Cl^--accumulating $Na^+/K^+/Cl^-$ co-transporter, NKCC1, and the Cl^--extruding K^+/Cl^- co-transporter, KCC2, and (2) the potential changes generated by the optical stimulation of glycinergic afferents in BFC neurons.

Using the Multiplex RNAscope technique, mRNAs levels of choline acetyltransferase (ChAT), NKCC1, and KCC2 were determined in five brains collected at different postnatal (PN) time points (PN1, 7, 14, 45). Signals were quantified with NIS Analysis at a single-cell resolution. ChAT mRNAs were detected first in PN7. Colocalization of the ChAT signal with the NKCC1 and KCC2 was already observed at this time point, and the mRNA signals of KCC2 in ChAT cells increased significantly from PN 7 to 14 and PN 14 to 45. No postnatal downregulation could be observed in NKCC1 expression; instead, a similar increase was detectable in its expression as it was observed for KCC2 in ChAT positive cells.

Transgenic animals (aged: 2–3-month-old) expressing GFP in ChAT cells, and channelrhodopsin 2 in glycinergic neurons were used to study the effect of glycine release on the synaptic inputs of cholinergic neurons in slice preparations. Our preliminary data indicate an increase in IPSC and EPSC frequency elicited by local optogenetic stimulation of glycinergic afferents. This effect was more pronounced on caudally located cholinergic neurons.

These results indicate that glycine release from local afferents of cholinergic neurons influences both excitatory and inhibitory inputs to cholinergic neurons, the latter is ensured by the increased expression of KCC2 in maturing cholinergic neurons.

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P4.39 Resolvins inhibit Transient Receptor Potential Vanilloid 1 and Ankyrin 1 ion channel activity via lipid raft modification

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Transient Receptor Potential Vanilloid 1 and Ankyrin 1 (TRPV1, TRPA1) cation channels are expressed in nociceptive primary sensory neurons, and regulate nociceptor and inflammatory functions. Resolvins are endogenous lipid mediators. Resolvin D1 (RvD1) is described as selective inhibitor of TRPA1-related postoperative and inflammatory pain in mice acting on the G protein-coupled receptor DRV1/GPR32. Resolvin D2 (RvD2) is a very potent TRPV1 and TRPA1 inhibitor in DRG neurons, decreases inflammatory pain in mice acting on the GPR18 receptor, via TRPV1/TRPA1-independent mechanisms. We provided evidence that resolvins inhibited neuropeptide release from the stimulated sensory nerve terminals by TRPV1 and TRPA1 activators capsaicin (CAPS) and allyl-isothiocyanate (AITC), respectively. We showed that RvD1 and RvD2 in nanomolar concentration significantly decreased TRPV1 and TRPA1 activation on sensory neurons by fluorescent calcium-imaging and inhibited the CAPS- and AITC-evoked ^{45}Ca-uptake on TRPV1- and TRPA1-expressing CHO cells. Since CHO cells are unlikely to express resolvin receptors, resolvins are suggested to inhibit channel opening through surrounding lipid raft disruption. Here we proved the ability of resolvins to alter the membrane polarity related to the cholesterol composition by fluorescence spectroscopy. It is concluded that targeting lipid raft integrity can open novel peripheral analgesic opportunities by decreasing the activation of nociceptors.

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P4.40 Induced pluripotent stem cell derived long-term in vitro neuronal culture on a microelectrode array

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Background: The broad aim of our research group is to model neurodevelopmental psychiatric disorders *in vitro* using induced pluripotent stem cells. To this end, it is necessary that we are able to reliably produce mature neurons and characterize their functions. In this study we present a successful attempt to differentiate cortical neurons and measure their electrical activity using a micro-electrode array system. The induced pluripotent stem cells originated from a healthy individual. We observed spontaneous network activity, and tested the effect of different receptor modulator substances, and culture media.

Methods: We used a small molecule supplementation protocol* modified in our laboratory. Neuronal progenitor cells were seeded onto the micro-electrode array to form a monolayer culture. The micro-electrode array measures the extracellular field potential with high temporal and spatial resolution. We were able to detect extracellular field potentials, manifesting themselves as spikes, bursts, or network bursts. The synchronisation of electric activity happened spontaneously, and it is a sensitive indicator of network connectivity in a differentiating neuronal cell population. We added chemical stimuli (glutamate receptor agonists and antagonists, GABA antagonist) and monitored their effect. We measured neuronal activity in different culture media and following electrical stimulation.

Results: Electric activity started with individual spikes around day 30, then drastically increased until around day 70, when activity reached a plateau phase. Individual spikes gradually formed asynchronous bursts, then synchronized into network bursts. We were able to inhibit most of the activity by the specific glutamate receptor antagonist CNQX, and increase the activity by adding kainate, a specific glutamate receptor agonist.

Future Goals: Based on this experience we aim to differentiate neurons from induced pluripotent stem cells, originating from schizophrenic patients, and compare the neuronal activity with a healthy control.

*****Li Y, Cao J, Chen M, et al. Abnormal Neural Progenitor Cells Differentiated from Induced Pluripotent Stem Cells Partially Mimicked Development of TSC2 Neurological Abnormalities. Stem Cell Reports. 2017;8(4):883-893. doi:10.1016/j.stemcr.2017.02.020

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P4.41 Robust somatic HCN channel-mediated facilitation of GABAergic basket cell input-output function in human compared to mouse supragranular neocortex

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Neurons in the mammalian brain exhibit evolution-driven species–related differences in their functional properties. Therefore, understanding the human brain requires unraveling the human neuron “uniqueness” and how it contributes to the operation of identified neuronal circuits. We show here that a highly abundant type of inhibitory neurons in the neocortex, GABAergic fast-spiking basket cell, in the human brain regularly exhibit hyperpolarization-activated cyclic nucleotide-gated cation (HCN) channels in their somatic compartment whereas HCN channels are rare or absent in their rodent neuronal counterparts. Somatic HCN channels in the human pvBCs set the depolarisation level of resting membrane potential and shorten action potential generation for excitatory inputs thereby regulating input-to-output fidelity and signal transmission speed in this common inhibitory GABAergic neuronal circuit in the human but not in rodent supragranular neocortex. Uncovering human neuron-related features like this are essential for developing more realistic computational models of human neuronal networks and to understand species-specific differences in effects of drugs.

P4.42 Persistent inflammatory pain induced upregulation of P2X4 receptor in rat spinal dorsal horn and lumbar dorsal root ganglia

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Long-lasting inflammation may result in persistent pain, which manifests as central sensitization at a spinal level. There is growing evidence that purinergic signaling play a pivotal role in central pain processing. Purinergic receptor activation may contribute to the release of several molecules (IL-1β, BDNF), which lead to spinal hyperexcitability. Over the last decade P2X4 got into spotlight in neuropathia, however its expression was scantily characterised in inflammation. Thus, we intended to analyse the receptor distribution within spinal dorsal horn and lumbar dorsal root ganglia (DRG) of rats suffering in inflammatory pain induced by complete Freund adjuvant (CFA). CFA induced peripheral inflammation was validated by mechanical and thermal behavioural tests. In order to ensure about the putative alteration of spinal P2X4 receptor gene expression qPCR reactions were designed, followed by immunoperoxidase and Western blotting experiments to assess changes at a protein level. Colocalisation of P2X4 receptor with neuronal and glial markers was investigated by performing double immunofluorescent labelings, which were subsequently analysed by IMARIS software. Transmission electronmicroscopy was also applied to study the ultrastructural localisation of the receptor with higher resolution. Beyond spinal dorsal horn in lumbar DRG cells we also carried out similar methodology to confirm our findings.

We observed enhancement of P2X4 transcript level within the spinal dorsal horn three days upon CFA administration. Elevation of P2X4 immunoreactivity within Rexed lamina I-II of the spinal gray matter was in synchron with mRNA expression, and verified by protein blotting. According to IMARIS calculation the robust protein increase was mainly detected on primary afferent axontermini of the spinal dorsal horn, in addition its abundant distribution on glial cells and neuronal somata, but not on postsynaptic dendrites. Lumbar DRG analysis also proved that peptidergic and non-peptidergic nociceptive subsets of ganglia cells were abundantly positive for P2X4 receptor in CFA model.

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P4.43 Prox1 immunoreactive amacrine cells and their relationship to electrical synapses in the mammalian inner retina

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Introduction and Objectives: The homeodomain protein Prox1 is expressed in horizontal cells, bipolar cells and AII amacrine cells of the adult mouse retina. Its role has also been established in the differentiation of horizontal cells. Here, we were interested whether the same expression pattern of Prox1 is found in cats and rats. Furthermore, we aimed to use Prox1 as a putative marker of AII amacrine cells. AII amacrines form an extensive, electrically coupled network in the ON sublamina of the inner plexiform layer (IPL). Extending earlier results, we measured the density correlation of Prox1 expressing amacrine cells and connexin-36 (Cx36) plaques, the light microscopic correlates of gap junctions in the ON and OFF sublaminas of the IPL in cats, rats, and mice.

Methods: We used flat-mounted retinas of mice (n =5), rats (n =4) and cats (n = 4) to include species with different patterns of regional specializations in the analysis. Prox1 immunohistochemistry was used in combination with calcium binding proteins parvalbumin (PV) and calretinin (CaR) allowing the identification of AII amacrine cells in cats and rats. In further retinas, the densities of Prox1 labelled cells and Cx36 puncta was measured at several retinal locations.

Results: Prox1 was found in PV-labelled AII amacrine cells in cat and rat, and in CaR expressing AII amacrines of the cat. In the rat retina, Prox1 was largely absent from CaR positive amacrines, which are known to be non-AII. Large Cx36 plaques were attached to about 8–10% of Prox1-positive amacrine cell somata in all three species. When analysing the regional changes in the volumetric density of Cx36-immunoreactive plaques, we found correlation with the density of Prox1-expressing amacrine cells in the ON (cat: $p = 0.001$, rat: $p = 0.013$, mouse: $p = 0.004$), but not in the OFF sublamina.

Conclusions: The results suggest that Prox1 is a conservative marker of AII amacrine cells in mammalian species. We also found that the relative contribution of electrical synapses to the ON- and OFF-pathways of the retina changes with retinal location, which may contribute to functional ON/OFF asymmetries across the visual field.

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P4.44 Inflammasome activation in primary astrocyte cultures

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Our previous experiments showed that interleukin-1 receptor type-1 (IL-1R1) is overexpressed in spinal neurons and IL-1R1 deficient mice show significant attenuation of thermal and mechanical allodynia during CFA-evoked inflammatory pain. The ligand of this receptor, interleukin-1beta (IL-1beta) is a pro-inflammatory cytokine, which plays central role in host defense, but it is also involved in several neurological disorders. Analysis of cellular distribution revealed that in lumbar spinal dorsal horn IL-1beta is dominantly expressed by astrocytes and its amount was significantly increased during CFA-evoked pain. The production of the bioactive form of IL-1beta is under the control of multiprotein complexes called inflammasomes. When mechanical allodynia was highest in the CFA model, we found overexpression of NOD-like receptor protein 2 (NLRP2) inflammasome sensor and its significantly elevated co-localization with GFAP astrocytic marker.

The currently available literature on NLRP2 expression and its activation is relatively limited, and several points are still unclear. Thus in the current study our aim was to investigate stimulatory agents which can induce increased expression of the NLRP2 sensor in cultured spinal astrocytes. We utilized such stimulatory signals which were shown earlier to be present or increase during chronic pain. Besides NLRP2 overexpression we detected the assembly and cellular re-distribution of apoptosis speck-like protein (ASC) monomers which serve as organizers of inflammasome complexes.

Together with other factors, chronic neuroinflammation plays an increasingly accepted role in the induction and maintenance of persistent pain states. One of the most prominent members of pro-inflammatory cytokines contributing neuroinflammation is IL-1beta. The release of mature IL-1beta can contribute to the maintenance of persistent pain by acting on its neuronally expressed receptor, which leads to altered neuronal excitability. Inflammasome research can provide unique tools to find tissue- or cell-type-specific molecular targets for the regulation of the IL-1 signaling pathway.

P4.45 Testing the sensitivity of virus injected ASAP and Glutamate sensors with two-photon imaging in mouse cortical neurons in vitro

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To understand how information is processed in the brain we need precise spatio-temporal recordings of electrical activity from individual neurons and neuronal circuits. Genetically encoded fluorescent sensors could be a promising solution for this, because of their suitability of optical recording of brain activity, as they allow monitoring of genetically defined neuronal circuits and do not require chemical access.

Here, we tested the sensitivity of virus injected accelerated sensor of action potential (AAV9.hSyn-ASAP3-WPRE, ASAP) and virus injected glutamate sensor (iGluSnFR.A184S, GluSn) with simultaneous two-photon imaging and electrophysiology in vitro in different animal models. With the usage of ASAP sensor, we can reliably detect single action potentials and subthreshold membrane potential events. Since the depolarization of the membrane causing a decreased fluorescence activity in ASAP expressing cells, we measured negative signal responses for electrophysiological and chemical (KCl) excitation during two-photon line-scanning. As far as the GluSn sensor expressing animals concerned, with the combined usage of glutamate puffing, patch-clamp stimulation and two-photon line-scanning we were able to detect spontaneous and evoked positive signals from cell populations, dendrites, and unique dendritic spines as well.

P4.46 In vitro examination of the effect of lipid raft disruptors on different cell properties

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Lipid rafts are specialized cell membrane microdomains rich in cholesterol, sphingolipids and gangliosides, and form functional complexes with different receptors and ion channels. Integrity of the raft regions can investigate with such compounds that can disrupt one or more constituent of the raft. Methyl-β-cyclodextrin (MCD) and a carboxamido-steroid compound (C1) – synthetized by ourselves – and the endogenous lipid mediators Resolvin D1 and D2 deplete the cholesterol from the membrane. Sphingomyelinase (SMase) is an enzyme which hydrolyze the sphingomyelin components, Myriocin – an enzyme inhibitor – block the de novo sphingolipid synthesis. In this study we examined the effect of these compounds on different cell properties.

First, we examined the effect of lipid raft disruptors on cell viability with two different method (CellTiter Glo and MTS assay) in a short period (30 min treatment) and in a longer period (24 h treatment). We also investigated the membrane fluidity – with Laurdan fluorescent spectroscopy – after treatment and in a third series of experiments we performed Filipin staining to visualize the effect of the compound on cell membrane modification.

None of the tested compounds changed significantly the number of living cell during both treatment period excludes MCD, which destroyed the cells in a concentration dependent manner during the longer (24 h) treatment. Compounds acting via cholesterol depletion – MCD, C1, RvD1 and RvD2 modified the membrane fluidity, however SMase and Myr did not changed significantly this parameter. Also, in the Filipin staining, disruptors actiong on cholesterol modified the membrane and change the sign of Filipin.

In conclusion our compounds did not change cell viability and modified the cell membrane properties therefore they can disrupt lipid rafts. These result in connection with our previous in vitro and in vivo findings suggest that targeting the lipid rafts can lead to receptor or ion channel modulation – e.g. TRP channel modulation – and therefore it can be a potential pharmacological intervention in many cases

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P4.47 RetaiN: Neuroimmunology and stress resistance in human ageing

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There is an urgent need to understand aging, since it affects all and has an enormous impact on our life and health. Changes of the immune functions and stress-protection pathways are detected in most of the diseases including age-related disorders, like neurodegeneration. The role and importance of the altered protective mechanisms in these disorders are still not fully understood. In this project we will investigate the immunological and stress-protection-related mechanisms of the neurons in differently aged healthy individuals, using the induced neuron (iN) model. iNs represent a novel tool to investigate human aging by direct reprogramming of fibroblasts into neurons. Uniquely, iNs retain the aging signatures of the donor. **RetaiN** can help to understand the role of immune- and stress-protection mechanisms in aging, through which we can identify novel targets to prevent age-related diseases and achieve healthy aging.

P4.48 Investigation of a pentapeptide carrier on culture models of the blood-brain and epithelial barriers

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Treatment of many diseases is difficult because biological barriers block the delivery of large biopharmaceutics at therapeutically relevant concentrations. Targeted delivery of protein drugs to the intracellular space and through biological barriers to achieve a better therapeutic effect is an actively investigated area. We demonstrated that a galectin-1 derived pentapeptide binds to GM1 ganglioside in nanomolar concentration and delivers large proteins into the intracellular space via lipid-raft mediated/caveolar endocytosis without localization in lysosomes (Imre et al 2020, PMID: 32099761). This peptide is small and does not influence the viability of cells, therefore, it is a promising candidate as a carrier for proteins to cross biological barriers. Since no data are available if this complex could be used as a shuttle through biological barriers, our aim was to compare the cellular entry of the peptide complexes into cells and their penetration across culture models of the blood-brain and different epithelial barriers. We observed that the peptide-antibody construct entered the epithelial and endothelial cells and localized in the cytoplasm. Incubation with the complexes did not alter the immunostaining pattern of ZO-1 and β-catenin tight junctional proteins indicating no effect neither on the epithelial nor on the endothelial barrier integrity. In case of the epithelial cells, both the peptide-antibody complex and the antibody complex alone penetrated across the barriers better at the four-hour time point compared to the one-hour time point in all cell lines. Our results showed that there was no difference between the penetration of the peptide-antibody and the antibody complexes across intestinal, lung and cornea epithelial models. In contrast, the permeability of peptide-antibody complex was higher across a human co-culture model of the blood-brain barrier. Furthermore, we confirmed that the peptide-antibody construct did not colocalize in lysosomes or ER in these barrier cells. Endocytotic pathways inhibitors decreased complex penetration across the human blood-brain barrier model. In conclusion, the pentapeptide can increase the entry of large protein complexes into epithelial and human brain-like endothelial cells and promote the transfer of a protein complex, but only across the blood-brain barrier.

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Poster session - Topic 5
Systems neuroscience

P5.01 Sampling motion trajectories during hippocampal theta sequences

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Efficient planning in complex environments requires that uncertainty associated with current inferences and possible consequences of forthcoming actions is represented. Representation of uncertainty has been established in sensory systems during simple perceptual decision making tasks but it remains unclear if complex cognitive computations such as planning and navigation are also supported by probabilistic neural representations. Here we capitalized on gradually changing uncertainty along planned motion trajectories during hippocampal theta sequences to capture signatures of uncertainty representation in population responses. In contrast with prominent theories, we found no evidence of encoding parameters of probability distributions in the momentary population activity recorded in an open-field navigation task in rats. Instead, uncertainty was encoded sequentially by sampling motion trajectories randomly in subsequent theta cycles from the distribution of potential trajectories. Our analysis is the first to demonstrate that the hippocampus is well equipped to contribute to optimal planning by representing uncertainty.

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P5.02 Contribution of top-down interactions to texture processing in the visual cortex

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In recent years we have seen a surge of modeling visual processing in the ventral stream of primates and humans with deep artificial neural networks (ANNs). The most successful one of these efforts, goal-driven modeling, promises to deal with the entire ventral stream at once by (1) specifying a hierarchical ANN topology, a loss function, and a learning rule, (2) training the model end-to-end for object categorization in a supervised manner, and (3) evaluating its biological relevance by sequentially pairing certain model hierarchy layers to cortical areas in the ventral stream. Such goal-driven models are able to predict a substantial portion (but far from all) of the response variance of neurons all along the ventral stream to naturalistic stimuli, but this task-specific all-in-one approach is limited in scope since the cortex is required to learn more flexible representations that can deal with multiple tasks. This inspires an approach that performs task-agnostic learning on natural images.

Here, we present a deep learning model of the lower ventral stream inspired by neuroscience in key details: it (1) is two-layered (model layers directly represent cortical areas V1 and V2, respectively), (2) incorporates both bottom-up and top-down signaling pathways, (3) represents stimulus noise and ambiguity with a probabilistic formulation, and (4) learns its model parameters from naturalistic images in a task-agnostic, unsupervised manner.

We found that our model fulfills basic expectations by learning Gabor-like representations at its V1 level and texture-like representations at its V2 level. Moreover, it reproduces classical electrophysiological findings on higher selectivity for stimulus identity at the level of V1 and higher selectivity for texture-like statistics at the level of V2, a hallmark of progressive compression. We also demonstrate several consequences of top-down signaling: (1) texture statistics become linearly decodable from V1 when top-down influences are present, (2) top-down signaling introduces noise correlations in V1 whose structure is specific to the high-level structure of stimuli, and (3) we demonstrate the presence of visual illusions with an in silico masking experiment.

Given these successes, we hope our model can be extended to account for key features along the entire ventral stream by finding the computational goals the brain evolved to follow.

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P5.03 Relevance of stimuli is represented in anterior cingulate cortex during a context-shifting task

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In complex tasks partially overlapping sets of stimuli can be relevant to different contexts. Ambiguity arises when a stimulus instructs a response in one context and an opposite response in another context. In such stimulus conflicts learning to categorize each stimuli as cue or distractor for a context resolves ambiguity. In both humans and monkeys anterior cingulate cortex (ACC) has been shown to play a key role in retaining outcome history, and resolving conflict. However, the neural underpinnings of its functions remained elusive. Here we investigated if ACC, as opposed to primary visual sensory area (V1), distinguishes stimuli based on contextual relevance in a multiple-cue visual-audio context-shifting paradigm. Mice were required to infer the relevant stimulus modality from recent reward history. We found that contextual relevance indeed influences how strongly a stimulus modality is decodable from cortical activity. In ACC, but not in V1, the respective irrelevant stimulus modality was suppressed in both contexts. Furthermore, throughout congruent trials, when both modality would indicate the same action, stimuli were continuously decodable. In conflicting trials, however, in ACC, but not in V1, accuracy of the decoded relevant stimuli dropped early in the trial and gradually increased thereafter. These findings suggest that suppression of conflicting irrelevant stimuli takes a toll on accumulating evidence for the relevant stimuli. We also investigated whether early and late representations were different: Our data suggests that an early stimulus-driven transient and activity once evidence accumulation is complete reside in population subspaces orthogonal to each other. Selecting the relevant stimulus requires contextual awareness. Indeed, context was decodable continuously in a subspace orthogonal to all stimuli. We found that representation of task relevant variables are significantly correlated with behaviour. In summary, relevant stimulus in conflicting situations is actively selected, gradually accumulates onto a subspace orthogonal to both an early stimulus transient and context-related activity, while requiring more evidence in conflicting trials.

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P5.04 Travelling slow waves in the thalamus of anesthetized rodents

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Brain oscillations that play an important role in brain functions, such as slow waves or sleep spindles, often behave like waves. Numerous in vivo and in vitro experiments have shown that travelling waves have a significant impact on the brain's computational performance and synaptic regulation. Nowadays, it is clear that propagating waves are also essential for sleep and also in wakeful functioning. The thalamus we study is essential for the generation of slow waves and sleep spindles. Propagation of sleep spindles has been observed previously in the thalamus of cats in vivo but travelling slow waves with thalamic origin have only been described in in vitro brain slices. The application of multi-shank and high-density silicon-based probes allows us to investigate the firing patterns of neuronal populations at high spatial and temporal resolution. Using this technique, we investigated in vivo whether propagating waves can be recorded in the thalamus of anesthetized mice (n=10) and rats (n=27). In these experiments we used either NeuroNexus multi-shank probes (having 64 or 128 recording sites) or high-density Neuropixels probes with 384 channels (selectable from 960 sites). In a single animal, we recorded thalamic activity from multiple insertions and at multiple depths to map the firing patterns of a high number of thalamic nuclei. Based on our preliminary results, we found that propagating slow waves only appear in some, mainly higher-order thalamic nuclei (e.g., Po or LDVL) and the properties of propagation might also depend on the actual brain state. In addition to slow-wave propagation, we observed that cycles of sleep spindles emerging during the active states of slow waves also propagate (e.g., in the first-order nuclei VPM) and that the strongly intertwined nature of these two types of oscillations results in the appearance of complex spatiotemporal propagation patterns. Besides investigating population activity, we also applied spike sorting on the collected dataset to analyze thalamic single-unit activity. Furthermore, we are also interested in how different anaesthetics (ketamine/xylazine, isoflurane and urethane) can affect the appearance of propagating waves. In summary, the propagating waves observed in the thalamus in vivo are significantly influenced by a number of factors including the depth of anaesthesia, the substance type or the degree of synchronization of the thalamocortical network.

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P5.05 Functional interactions within the thalamus

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The thalamic relay cell-reticular cell loop is the origin of diverse oscillations, most notably sleep spindles and absence seizures. Though well characterized in vitro, the direct excitatory and inhibitory connections between the two cell types have not been detected in extracellular population recordings. Here we performed high-density multichannel silicon probe recordings from primary- and higher order somatosensory thalamus of anesthetized and head-restrained mice. Thalamocortical (TC) cells and axon terminals of locally projecting thalamic reticular (nRT) cells were distinguished by their characteristic waveform and autocorrelogram (as reported in Barthó et. al. 2014).

TC and nRT units most commonly showed and indirect relationship (nRT cells broadly increasing their firing ~20 ms from TC spikes) likely due to both cells taking part in spindle oscillations. We also found, however, short latency narrow peaks and troughs on several cross-correlograms, indicative of monosynaptic excitation and inhibition, respectively. Both monosynaptic excitation and inhibition occurred between TC and nRT units, never between cells of the same type. In some cases, reciprocal excitation-inhibition was observed between coupled units. On the other hand, TC-TC and nRT-nRT pairs usually showed synchronous activity on a varying time scale. The probability of monosynaptic connections and the degree of synchrony both decreased with physical distance, indicating a strict topography of these connections. Units in the VPM/VPL exhibited predominantly indirect relations and/or monosynaptic excitation, whereas clear inhibitory interactions were more pronounced in higher order nuclei, such as Po.

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P5.06 Putative inhibitory projection neurons in the spinal dorsal horn of mice

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Inhibitory neurotransmitter containing neurons that, contrary to the classical interneurons, project to distant targets have been previously described in several CNS areas of rodents (e.g. hippocampus, entorhinal-, auditory and motor cortex). These GABAergic, long-range projecting neurons (PN) are capable of forming both uni- and bidirectional connections, and thus by synchronization of distant brain areas might contribute to certain cognitive functions. However, similar PNs in the spinal cord have not been described yet.

In a set of experiments we retrogradely labelled spinal dorsal horn PNs from the lateral parabrachial complex (LPB) using a retrograde AAV9-viral vector to induce TdTomato expression in VGLUT2::EGFP (n=4) and VGAT::EGFP (n=5) hybrid mice. One week after the stereotaxic surgery the lumbar spinal cord segments were sectioned in the sagittal plane to analyse the local targets of PN axon collaterals in confocal z-stacks of randomly selected areas.

Unexpectedly, the 32,95% of the identified PN somata showed a clear EGFP signal in VGAT::EGFP hybrids and 10,10% of the PN somata in VGLUT2::EGFP hybrids lacked EGFP, suggesting the previously unreported presence of inhibitory PNs in the mouse spinal dorsal horn.

The soma of the putative inhibitory PNs were located in lamina I and in the dorsolateral funiculus, presumably in the lateral spinal nucleus (LSN). While, their soma size and shape varied, those with strong VGAT positivity showed distinct mediolaterally elongated disc like appearance. Most of the putative inhibitory PNs exhibited dendrites oriented along the rostrocaudal axis. The confirmation of the genuine inhibitory nature of these PNs will require further morphological and functional experiments.

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P5.07 Dissecting the amygdalar microcircuitry

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The amygdala is a central hub in the brain circuit that controls behavioral responses to the environmental changes via the well-accepted lateral (LA)/basal (BA)→central amygdala (CeA) route. Accordingly, CeA controls a line of motor and autonomous brain centers regulating behavioral responses. In addition, recent discoveries have refined this canonical functional organization by identifying valence-specific LA/BA pathway avoiding the CeA suggesting that CeA is not the only output region of the amygdala. Therefore, our main goal was to provide a comprehensive anatomical map with connectivity principles of the amygdalar subregions participating in emotional response.

First, we combined antero- and retrograde tracer microinjections with fluorescent immunohistochemistry to investigate the afferent and efferent connections of different amygdala subnuclei. We selectively labelled the LA, BA, basomedial (BM), centrolateral (CeL) and centromedial (CeM) subnuclei, as well as the amygdalostriatal transition area (Astr) and the intercalated inhibitory cells.

Then we investigated the localization of labelled axons and neurons and compared their relative densities in each subnuclei. Our results indicate that there are several, rather non-overlapping pathways within the amygdala which contradicts the traditional serial information flow model. For example, there is no prominent connection between LA and BA, and their intra-amygdalar targets are also different (BM, Astr and CeM, respectively). Notably, both LA and BA projections avoid CeL and only weakly target CeM, suggesting a limited intra-amygdalar connection between the cortical-like and striatal like amygdala.

Next, we investigated the major excitatory, namely the cortical and thalamic inputs to the amygdala. Furthermore, using cortical layer- and thalamic subnuclei-specific Cre mouse lines, we further detailed these pathways in a cell type-specific manner.

We found that the cortical and thalamic afferents selectively innervate amygdalar subnuclei, suggesting the existence of functional link between cortical/thalamic input and the separate intra-amygdalar routes. Specifically, LA and BA as well as CeL and CeM connectivity largely differ.

Taken together, our findings highlight the existence of separate (rather than serial) information flow within the amygdala defined by their intra-amygdalar and cortical/thalamic innervation which could provide a framework for future studies on understanding amygdalar function.

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P5.08 The cellular and synaptic connectivity of the colliculo-thalamic network

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Associative learning is essential for adaptation in a constantly changing environment. When a neutral (conditioned) stimulus (e.g. sound; CS) appears along with an affective (unconditioned) stimulus (e.g. pain; US), a memory trace about the conditioned signal is formed. Then, at a later time-point, a fear response is elicited even if only the CS (the former neutral stimulus) is presented. This phenomenon is called associative fear learning. The anatomical background behind CS-US pairing contains a network, where these two pieces of information are able to converge on a single neuron. Our recent data suggests that this occurs before the conventionally accepted brain area, the lateral amygdala (LA), in the tecto-thalamic circuit, at the level of the LA-projecting calretinin-expressing lateral thalamic (CR+LT) cells. However, the exact anatomical evidences for this theory are missing. The tectal part consists of the midbrain's paired structures, the inferior (IC) and the superior colliculus (SC). They can transmit uni- and multimodal information (auditory, visual, somatosensory, etc.) to the thalamus. To investigate the organization principles of dual tecto-thalamic circuits, we used classical and viral tracing combined with immunohistochemical approaches in mice. We demonstrate that, although the colliculo-thalamic innervations have different topography, both are positioned to form synaptic contact on the same CR+LT cell. This convergence is likely to be present both between glutamatergic and the GABAergic collicular cell populations. Our findings show that multisensory information mediated by the SC and the IC can be converged on the same CR+LT cell. In summary, the presented tecto-thalamic pathways can jointly inhibit or excite the CR+LT cells and these complex synaptic transmissions can shape thalamic signal integration during the associative fear learning process.

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P5.09 The dorsal midline thalamus effect over prefrontal cortex by different parallel pathway

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The dorsal midline thalamus (DMT) has a key role in regulation of cognitive processes through frontal thalamo-cortical (FTC) interactions. Our previous data showed that the DMT can be separated into calretinin (CR) expressing (CR+) and non-expressing (CR-) cell groups, which have distinct arousal-related activity (Matyas, Komlosi et al, 2018). This suggests a functional dichotomy in FTC; however, the cellular and network features as well as the effector mechanism(s) of the CR+ and CR- pathways are poorly understood. Here, by integrating in vivo acute and chronic electrophysiological recordings, anatomical and optogenetic approaches in mice we demonstrate that the CR+ and CR- DMT populations have qualitatively and quantitatively different cortical and subcortical input/output organization and possess diverse cortical effect. In general, CR+ DMT neurons (homologue with paraventricular thalamic cells) have global efferent connections and persistent cortical activation. In contrast, CR- DMT (homologue with mediodorsal thalamic (MD) cells) cells have much fewer brain targets and their cortical effects is rather local. In addition, the proportions of the CR+ and CR- DMT activated principal cells and interneuron are different in the prefrontal cortex. Notably, the CR- DMT cells do not form a homogeneous population either; rather, there is a topographic segregation of distinct CR- neuronal groups (which could be analogue with medial, central and lateral MD). Furthermore, our preliminary data also suggest that majority of the CR+ DMT cells show general arousal-mediated activation pattern, while CR- DMT cells only follows the arousal changes. Finally, activation of the CR+ network can be transmitted to the CR- neurons, via indirect antero-medial thalamic reticular nuclear and the prefrontal cortical loops. These findings indicate that, although the CR+ and CR- FTC networks are anatomically and functionally different, they form sequentially activated 'inter-loop' system. Building on each other, they can collectively mediate complex, arousal-dependent brain functions.

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P5.10 Age-dependent role of midline thalamus in learning

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It is well-documented that arousal–dependent cognitive functions like memory processes, sleep-wake cycles and stress management are age-dependent. The thalamo-frontal network goes through age-related changes, which could be responsible for these behavioral alterations. However, the exact causal neuronal mechanism is not fully understood. Previously, the calretinin (CR)-expressing thalamocortical cells in the dorsal midline thalamus (DMT) were identified as an important neuronal network element for cortical arousal. Furthermore, DMT cells were also shown to modulate associative learning. Thus, we aimed to clarify the age-dependent role of the CR+DMT neurons in fear learning processes. First, we found that the bi-directionally control of CR+DMT activity differently altered associative fear learning in young (<6 months old) and aged (>18 months old) mice. DMT (most probably the CR+ cell population) provides most of the thalamic brain derived neurotrophic factor (BDNF) for the cortex, which is a key factor in many cognitive functions. Furthermore, the BDNF level in the brain shows age-dependent alteration; thus, we measured the control and the fear conditioning evoked thalamic BDNF levels in young and old mice. We found that the evoked BDNF levels were also changed by age. Notably, both the mature- and the pro-BDNF levels also increased in the thalamo-frontal circuit of the young but not the old mice.

Taken together, our preliminary research proposes that the dorsal midline thalamic BDNF can be a key regulator for age-dependent changes in learning and in other arousal-related behavior. Currently, we are investigating the age-dependent effect of DMT-selective BDNF deletion in various cognitive functions.

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P5.11 Analysis of ultrasonic vocalizations (USV) in mice

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Ultrasonic vocalizations (USVs) are fundamental forms of communication between conspecifics to promote social interactions and survival. Still, the underlying neural mechanisms of this communication channel are mostly unknown. Thus, we first recorded ultrasonic vocalizations of the male and female laboratory mice in their home cage and during several diverse stressful situations (separation, shock, restraint, cat fur). The recorded USV signals are acoustically analysed, with the help of the individual call extractions. These individual calls were further processed with noise filtering and principal component analysis for call characterization. In our analysis we could group mice USV calls (n = 459) into 5 distinct clusters. One of the clusters' frequency range reached 120-150 kHz and was only recorded during restrain stress. As this high frequency USV has not been identified in the literature, we have started to investigate its behavioural effects and the related underlying neuronal circuitries. Thus, we used an early-immediate gene(c-Fos) approach to identify those brain regions which are activated by the replay of this distress USVs (dUSV). Especially, we were interested whether the calretinin-expressing lateral thalamic neurons, which plays an important role in learnt auditory fear responses (Barsy, Kocsis et al, 2020), also take part in the process of these calls. Furthermore, we are planning to analyse the dUSV-evoked activation pattern in vivo. Taken together, our date can shed light on a novel communication channel between mice in stressful conditions. Furthermore, our research may reveal a neuronal network mechanism in which evolutionary conserved as well as experience-dependent auditory signals can be processed.

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P5.12 Adeno-associated virus infection patterns in the brain with different delivery methods in cats

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Neural activity modulation is crucial to map neural circuits, to cure or to understand diseases, especially in large animals from where the results can be easily translated into human aspects. To reach the desired cell types or cell populations a specific targeting and an efficient delivery method is required. In our work we aim to test the efficiency of intravenous and intrathecal injections in cats by describing the expression pattern of recombinant AAV viruses. The results are obtained from native and immunohistochemically stained samples.

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P5.13 Functional ultrasound imaging of deep visual cortex and beyond in awake cats

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Optical imaging has become the imaging method of choice as it is compatible with genetically specified access to brain activity across the meso- to the subcellular scale. Yet, to access brain tissue beyond mm depth, optical methods invoke invasive solutions like implantation of GRIN lenses or prisms. Functional ultrasound imaging (fUSI) is a novel technique, which enables access to brain activity in deep layers of the cortex or even subcortical structures via imaging changes in the haemodynamic signal. We imaged deep cortical and subcortical sensory-evoked responses in Brodmann areas 17, 18 and 19 of awake and anaesthetized cats using fUSI. We achieved signal-to-noise ratio high enough to reliably detect single-trial responses with imaging depth reaching 1.6 cm. Using a custom motorized imaging chamber design, we were able to construct 3D activity maps in a large volume, while limiting the length of imaging sessions to an amount well-tolerated by awake subjects. This method bridges the gap between functional magnetic resonance imaging and optical imaging techniques as a versatile mesoscale activity imaging option that does not rely on head fixation or anaesthesia-based immobilization.

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P5.14 Brainstem can recall fear memory via hippocampal somatostatin interneurons

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Contextual fear memories are encoded by a sparse population of hippocampal principal neurons that are selected based on their inhibitory-excitatory balance during memory formation. Yet the details of this mechanism are still unclear. Disinhibition of principal neurons could facilitate their selection and then also their re-selection during memory recall. Using optogenetic behavioral experiments, we found that if a subpopulation of dendrite targeting somatostatin (SOM) positive interneurons in the dentate gyrus (DG) of the hippocampus are inhibited during fear conditioning, their re-inhibition can recall fear memory even in a novel environment. We discovered that SOM cells are selectively innervated by brainstem nucleus incertus (NI) GABAergic cells. We also found that if NI GABAergic cells or their fibers in DG are stimulated optogenetically during fear conditioning, their re-stimulation can recall fear memory via hippocampal SOM cells. Using c-Fos immunostaining, we found that NI neurons showed correlated activity with DG principal neurons during contextual fear memory recall, but not during rest. Furthermore, we observed that inhibition of NI GABAergic cells impaired contextual fear memory recall. Finally, we found that NI hippocampal-projecting cells are strongly innervated by memory-related neocortical centers. Our data suggest a key disinhibition-based memory mechanism in the hippocampus that is supported by local SOM interneurons and their brainstem inputs.

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P5.15 An alternative cholinergic innervation of the hippocampus

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Cortical functions are highly regulated by ascending subcortical pathways, some of which originate from the basal forebrain. Cholinergic cells of the medial septum (MS) and the horizontal diagonal band (HDB) in the basal forebrain play a vital role in the regulation of attention and memory formation. While the cholinergic innervation of the hippocampus is known to originate from the MS, we discovered an additional cholinergic pathway from the HDB. Using tracing techniques combined with immunohistochemistry and electron microscopy, we found that HDB cholinergic cells mostly target hippocampal layers that are only sparsely targeted by the MS and HDB preferentially target the hilar mossy cells. Our preliminary chemogenetic behavioral experiments suggest that HDB cholinergic cells drive hippocampal novelty detection and memory formation via the mossy cells. Our results provide new insights into the regulation of memory formation and may help better understand cholinergic system-related neurodegenerative diseases.

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P5.16 Cholinergic neuron firing patterns in the Lateral Septum during a probabilistic Pavlovian learning task.

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The forebrain cholinergic system, through its wide spread projections to a multitude of cortical regions and higher order emotional processing centres (e.g. amygdala), has been implicated in arousal and central cognitive processing aspects such as associative/reinforcement learning and decision making. The basal forebrain and the medial septum cholinergic systems, with projections primarily to wide regions of the neocortex and to hippocampal and para-hippocampal circuits respectively, are the most studied loci of cholinergic neurons in the forebrain. A rather overlooked cholinergic population that has not been investigated functionally and only scarcely anatomically is that of the lateral septum (LS). The lateral septum is an important hub of the limbic system conveying contextual information received from the hippocampus to sub-cortical structures such as various hypothalamic nuclei and also emotional centres as the amygdala.

Here, we use optogenetics combined with chronically implanted tetrodes to identify and record LS cholinergic neurons specifically in mice during conditioning with a probabilistic Pavlovian learning protocol. Water-restricted, head-fixed mice are presented 2 different pure tones paired with reward (water droplet through a lick port) or punishment (mild air-puff). In mice with behaviour consistent with Pavlovian learning, we observed cholinergic functional profiles markedly different from those recorded in the basal forebrain or basal ganglia under similar protocols. Recorded neurons to date exhibit stable rhythmic firing at a frequency of 4-5Hz and their instantaneous firing rates (FRs) show selective inhibition after both reward-predicting stimuli and rewards. On the contrary, punishment-predicting stimuli do not elicit any increase or decrease in FRs whereas punishments lead to stimulus-locked bimodal (2 peak) increases closely following the start and end of negative reinforcement delivery.

The functional variability of the LS cholinergic population, as well as putative sex (male-female) differences, will be assessed by repeated experiments increasing the sample of opto-tagged neurons. Additionally, optogenetic approaches along with qualitative learning assessment will be used to assess learning under perturbations of normal firing patterns under the current protocol.

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P5.17 Simultaneous examination of neuromodulatory systems by fiber photometry and electrophysiology

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The essential role of neuromodulators such as acetylcholine and dopamine in learning is supported by a number of studies. However, the relationship between different neuromodulatory systems and possible synergistic or antagonistic effects are almost completely unknown. Furthermore, the known association of the degeneration of cholinergic and dopaminergic neurons with neurodegenerative diseases such as Alzheimer's and Parkinson's lends special importance to studying these systems. Recent findings suggest that reward prediction error, previously associated exclusively with dopamine, may also be encoded by the cholinergic system. It is hitherto unclear what similarities and differences the information represented by the two systems show. In this study, we investigated the two systems simultaneously in a special auditory operant conditioning task using fiber photometry and electrophysiological methods. In the fiber photometry experiments, acetylcholine and dopamine sensors were used to measure the release of the respective neuromodulators. In electrophysiological experiments, specific light-sensitive ion channels were expressed in cholinergic and dopaminergic cells, providing an opportunity for their optogenetic identification. Examining the temporal relationships of the defining events of learning-the cellular response to the sound predicting reward and punishment, and the neural response to the delivery of reward and punishment-we found groups of neurons following different characteristic firing patterns. Of these, dopaminergic and cholinergic populations were identified among several other groups with unidentified neurochemical identities. We found that dopaminergic neurons responded earlier than cholinergic cells after reward-predicting tones, while neither of them responded to punishment-predicting tones. More activity was observed after the delivery of unexpected than expected reward in both cell types. Cholinergic neurons responded earlier than dopaminergic neurons to both expected and unexpected reward. Dopaminergic neurons were heterogeneous in their responses to punishment, being either suppressed or activated. A group of cholinergic neurons responded to punishment with an extremely fast and precise activation, while some neurons did not respond. Our results show that the information encoded by the two systems correlates but evolves over different time scales. These results hint at a complex relationship between the two systems.

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P5.18 Synaptic communication within the microcircuits of pyramidal neurons and basket cells in the mouse prefrontal cortex

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The diverse population of neurons in the prefrontal cortex is comprised of excitatory pyramidal neurons and inhibitory GABAergic cells evolved for performing distinct network functions. Among the inhibitory interneurons, parvalbumin- and cholecystokinin-containing basket cells (PVBCs and CCKBCs) are known for preferentially targeting the perisomatic region of other neurons, enabling them to efficiently control the spiking of their postsynaptic partners. Here, we investigated the intrinsic electrophysiological features and synaptic properties within the networks of basket cells and pyramidal neurons in the mouse prefrontal cortex to determine the organizational principles of their microcircuits.

In vitro whole-cell recordings were performed in brain slices prepared from transgenic mice - BAC_Pvalb/GFP and BAC_Cck/dsRed mice - in which PVBCs and CCKBCs express fluorescent proteins, allowing us to target them under visual guidance. Single-cell features of basket cells showed major differences regarding both their active and passive membrane properties. Paired recordings were obtained to reveal the properties of connections within the microcircuits formed by pyramidal neurons and basket cells. Our results uncover that PVBCs receive larger and faster excitatory postsynaptic currents from pyramidal neurons than CCKBCs and PVBCs provide stronger and more reliable synaptic inputs onto pyramidal neurons than the other basket cell type. In addition, the amplitude of postsynaptic responses between PVBCs were found to be larger than those observed between CCKBCs. Short term-dynamics of connections were also dependent on and characteristic for the cell type of synaptic partners. Moreover, we aimed to assess whether PVBCs and CCKBCs are interconnected with each other: our anatomical data together with electrophysiological recordings combined with pharmacology and optogenetics revealed that there is indeed synaptic communication between the two types of basket cells.

Taken together, our data provide the first detailed description of connectivity within the microcircuits of pyramidal cells and basket cells in the prefrontal cortex, which means a crucial step in the direction of understanding the activity of this complex network.

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P5.19 Anatomical and in vivo electrophysiological characterization of neurons responding to noxious stimuli in the basolateral amygdala

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Amygdala refers to a cluster of structurally distinct nuclei in the brain. Its core unit is the basolateral amygdala complex (BLA), proposed to be the site of associative learning during Pavlovian fear conditioning. In associative learning subjects learn to pair a neutral conditioned stimulus (CS) with an aversive unconditioned stimulus (US). Lateral amygdala (LA) was shown to be the nucleus for plasticity during this process. Although, Pavlovian fear conditioning is one of the most studied learning paradigms, little information is available on how each amygdalar neuron type responds to mild electrical shock used as an unconditioned stimulus. Therefore, we set up experiments to reveal how principal neurons in the BLA change their firing upon noxious stimulation and whether the responsiveness correlates with their morphology.

The spiking responses of the BLA neurons on the US were monitored by juxtacellular recording technique in anesthetized animals. After recordings, neurons were filled with neurobiotin, which was visualized with immunostaining, allowing us to identify the soma location as well as reconstruct the axonal and dendritic arborizations of well-labelled neurons *post hoc*. The 45 successfully measured and labelled neurons were clustered based on the response latency to the pain stimuli and 20 of them were reconstructed in their entirety. We found that most of the recorded neurons elevated their firing activity upon the US delivery. Importantly, we identified a principal cell population in the basal amygdala (BA) that responded to the noxious stimulus with a latency of less than 30 ms, similarly to that observed in the LA. In addition, two principal neuron groups were defined in the BA: one of them projected to the central amygdala, whereas the other had no axons in this amygdala region.

Our results support the hypothesis that US may be processed in the BLA in a parallel manner, and BA has at least two functionally distinct cell groups based on their axonal arborization.

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P5.20 The role of calretinin positive midline thalamic neurons in stress induced behavioural changes

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Exposure to severe stress could lead to the emergence of stress-related psychiatric disorders, which pose a considerable socioeconomic burden to society, however its underlying neuronal mechanisms remained unresolved. The paraventricular thalamic nucleus is one of the midline thalamic nuclei encompassing calretinin expressing neurons (PVT/CR+) which play fundamental roles in sleep, fear and anxiety regulating circuit operations. We tested how the post-stress activity of PVT/CR+ neurons contributes to stress-related behavioural dysfunctions induced by an exposure to a natural stressor (fox odour, 2MT). We examined how optogenetic inhibition of the PVT/CR+ cells after the stress event affects nesting behaviour, locomotion, sleep, stress hormone levels, and c-Fos activity at the projection areas of the PVT/CR+ cells. PVT/CR+ neurons were inhibited using the inhibitory step-function opsin SwiChR. After five pre-stress days both inhibited (SwiChR) and control (EYFP) mice were subjected to 2MT (10 min). Immediately after the termination of stress exposure mice were photo inhibited in their homecage. During the stress exposure, both the control and SwiChR groups showed similar levels of defensive and escape behaviours. Following the stress exposure, the control group exhibited increased EMG activity, disturbed behaviour in the nest, altered slow-wave sleep, elevated corticosterone levels and increased c-Fos expression in the PVT/CR+ cells. The behavioural changes were altered for five days following the stress exposure. With the exception of corticosterone levels, photoinhibition of PVT/CR+ cells after the stress exposure (1 hour) prevented all these changes, behaviour of the SwiChR group remained unaltered for 5 days after the stress event. This suggests that acute photoinhibiton of PVT/CR+ neurons did not affect the hypothalamic-pituitary-adrenal stress response but had long-term effects on post-stress behaviour. We also tested if post-stress photoinhibition of PVT/CR+ cells is able to reverse elevated c-Fos expression in its major postsynaptic targets such as nucleus accumbens (NAc), central amygdala (CeA), basolateral amygdala (BLA) and prelimbic cortex (PrL).

Collectively, our findings indicate that post-stress activity of PVT/CR+ neurons plays an instrumental role in the emergence of stress induced behavioural changes, and post-stress photoinhibition of PVT/CR+ cells is sufficient to prevent these changes.

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P5.22 Sleep effect of bromocriptine-evoked prolactin release suppression during the reproductive cycle

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Sleep characteristics were addressed in female rats after bromocriptine treatments for 72h applied during the different stages of the estrus cycle and for 24h in the early- and middle postpartum period. Sleep changes after bromocriptine injections showed strong dependency on the estrus cycle phase of the drug application. Strongest wakefulness elevation and slow wave sleep- and rapid eye movements (REM) sleep reduction appeared during diestrus-proestrus and middle postpartum treatments.

Stronger sleep-wake effects appeared in the dark phase in case of the estrus cycle treatments, but in the light phase in postpartum treatments. Slow wave sleep and REM sleep loss in case of estrus cycle treatments was not compensated at all and sleep loss seen in the first day post-injection was gained further later. In opposition, slow wave sleep loss in the light phase after bromocriptine injections showed compensation in the postpartum period treatments.

These results can be explained by the interplay of dopamine D_2 receptor agonism, lack of prolactin release and the spontaneous homeostatic sleep drive being altered in the different stages of the estrus cycle and the postpartum period. The interplay of these factors is present in physiological sleep-wake stages although the significance of D_2 agonism may be different due to the systemic bromocriptine injections applied in this study.

Our results emphasize the role of D_2 agonism and PRL in the homeostatic sleep regulation.

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P5.23 Effects of arctigenin and trachelogenin on the hippocampus and rat ileum ex vivo

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Arctigenin and trachelogenin are dibenzylbutyrolactone lignans found in *Arctium lappa* and *Cirsium arvense*, which were used to treat gastrointestinal disorders in traditional medicine. They exhibit various biological activities, such as neuroprotective, anticancer, antioxidant, anti-inflammatory, antiviral activity, and calcium channel inhibition. The neuroprotective effects of arctigenin have been demonstrated in the neocortex of the rat brain via inhibition of AMPA/KA receptors. The aim of this study was to determine the effects of arctigenin and trachelogenin, on CA1 hippocampus and isolated ileal segments in rats.

Test lignans were isolated from *Arctium lappa* and *Cirsium arvense,* respectively, and formulated in DMSO and diluted in ACSF and Tyrode to final concentrations [1, 10, 20 and 40 microM for arctigenin] and [0.5, 1, 10 and 20 microM for trachelogenin] for brain and intestinal measurements, respectively. 400-micron thick horizontal brain slices and 1-1.5 cm long segments of distal ileum were obtained from adult male Wistar rats. Test lignans were perfused onto brain slices placed in a recording chamber and onto the intestinal segments suspended vertically in a well-aerated organ bath. In brain slices, electrically evoked field potentials were recorded, while in intestinal segments, spontaneous peristaltic movement activity was tested. Cholinergic, glutamatergic, and adrenergic antagonists, as well as compounds inhibiting nitric oxide synthase and L-type calcium channels were tested to investigate their mechanism of action in the ileum.

Arctigenin and trachelogenin showed a dose-and time-dependent decrease in the amplitude of population spikes and EPSP slope, Furthermore, they dose-dependently decreased the frequency of contractions in the ileum ($p < 0.05$). Arctigenin [20 microM] also inhibited long-term potentiation in the CA1 hippocampus ($p < 0.001$). Trachelogenin had a higher potency in both systems, as the effects of trachelogenin [20 microM] and arctigenin [40 microM] were similar. At these concentrations, there was a marked alteration in spontaneous contraction in the ileum with a significant increase in period time ($p < 0.001$).

The activity in the hippocampus is mediated via inhibition of AMPA/KA receptors, while in the ileum, it may be mediated via blockade of the L-calcium ion channel. Arctigenin and trachelogenin could serve as lead compounds for the development of alternative neuroprotective drugs.

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P5.24 Effect of Fusarium mycotoxins on glutamate receptor density and neuronal network activity after subchronic exposure in rat

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Microscopic fungi belonging to the genus Fusarium can cause serious worldwide economic and health damage. The mycotoxins that they produce can enter the human body directly or indirectly, by entering the food chain: accumulating in the contaminated forage in farm animals or consuming contaminated grain-based foods can be a serious risk to human health. Because the toxins enter into the human organism, they can disrupt several molecular processes and they can cause cell- or tissue-level changes.

During our work, we investigated the effects of 3 Fusarium mycotoxins on the central nervous system. Fumonisin B1 (FB1) impairs the membrane function by inhibiting sphingolipid synthesis. Deoxynivalenol (DON) inhibits protein synthesis, while zearalenone (ZEA) acts on estrogen receptors. These mycotoxins usually can be found together because the fungi can produce several toxins at the same time.

We investigated the effects of the three mentioned mycotoxins individually and in combination, on rat horizontal brain slices. We used Wistar rats of both sexes and they were treated for 28 days via gavage at the following mycotoxin doses: individually FB1 (50 and 500 µg/bw kg), DON (20 and 200 µg/bw kg), ZEA (20 and 200 µg/bw kg) and in combination in lower doses. We used the immunohistoblot method in the hippocampal, somatosensory and entorhinal cortex region, to compare the regional allocation and expression levels of several glutamate receptors. The particularity of this method is that - unlike with the immunohistochemistry method - no cell permeabilization is performed, however it still provides high sensitivity and good consistency, due to the parameters of the unfixed preparations, which give us an opportunity to analyze the expression level of different proteins in a semi-quantitative manner. In addition, field potential recordings were performed in the abovementioned brain areas.

In hippocampus, FB1 alone and in combination with DON had significant inhibitory effects on network excitability in male rats. ZEA mostly affected the pattern of seizure-like activity in neocortical networks in both sexes. The analysis of immunohistoblot data is still under way. In conclusion, mycotoxins may affect brain functions after subacute exposure and these effects are sex-dependent. The effect of pairwise combinations is stronger than that of individual toxins.

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P5.25 Lateral septum affects maternal adaptation via a parathyroid hormone 2 neuropeptide-containing pathway arising from the thalamus

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Parental care is a special form of social behaviour, which increases the survival of the offspring. Our research group previously described that neurons in the posterior intralaminar thalamic nucleus (PIL) express an excitatory neuropeptide, parathyroid hormone 2 (PTH2), whose expression is induced in mothers. Here, we demonstrate that PIL neurons expressing PTH2 send projection to the lateral septum (LS). Furthermore, we confirmed that lateral septal neurons show significantly elevated number of c-Fos-activated neurons in mother rats following pup exposure compared to control mothers without pup interaction. Nerve terminals of LS-projecting PTH2+ fibres arising from the PIL show the same distributional pattern within the LS as pup-activated c-Fos+ neurons. Therefore, we made further examinations and found that PTH2+ terminals closely appose c-Fos-activated septal neurons. Furthermore, PIL neurons projecting to the LS show c-Fos-activation in mother rats following interaction with the pups. Beside the LS, PTH2-expressing neurons of the PIL also send dense projection to the medial preoptic area (MPOA), therefore, we further examined this neuronal pathway. We determined that projections to LS and MPOA origin from the calbindin-positive neuron population of the PIL. By injecting different retrograde tracers into the LS and MPOA of the same rats, we demonstrated that PIL neurons projecting to the LS send axon collaterals to the MPOA, too.

We conclude that calbindin-positive PIL neurons project both to the lateral septal neurons and MPOA neurons. We investigated the septal projections where the terminals contain PTH2 neuropeptide, and innervate lateral septal neurons. Since pup exposure activates neurons in all 3 brain regions, our data suggest that PIL neurons convey the stimulatory signal of pups to both the lateral septum and the MPOA thereby contributing to their role in the maternal adaptation.

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P5.26 Inhibitory calbindin neurons of the lateral septum are involved in maternal care

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The lateral septum (LS) is a forebrain area involved in the regulation of social behaviors including maternal care. Previous studies reported that LS show elevated number of activated neurons in lactating rats. However, the mechanisms how LS neurons control social behaviors are not well understood.

To determine the type of maternally activated neurons, we addressed their distribution in mouse dams in relation to calbindin neurons. We observed a discrete group of activated calbindin neurons throughout the intermediate and ventral part of the LS. The ratio of calbindin neurons in c-Fos activated neuron population was significantly higher in the presence of the pups compared with control mothers. By using VGAT-Cre-ZsGreen reporter mice, we showed that the neurons activated by pup-exposure are all inhibitory GABAergic cells. Moreover, both calbindin and GABAergic neurons are closely surrounded by terminals containing a maternally-induced neuropeptide, parathyroid hormone 2 (PTH2) supposing a synaptic connection, which was indeed confirmed in case of GABAergic neurons by electron microscopy. We also revealed that calbindin neurons are a part of the GABAergic inhibitory population in the LS. Finally, we found that neurons of the medial preoptic area (MPOA), the major regulatory region of maternal behaviour, receives prominent input from septal inhibitory neurons. We confirmed LS to MPOA projection by retrograde tracing and observed that a relatively high number of MPOA-projecting septal neurons show calbindin positivity.

In conclusion, the results suggest that GABAergic calbindin neurons of the LS innervated by PTH2+ terminals are involved in maternal adaptation processes through their projections to MPOA neurons.

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P5.27 The localisation and potential functions of the Parathyroid hormone 2 receptor in mice brain

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The parathyroid hormone 2 receptor (PTH2R) is a G-protein coupled receptor whose endogen ligand is the tuberoinfundibular peptide 39 (TIP39). The PTH2R and TIP39 form a neuromodulator system, which plays a role in maternal behaviour, and also in the regulation of depression-like behaviour in rodents. We pursued the goal to visualise the PTH2 receptors in the brain, also describe their activity during maternal behaviour, and induced depression. The other direction of my research was to understand, how the receptor expression is related to brain oxytocin, and dopamine circuitry.

To locate the PTH2R expressing cells, we created a new mice strain, by crossing a PTH2R-Cre recombinase and a Reporter mice strain. The offsprings of these mice expressed a fluorescent protein, called ZsGreen in the PTH2R expressing cells. With this strain, we were able to map the PTH2R expressing neurons, and label them with different markers. I found PTH2R-ZsGreen cells in numerous brain regions, however, the expression density was the highest in the Arcuate nucleus (Arc) and the paraventricular nucleus of the hypothalamus (PVN). The PTH2R-ZsGreen neurons were abundant in regions, known for high expressions of oxytocin and dopamine. Therefore, we examined the relation between the receptor and these markers. I found a number of PTH2R-ZsGreen neurons in the supraoptic nucleus and in PVN, which also expressed oxytocin. In turn, in the medial preoptic area, co-localisation was found between PTH2R-Zsgreen cells and tyrosine hydroxylase (TH) labelled cells. In regions, such as the Arc and VTA, both the PTH2R- and TH-expressing cells were abundant, we still found a clear distinction in their localisation.

We described the PTH2R expressing cell activation in virgin female mice, following induced maternal-, and depression-like behaviour. We found a vast amount of activated PTH2R-ZsGreen cells in the periaqueductal grey, also in the Arc, following pup exposure. In response to forced swim test, we found extensive amount of co-localisations in the medial amygdala and the Arc.

We suspect, that the TIP39 exerts its effect trough the PTH2R-expressing cells, causing antidepressant-like behaviour, and maternal motivation. The neurons taking a part in the regulation of these behavioural pattern, are likely those, showing Fos activation following a relevant stimuli.We were able to discover more specific localization examining the dopaminerg, and oxitocinerg neurons in relation to the PTH2R.

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P5.28 Controlling pathological fear expression through closed-loop brain stimulation

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Traumatic experiences can trigger neuropsychiatric diseases such as posttraumatic stress disorder (PTSD). Mnemonic features like fear generalization and resistance to the extinction, prevents the effectiveness of psychotherapy and pharmacological treatments. Sharp-wave ripples (SWRs) and thalamocortical spindles (TCS) are well known to play a critical role in spatial memory consolidation. However, how SWRs and TCS mediates the consolidation of emotional memories is poorly understood. In this study, we evaluated the relationship between SWRs, TCS and memory consolidation after extinction sessions, a highly context-dependent process. Using Long Evans rats, we applied high intensity cue fear conditioning training to induce fear generalization and a phenotype resistant to extinction. 24h later, rats underwent extinction sessions once per day until achieving remmission (i.e. 85% reduction in freezing). Immediately after extinction, animals received 1 hour closed loop (CL) intervention by the electrical stimulation of the medial forebrain bundle (MFB) triggered by SWRs. Since the MFB stimulation could condition place preference, we hypothesize that pairing SWRs occurrences with MFB stimulation could change the emotional valence in a fear memory trace. After remission, animals were submitted to renewal test in a hybrid context 24h and 25 days later. We found that CL-SWRs stimulation, compared with open-loop and non-stimulated groups, significantly decreases the number of days to achieve a successful extinction. The low fear expression is maintained in the renewal test. This effect was prevented by the inhibition of Rac1 in the basolateral amygdala (BLA), a key-protein of dendritic spine remodeling and also by the antagonism of amygdalar D2 dopamine receptors. Finally, we reproduce our CL effect using TCS triggering MFB stimulation, suggesting that patterns detectable by non-invasive methods can also be suitable to trigger this intervention. We consider these finding as a potential strategy for the non-pharmaceutical treatment of PTSD.

P5.29 Closed-loop stimulation of infralimbic cortex reduces anxiety and prevents fear generalization during memory consolidation and reconsolidation

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Fear memory generalization is a central hallmark in the broad range of anxiety and trauma-related disorders. Recent results from our laboratory suggest that fear memories can be attenuated using closed-loop (CL) neurostimulation guided by the real-time detection of dorsal hippocampal sharp-waves ripples (dSWRs) during extinction. In this study, dSWRs were used to trigger the infralimbic cortex (IL) stimulation, a well-known cortical structure implicated in the inhibitory control of the basolateral amygdala (BLA). Using Wistar rats, we applied high-intensity cue fear conditioning training to induce fear generalization. Immediately after learning or a short memory reactivation, animals received 3h intervention using a real-time dSWRs detection inducing the IL electrical stimulation (0.2-ms pulse width, 100 µA, 100 Hz). 24h later, rats underwent a test to the conditioned stimuli (CS+) or a neutral one (CS-). In order to evaluate the effect of our intervention during fear extinction, animals were exposed to 20 non-reinforced CS+ and 24h later to a renewal test. We found that animals with CL stimulation immediately after training expressed fear to the CS+ but not CS- suggesting discrimination while open-loop (OL) and non-stimulated groups expressed fear generalization. CL stimulation lost efficacy if applied 48h after training, however, the effect can be recovered using a short memory reactivation before the CL intervention. This short reactivation promotes protein-synthesis-dependent reconsolidation since fear expression could be disrupted by intra-BLA infusion of anisomycin. The fear discrimination induced by CL intervention also contributes to fear reduction during extinction, since we found a strong correlation between discrimination and low fear expression during renewal. Both closed and open-loop IL stimulation reduced anxiety-like behavior in the elevated plus-maze. Our results suggest that dSWRs are implicated in the discrimination of cued fear memories and can be used to trigger the stimulation of cortical structures related to emotional regulation in order to enhance fear attenuation and manipulate the memory quality using memory reconsolidation-based approaches.

P5.30 Role of higher order thalamic nuclei in the cortical generalisation of Spike and Wave Discharges

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Spike and wave discharges (SWDs), generated by the cortico-thalamo-cortical (CTC) network, are pathological oscillations and the hallmark of absence seizures. SWDs begin in cortical initiation networks in both humans and animal models, including the Genetic Absence Epilepsy Rats from Strasbourg (GAERS), where they initiate in the primary Somatosensory cortex (S1). The mechanisms of how SWD rapidly spread across the brain has not been explored in detail and it remains unknown how trans-thalamic corico-cortical communication contributes to this process. We have now investigated the role of higher order thalamic (HO) nulcei in the generalisation of SWDs in freely moving GAERS. The diffuse connectivity of HO nuclei, their known anatomical interactions with S1 and their altered synaptic anatomy in GAERS make these nuclei potential candidates for the rapid cortical spreading of SWDs. Local field potential recordings of different HO nuclei and different cortical regions revealed a novel feature of SWDs: the synchrony in cortical regions located far from S1 (such as primary Visual cortex, V1) transiently disappeared, i.e. there are short breaks in SWD. Additionally, relatively brief SWDs would occur only in S1 or in S1 and the neighbouring primary Motor cortex (M1), but not elsewhere. These spontaneous events provded a unique mechanistic insight since they represent unsuccessful maintenance and generalisation of SWD. Local inhibition of different HO nuclei with muscimol increased the delay of SWD propogation and the occurance and duration of SWD-breaks. Moreover, HO nuclei single unit recordings revealed heterogeneity of burst firing dynamics during SWD of putative excitatory neurons. Noteably, all neurons exhibited a switch from tonic to burst firing before the generalisation of SWD, but responded differently during ictal periods, V1 SWD-breaks and non-generalised SWD events. Selective activation of HO TC neurons resulted in altered generalisation of SWDs. These results support the view that trans-thalamic cortical communication is utilised in the initial propogation of SWD and has an active role in maintaining cortical sychrony throughout the paroxsysmal activity.

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P5.31 Gap junction mediated ganglion cell population code serves equalization of response kinetics and corresponding visually guided behavior in the retina.

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Human vision is responsible for receiving ~80 % of environmental information, perceived and preprocessed by a complex neural network in the eye, the retina. Several parallel signaling pathways arise from photoreceptors (PRs) and diverge prior to reaching the retina's output, the retinal ganglion cells (RGCs) which summate input information to an action potential (AP) pattern, which is the code interpreted by higher visual brain centers. Scientists distinguish 20-30 RGC types, however, the number of visual aspects exceed this number. The retinal neural network solves this problem by allocating a single RGC subtype to encode more than one visual aspect. Accordingly, in addition to generating an individual code, some RGCs are also involved in generating a population code to inform the brain about multiple visual aspects. The population code requires correlated activity of cells, which is often achieved in the retina by electrical coupling of neighboring same subtype RGCs and/or ACs. One such RGC is the Transient OFF Alpha (OFF_TAlpha) that has recently been identified as the approach detector that upon activation triggers escape behavior. We combined multielectrode-array electrophysiological recordings, Ca^{2+}-imaging, gap junction blockade pharmacology (meclofenamic acid – MFA), Neurobiotin tracer injections and behavioral tests in both wild-type and GMO mice to examine how electrical coupling of neurons in the OFF_TAlpha RGC array underlie the approach detection and the corresponding escape behavior of mice. We found that the MFA induced GJ block, in general, reduces the length of RGC responses and more importantly increases the variability in response transience values for cells in the OFF_TAlpha RGC array. Since response transience is a foremost signal feature, we assumed that the observed GJ mediated transience equalization is essential in performing the OFF_TAlpha RGC function, the approach detection. In fact, our behavioral tests indicate that the approach detection and the corresponding escape behavior are suppressed by the blockade of GJs. As GJ coupling strength and thereby the above signal equalization effect can easily be altered, we hypothesize that the visual system utilizes GJ gating to switch the encoding dominance between approach detection and contrast detection by opening and closing GJs, respectively.

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Poster session - Topic 6
Cognitive neuroscience

P6.01 Resting-state delta- and theta-band EEG functional connectivity in schizophrenia

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Background: Schizophrenia is a serious and complex mental disease, known to be associated with various subtle structural and functional deviations in the brain. Recently, increased attention is given to the analysis of brain-wide, global mechanisms, strongly altering the communication of long-distance brain areas in schizophrenia.

Methods: Altogether, 37 patients with schizophrenia and 33 matched healthy control subjects were enrolled in our study. Two 2 minutes long 64 channel EEG recordings were registered during resting (in eyes open and eyes closed conditions respectively). Average connectivity strength was estimated with Weighted Phase Lag Index (wPLI) for delta (0.5-4 Hz) and theta (4-7 Hz) frequency bands. In order to analyze functional network topology Minimum Spanning Tree (MST) algorithms were applied.

Results: Results show that patients have weaker functional connectivity in both delta (eyes closed condition) and theta (eyes open condition) frequency bands. Concerning network differences, the result of lower diameter, higher leaf number, and also higher maximum degree and maximum betweenness centrality in patients suggest a star-like, more random network topology in patients with schizophrenia.

Conclusion: Our findings of disturbed global functional connectivity in patients are in accordance with some previous findings based on resting state EEG (and fMRI) data, suggesting that MST network structure in schizophrenia is biased towards a less optimal, more centralized organization.

P6.02 Unique and Shared Neural Codes in Familiar Face Perception

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The neural processing of familiar and unfamiliar faces received much attention in recent years, with the nature of shared and unique information processing being a matter of intense research [1, 2]. Here, we used two independent methods, neural fingerprinting, and cross-participant multivariate pattern analysis (MVPA), to test the contributions of unique and shared patterns of activity in response to familiar and unfamiliar faces in the EEG evoked response.

MVPA uses machine learning classifiers to probe the presence of information in the neural signal patterns useful to distinguish between stimulus categories. EEG fingerprinting on condition-averaged whole-epoch data has recently been demonstrated to be capable of identifying individual participants based on their unique correlation patterns across electrode pairs [3]. In this study we used both methods in a time-resolved manner to test the evolution of these factors across the time-course of the signal.

We re-analyzed EEG data from four experiments, (overall n = 82) published in two studies by Wiese et al. [4, 5] in which participants were exposed to trial-unique photographs of an unknown and a long-term personally familiar person.

The unique neural patterns across conditions identified individual participants remarkably well, most prominently in the first 400 ms after stimulus onset. We also observed very high decoding accuracies for long-term familiar and unfamiliar faces across participants, which further supports a shared neural code for face-familiarity, even for long-term, pre-experimental personal familiarity. The time-course of this signal was consistent with those reported in previous studies [1, 5, 6], showing a rapid ramp-up around 200 ms post-stimulus, simultaneously with the tapering off of the unique component. Significant unique and shared signals overlapped in the 200 to 400 ms time window.

These results show that time-resolved neural fingerprinting can supplement other methods to probe the information present in neural time-series signals.

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P6.03 Multisensory information improves the performances in associative learning in healthy children

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In healthy adults the visually and multisensory (audiovisual) guided associative learning is similarly effective, thus these processes seem to be independent from the stimulus modality. The multisensory processes are changing during development and that is an interesting question, whether the multisensory information could help to children and adolescents in associative learning. In this study, visual and audio-visual equivalence learning in 157 healthy participants younger than 18 years were investigated. Visually and audio-visually guided acquired equivalence learning paradigms were applied. The performances in the acquisition phase (building of associations), which primarily depends on the function of the basal ganglia were significantly better in the multisensory paradigm but there was no difference between the reaction times in this phase. The performances in retrieval part of the test phase, where the earlier learnt associations were asked were also significantly better and the reaction times were significantly shorter in the multisensory paradigm. The performances in the transfer (generalization) part where hitherto not learnt but predictable associations were asked was not enhanced by the multisensory stimuli but the response latencies were shorter here, too. The members of both age-groups below and above 12 years old learnt and recalled the multisensory associations more effectively than the unimodal visual one. Our results demonstrated that the performances in associative learning and the connected memory processes are facilitated by the multisensory stimuli, which was most obvious during building and retrieval of associations. Thus, the multisensory information and the connected multisensory integration in behavioral level could significantly enhance the performance in sensory guided equivalence learning in healthy children and adolescents.

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P6.04 Age-related functional disconnection between the anterior and posterior regions: evidence from cross-frequency coupling and directed connectivity measures

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Neural interactions between brain regions were modeled by the strength and direction of the information propagation within and between distinct frequency bands. A common feature of these models was to characterize aging as a functional disconnection between the anterior and posterior brain regions. However, previous methods to characterize the changes of age-related information propagation based on EEG signals were limited to undirected and within frequency connections. In the current study, we applied the generalized orthogonalized partial directed coherence (goPDC) to measure within frequency directed connections and the modulation index (MI) to measure between frequency phase-amplitude coupling. Eyes closed resting-state EEG was recorded in young (N=22; mean age= 22.4 ±3.1) and elderly (N=19; mean age= 66.3 ±3.9) healthy subjects. The EEG was filtered to distinct frequency bands: theta (4-8 Hz), alpha1 (8-10 Hz), alpha2 (10-13 Hz), beta (13-30 Hz) and gamma (30-45 Hz) and connectivity was measured between the anterior (pre-frontal, frontal) and posterior (parietal, occipital) regions. In the elderly compared to the young an increased delta and decreased apha1, alpha2 and beta anterior to posterior goPDC connectivity was found, with a decreased gamma posterior to anterior goPDC connectivity. In the elderly compared to the young a decreased modulation of the posterior beta amplitude by the phase of the anterior lower frequency bands was found. Also, decreased modulation of the gamma amplitude of the anterior regions by the beta phase of the posterior regions was observed in the elderly compared to the young group. These results emphasize that age is predominantly associated with decreased anterior to posterior directed connections.

P6.05 Functional connectivity mapping of sensory pathways using flavoprotein imaging

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The general anatomy of somatosensory or auditory pathways are well known, the nuclei of brainstem, diencephalon involved in sensory processing were identified several decades ago. The primary sensory areas of the brain – receiving this information – show columnar organization, which makes the analysis of afferent information possible based on the different aspects of it. Due to this organization of cortical regions the tactile information of individual whiskers, or sensation of low and high frequency sounds separates well in corresponding sensory area. The anatomical background of somatotopic or tonotopic information processing were characterized in detail, however there are huge variances in the organization of these pathways among individual animals, making the identification of a given cortical column difficult. The aim of the given project is to provide a method for fast mapping of columnar organization

Experiments were carried out on C57BL/6 wild type adult mice, under urethane anesthesia. Electrophysiological recordings were made by silicon probes from somatosensory and auditory cortices, while for flavoprotein imaging (FI) continuous 480nm light illumination was used for optical data acquisition. Single whiskers were stimulated by air puffs, and in another set of experiments 12 or 25 kHz sinusoid sound waveforms were used to induce event related responses of corresponding sensory cortices.

The flavoprotein imaging as method for mapping cortical activity over a large region of brain surface proved to be useful to identify the primary sensory field of applied stimuli. In the somatosensory system the somatotopic organization was clearly visible on activation maps provided by FI, subsequent stimulation of 2-3 whiskers showed intense optical signal in non-overlapping small, barrel-like regions, in high proximity to each other. In the case of auditory stimulation the tonotopic organization was also visible, but overlapping of sensory fields were also present. Silicon probe recordings of high optical activity areas showed immediate and robust response in multi-unit activity, while after clustering, a small number of neurons with high modulation index were also found. The injection of cholera toxin subunit B into optically active barrels proved that the sensory information was originated from ventral posteromedial nucleus of thalamus, and multi- or single unit activity of this thalamic region also showed direct lemniscal input.

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P6.06 Development of mental fatigue detection headset

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In our highly technological and information-oriented society many people frequently experience fatigue after spending a considerable amount of time on mental tasks. Opposed to physical fatigue, mental fatigue is not necessarily accompanied by low energy levels but by drowsiness, difficulty in concentrating, decreased alertness, slower reaction times, reduced work efficiency, and it can lead to critical errors resulting in major financial costs or even in the loss of human lives. It's early and accurate detection is therefore of upmost importance to corporations involved in complex decision making with high risks, and to humanity in general.

It is well known that mental fatigue can be identified using a standard scalp 10-20 EEG, but this method has considerable technical difficulties that prevent it from widescale usage. We set out therefore to create a custom 3D-printed headset with platinum electrodes, that has more potential for application. For this end, we needed to find out which electrodes are best for detecting mental fatigue. After gathering data from publicly available EEG databases recorded during lengthy simulator driving sessions, we developed a genetic algorithm with linear SVM and logistic regression classifiers using fuzzy entropy and power spectrum measures for feature selection. We found that electrodes FP1, FT7, T4, TP7, Oz and O2 were the best, most robust combination for detecting mental fatigue, with an average 90% accuracy.

We also designed an experiment based on a NASA Cognitive Performance Assessment Tool, consisting of a simple reaction test, a motor skill evaluator, a BART risk decision making task, a matrix reasoning test and a memory exercise, with resting states at the beginning and the end. EEG will be recorded with our headset throughout the whole experiment. Mental fatigue will be estimated based on performance scores and self-reports. This experiment will be performed with multiple subjects, once in the morning when they are well-rested, and once in the afternoon after an arduous day. After gathering and analyzing the data, we hope to develop a reliable fatigue detection package (hardware and software), intended for common use, and if the results are promising then even industrial application cannot be ruled out.

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P6.07 Visually guided associative learning and related memory processes in pediatric and adult migraine patients

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Migraine is an attack like recurring unilateral throbbing headache, which can be accompanied by vomiting, nausea, sensitivity to light and sound. Cognitive performance deteriorates during migraine attacks but the long-term effects of the disease on cognitive functions are less known. In the present research, we aimed to compare the learning capabilities of pediatric and adult migraineurs in a special visually guided associative learning paradigm.

Rutgers acquired equivalence test (RAET or Fish-Face test) was applied to check the associative learning in pediatric and adult migraine patients. In the first, acquisition phase of the paradigm the patients had to learn different pairings between the cartoon faces and different colorful fishes based on the feedback information of the program. This learning process is connected primarily to the function of the basal ganglia frontal lobe loops. In the test phase which is primarily connected to the hippocampus-mediotemporal lobe without any feedback information, the participant had to recall the already learned pairs (retrieval part) and had to build hitherto not seen but predictable associations (generalization or transfer part). In the present study 27 migraine pediatric patients, 27 gender, age and intelligence range matched healthy children, 22 adult migraineur and 22 age, gender and level of education matched control participants were involved.

The adult patient population were less efficient in forming associations [P= 0.04] so as in the generalization [P<0.01] then the healthy control group. In contrast, the retrieval part was not affected by adult migraineours. On the other hand, the performances of the pediatric patients were not worse than those of the healthy children population.

Our results indicated that the chronic migraine by reaching adulthood could influence both the basal ganglia and the hippocampus-medial temporal lobe function in associative learning. In contrast, these cognitive deficits can not be detected in pediatric patients. The deficit of equivalence learning is not an inherent feature of the migrainous cognitive profile, rather the result of the attacks' interference with the development/function of the underlying structures.

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P6.08 Novel experimental paradigm for testing palatability-driven intertemporal food choice of nonhuman primates

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Maintaining a healthy diet entails acquiring and consuming food that meets physiological and hedonic needs in a balanced manner. In humans, all instantaneous decisions of whether and what to eat have to be made with subsequent meals of the day in mind, thus requiring (either 'instinctive' or deliberative) intertemporal choice. For animals, foraging in complex natural habitats arguably involves similar decision making – in spite of that, self-regulation and intertemporal choice in animal feeding behavior on the time scale of daily/weekly feeding schedules has rarely been addressed.

We studied daily operant food intake (FI) in five young adult male rhesus macaques in two sessions (S1: from 9 to 11 am, and S2: from 12 to 1 pm) with four types of 'diet' composed of two types of food pellets with differing palatability. Food preference was previously found to be uniform in all five subjects: banana flavoured pellets (1gm, 3.35 kcal/pellet) as low-palatability meal and 'very berry' flavoured pellets (1gm, 3.46 kcal/pellet) as high-palatability meal. Food intake in all possible combinations of the two types of pellets were assessed: S1 banana / S2 banana, S1 banana / S2 very berry, S1 very berry / S2 banana, S1 very berry / S2 very berry. Each individual pellet combination plan was offered for 5 consecutive experimental days, from Mondays to Fridays.

The animals consumed significantly more from the more palatable meal in S1. Intriguingly, FI in S1 strongly depended on the food offered that week in S2: animals ate significantly less in S1, when S2 meal was highly palatable, compared to weeks with low-palatability S2 meals. Importantly, this modulation of S1 FI depending on S2 palatability was only present from Tuesdays to Fridays but not on Mondays, when the weekly diet schedule was not yet known by the animals. In general, the animals decreased their FI in S1 to consume the more palatable meal later in S2 (reward comparison effect).

The present results suggest that macaques may well anticipate their daily feeding schedule based on the experienced stable temporal contingencies on the palatability of the available food. Downregulating instantaneous food consumption in anticipation of more palatable food hours later represents a case of intertemporal choice with an unprecedented long time scale in macaques. Notably, this pattern of behavior is consistent with dietary self regulation in humans.

P6.09 Characterizing the multifaceted interference phenomena in non-human primate object-location working memory

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Interactions between multiple items to remember are usually detrimental to memory performance - that is, they cause interference. Interference is arguably the primary limiting factor of short-term memory in humans and animals alike. Most human research on interference use word lists which is not feasible for animals, while animal studies frequently employ e.g. the same-different task that would be too easy for a neurotypical human. To remedy this and foster the translatability of working memory research, here we studied interference in a short-term memory task requiring object-location binding that can be used in both humans and non-human primates.

In each trial of the Paired Associates Learning task (PAL) the subject has to recall the locations of several distinct sequentially presented schematic visual stimuli. After extensive training, 12 young male adult rhesus macaques were able to perform the task on a 67-stimulus set with up to 3-7 stimuli per trial. First, we show that these animals exhibited strong recency but very weak primacy effects that were also not amenable to training. Second, we show that the animals generalized their task performance skill in just 3-4 sessions to previously unseen stimuli from a large (n=670) new stimulus set. The new large stimulus set was introduced to better control between-trial interference. Third, when we introduced stimulus recurrence from the previous trial, a very strong between-trial proactive interference effect emerged leading to 20-30% performance decrement for the affected memory item, which gradually tapered off if the interfering memory came from earlier trials. Importantly, this interference effect was very stable even after months of daily training in the task, indicating that the interference effect measured here represents an important inherent limitation in the animals' memory performance.

To conclude, the observed serial position effects suggest that on the within-trial level, object-location working memory performance in macaques may primarily be affected by retroactive interference and minimally by proactive interference. In addition, the very strong and stable between-trial proactive interference effects underline the importance of interference as a limiting factor in non-human primate short-term memory. Our results confirm that interference effects in macaques that are analogous with human list memory effects can be successfully studied using a visual object-location short term memory task.

P6.10 History of early-life stress influence face emotion recognition in depressed patients: A functional magnetic resonance imaging study

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BACKGROUND: Childhood adversity is a strong risk factor for the development of various psychopathologies including major depressive disorder (MDD), however, not all depressed patients experience early life stress (ELS). Functional magnetic resonance imaging (fMRI) studies using face emotion recognition (FER) tasks have documented altered blood-oxygen-level-dependent (BOLD) responses in specific cortico-limbic networks both in MDD patients and in individuals maltreated at an early age. Therefore, a history of ELS may represent a key modulating factor responsible for the altered processing of socio-affective cues.

OBJECTIVE: The aim of our present study was to investigate the impact of ELS on brain activity during a face emotion recognition task in MDD patients with and without the history of ELS.

METHODS: ELS was assessed with the 28-item Childhood Trauma Questionnaire. MDD patients with ELS (MDD+ELS, *n*=21), MDD patients without ELS (MDD, *n*=19), and healthy controls (HC, *n*=21) matched for age, sex and intelligence quotient underwent fMRI while performing a block design face emotion recognition task with images portraying negative emotions (fear, anger and sadness).

RESULTS: In MDD+ELS patients, accuracy to recognize face emotions, especially sad faces, was impaired. We found no between-group difference in positive BOLD response during the FER task, but analysis of negative BOLD responses revealed significant between-group differences. MDD+ELS subjects had significantly reduced negative BOLD signals in their right accumbens, subcallosal cortex, and anterior paracingulate gyrus compared to controls. Also, MDD+ELS patients had a reduced negative BOLD response in their anterior paracingulate gyrus relative to the MDD group. MDD+ELS subjects had a significantly increased negative BOLD response in their right precentral and postcentral gyri compared to controls.

CONCLUSIONS: Our data support the concept that ELS can have a long-lasting effect on the functioning of key reward-related fronto-striatal as well as somatosensory neural circuits and that these alterations contribute to the impaired emotion processing of individuals with major depression and ELS.

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P6.11 Validation of non-invasive pharmaco-electroencephalography in rhesus macaques performing a simple fixation task

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Resting-state electroencephalography (EEG) in non-human primates (NHPs) is traditionally recorded invasively and often in a sedated state, whereas human recordings are conducted in an awake state under continuous fixation. To address this gap and realise a translationally valid experimental setting for NHPs, we created a trial-based simple visual task and trained two male rhesus macaques (*M. mulatta*) to fix their gazes on a cross-shape stimulus on the screen for a certain duration in each trial. Animals were seated in a primate chair without head fixation and positive reinforcement training (PRT) was used to minimize movements, wherein head motion and gaze stability criteria (measured by infrared eye-tracking) were progressively narrowed while fixation time within the trial was gradually prolonged during PRT. After systemic application of behaviourally relevant doses of psychopharmacological agents, pupil diameter and high-density telemetric EEG (from 27 active scalp electrodes) were simultaneously recorded.

Diazepam, a positive allosteric modulator to the $GABA_A$ receptors, induced a marked increase in beta oscillatory power and decrease in gamma power even compared to baseline spectra (non-treatment days, n=15). Scopolamine, a muscarinic acetylcholine receptor antagonist, induced similar effects in beta and gamma band activity, however, the observed spectral change was of a smaller scale, closer to the variability of the baseline days. In addition, for scopolamine, tonic pupil diameter increased in a dose-dependent manner, but task-related (phasic) pupillary responses were reduced.

Furthermore, pharmacodynamic effects were investigated as a function of post-injection time. While the spectral EEG changes for diazepam were relatively stable within each session, scopolamine effects peaked around 50-60 min. Task performance showed similar temporal dynamics, but, unexpectedly, reached its lowest level approximately 10 min earlier.

The robust EEG effects of diazepam with a frontal focus were consistent with the literature. Interestingly, the effects of scopolamine were in line with previous NHP results but not with human data, which might be due to the inconsistency of keeping the eyes open or closed during EEG recording. To sum up, we have successfully developed a non-invasive, minimal-restraint NHP recording setup coupled with a PRT framework that may open new avenues for preclinical pharmaco-EEG research with high translational potential.

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P6.12 ELVISort: Encoding Latent Variables for Instant Sorting

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Introduction. The growing number of recording sites of silicon-based probes means that an increasing amount of neural cell activities can be recorded simultaneously, facilitating the investigation of underlying complex neural dynamics. In order to overcome the challenges generated by the increasing number of channels, highly automated signal processing tools are needed.

Aims. Our goal was to build a spike sorting model that can perform as well as offline solutions while maintaining high efficiency, enabling high-performance online sorting.

Method. We present ELVISort, a deep learning method that combines the detection and clustering of different action potentials in an end-to-end fashion. The architecture of ELVISort is based on a special type of autoencoder, a beta-variational autoencoder. During training, the model has 3 output branches, with different loss functions, combining supervised learning with unsupervised one.

Results. The performance of ELVISort is comparable with other spike sorting methods that use manual or semi-manual techniques, while exceeding the methods which use an automatic approach: ELVISort has been tested on three independent datasets and yielded average F1 scores of 0.96, 0.82 and 0.81, which are comparable with the results of state-of-the-art algorithms on the same data. We show that in addition to the good performance, ELVISort is capable to process data in real-time: the time it needs to execute the necessary computations for a sample of given length is only 1/15.71 of its actual duration (i.e. the sampling time multiplied by the number of the sampling points).

Conclusion. ELVISort, because of its end-to-end nature, can exploit the massively parallel processing capabilities of GPUs via deep learning frameworks by processing multiple batches in parallel, with the potential to be used on other cutting-edge AI-specific hardware such as Tensor Processing Units, enabling the development of integrated, portable and real-time spike sorting systems with similar performance to offline sorters.

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P6.13 Saliency-map-based feature selection for electrocorticography-based brain–computer interfaces

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In brain–computer interfacing (BCI), electrocorticography (ECoG) is mainly used for capturing fine motor-imagery-related cortical patterns. In practice, the majority of ECoG electrodes are implanted into epileptic patients scheduled for neurosurgery and serve diagnostic purposes reflected in the electrode placement, as well. Thus, a large number of ECoG channels prove to be redundant during signal processing and is beneficial to be removed—opening a niche for feature selection algorithms.

We implemented a two-dimensional convolutional neural network (2D-CNN) and a smaller dense network (DNN). The former was trained on the data of 16 subjects performing simple, repetitive hand, foot and tongue movements (the recordings were acquired at the National Institute of Mental Health, Neurology and Neurosurgery). As training and test samples, the amplitude spectrum of 1-second-long data chunks restricted to the (0, 200] Hz range were applied. After the training, saliency maps for each movement type were produced using the test data: these maps provide a quantitative measure of the importance of a specific feature (i.e. a frequency component on a particular channel) during classification. The most salient features were selected and the small DNN was trained using them (we applied the 1, 10, 100 and 1000 best features for each category). We performed 3 training/test sessions for each subject.

The accuracy of the 2D-CNN applied as baseline was above 82.5 % for each subject (with an average of 95.6 %). Using only 1 feature per class, the accuracy of the DNN was insufficient, only 51.5 % on average (53.6 % of the baseline). 10 features per class yielded an average accuracy of 69.1 % (72.1 % of the baseline). 100 features per class gave the greatest accuracy, 81.9 % (85.8 % of the baseline); a slightly smaller value, 81.6 % (85.3 % of the baseline) was obtained using 1000 features per class.

The 2D-CNN uses 12288 features in average; our finding supports the thesis that satisfactory performance can be obtained applying a much smaller network using the appropriate features that can be more efficient in terms of runtime and hardware resources.

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P6.14 Impaired multisensory integration in pediatric OCD patients in association learning at behavior level

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Several neurobiological differences can be found in patients with obsessive compulsive disorder (OCD), i.e. higher activation in the limbic frontal and the frontal associative cortex and the connected deep brain structures in the basal ganglia. Thus, the cortico-basal ganglia-cortical loops seem to be involved in the pathogenesis of OCD. While the multisensory integration is an important function of the basal ganglia we have aimed to check whether this is influenced at behavior level in OCD patients.

The visual and multisensory Acquired Equivalence Learning Tests were applied to observe the visual and multisensory learning in pediatric OCD patients. The paradigms have two main phases, the acquisition and the test. In the acquisition phase, which depends primarily on the basal ganglia, patients had to build different associations between cartoon faces and colorful fishes (visual paradigm), or between sounds and cartoon faces (multisensory paradigm) based on the feedback of the computer. In the test phase, which depends primarily on the hippocampi, the participant had to recall without any feedback information the already learned pairs (retrieval part) and had to build hitherto not seen but predictable associations (generalization or transfer part). In the present study, 28 pediatric OCD patients were involved.

The multisensory information could not improve the learning effectivity of the pediatric OCD patients in the acquisition phase. Similarly, the performances in the retrieval and the transfer parts of the multisensory test were not better than those in the visual test. On the other hand, the reaction times were significantly shorter in the retrieval and generalization parts of the test phase in multisensory paradigm.

Previous results demonstrated that multisensory information could facilitate the performance of healthy children in acquired equivalence learning paradigm. However, the present results could indicate that the pediatric OCD patients use less effectively the multisensory information (most probably from the weaker multisensory integration) than the healthy control children.

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P6.15 Familial risk factors of amblyopia and amblyogenic conditions

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Background: Amblyopia ('lazy eye') is a neurodevelopmental disorder of binocular vision which is the leading cause of visual loss in childhood. The amblyogenic conditions (such as anisometropia, strabismus, hyperopia) are accompanied with the impaired or lack of binocular depth perception, which potentially can lead to amblyopia without treatment. The aim of this study was to identify the risk factors of amblyopia and amblyogenic conditions in children.

Materials and methods: A total of 129 children aged 4-14 years (mean 8.14) were went through routine ophthalmologic examination and the family histories of their parents and siblings were gathered with a short questionnaire at the Department of Ophthalmology, University of Pécs, Hungary and Vithas Medimar Internacional Hospital of Alicante, Spain.

Results: Amblyopia was identified in 16% of the participants while amblyogenic conditions in 22%. The hyperopia in family history showed correlation with diagnosis of hyperopia ($p < 0.001$), strabismus ($p = 0.018$) and strabismic amblyopia ($p = 0.049$). There was also correlation between the strabismus in close relatives and the amblyogenic conditions in examined children ($p = 0.034$). Negative relationship was found between the myopia in family history and the tested children with strabismus ($p = 0.009$) and strabismic amblyopia ($p = 0.005$).

Conclusions: Our data support those previous observations that amblyogenic conditions show familial accumulation. Hyperopia or strabismus in family history increased the chance of emergence of strabismic amblyopia and amblyogenic conditions. The negative correlation between the myopia and strabismus/strabismic amblyopia can be explained by the fact that myopia and the hyperopia mutually exclude each other on the same eye. If it occurs on a separate eye in that case the diagnosis is anisometropia. Thus myopia by itself is not an amblyogenic condition.

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P6.16 Beta activity during implicit, visual statistical learning

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The ability to grasp relevant patterns from a continuous stream of environmental information and build them into an internal representation of our surrounding is called statistical learning (SL). SL has been demonstrated in visual, auditory, haptic, and multimodal paradigms, but very little is known about the neural background of its emergence. We aimed to examine the cortical activity during the emergence of implicit, visual SL.

Twenty-two healthy, right-handed volunteers with correct or corrected-to-normal vision were involved in our study (female: 13, mean age: 25.1). Three runs, each with 16 images of objects (4 associated stimulus pairs and 8 control, single stimuli) were used in the experiment. The runs had two parts: in the first part, every image was presented 10 times in a completely random sequence and then in the second structured part, the stimulus pairs and the control stimuli were shown 15 times. The parameters of our paradigm were determined based on previous behavioral studies, where the sequence was generated in the same way and the SL was demonstrated. During the task, 64 channel EEG was recorded and afterward, time-frequency (TF) analysis was performed.

We examined the TF representation of all trials across the whole scalp and found a power elevation in the 10 to 30 Hz frequency range, at 800 to 1300 ms after stimulus presentation. The first and second members of the associated pairs were compared to the control stimuli. We performed a permutation-based z statistic in the TF window explained above with a significance threshold of 2.5% to determine clusters. This way, we found a cluster in the frequency range 13 to 30 Hz in the time window of 1000 to 1100 ms after stimulus presentation. This difference was presented only between the first member of the pair and the control stimulus. To localize where this difference emerges from, we compared the average values of the TF window of interest in each channel. These values showed a significant difference in the frontal electrodes near the midline and the left parietal electrodes.

In conclusion, a beta-band power difference can be found during the late processing period of the first member of the associated stimulus pairs, which overlaps with the prestimulus interval of the predictable, second stimulus. This beta activity difference can represent anticipation for the successive stimuli.

P6.17 The effect of stimulus complexity on acquired equivalence learning

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The Rutgers Acquired Equivalence Test (RAET) applies complex stimuli, which could help the participants to learn the associations. To avoid this effect on associative learning we have developed a modified equivalence-learning test (Polygon) with the same structure as the original RAET. The only difference in the Polygon test is the application of simple white-gray-black geometric forms instead of faces and fishes in RAET. In the present study we have compared the psychophysical performances of the same healthy volunteers in equivalence learning and the connected memory processes in RAET and Polygon test. The equivalence learning, which is connected primarily to the function of the basal ganglia, is strongly influenced by the stimulus complexity. The simpler the stimuli the worst are the performances here. On the other hand, the stimulus complexity had no effect on the performances in the retrieval and transfer phases of the test part, which is primarily connected to the function of the hippocampi. Because of the increased sensitivity of the Polygon test in equivalence learning it might be a suitable tool to find such weak or beginning learning disabilities in disorders connected to the dysfunction of the basal ganglia, which were hitherto not detectable with the original RAET.

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P6.18 Neuropeptide QRFP improves memory in rats

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The worldwide increasing incidence of dementia and other psychiatric diseases, associated with cognitive disorders, led to high research interest. A deep understanding of brain neuropeptides' actions and interactions would open new possibilities in treatment and diagnostic techniques. Several members of the RF-amide peptide family have been implied to modify memory and learning processes in different species. Nevertheless, almost nothing is known regarding the cognitive effects of recently discovered neuropeptide QRFP. The present study was designed to investigate the possible effects of QRFP on spatial memory.

While QRFP binding sites are widely spread within the CNS, the QRFP-synthesizing neurons are almost exclusively present in the hypothalamus. The medial hypothalamic area (MHA), including dorsomedial and ventromedial nuclei, and the lateral hypothalamic area (LHA) were chosen for treatment. The effects of two doses (200 ng and 400 ng) of QRFP on spatial memory were investigated in the Morris water maze paradigm. After that receptor antagonist BIBP3226 was applied to elucidate whether it can prevent the effects of QRFP. To reveal possible changes in anxiety level, animals were tested in the Elevated plus-maze.

Application of QRFP (400 ng) both into the MHA and the LHA improved short-term memory consolidation in the Morris water maze. Pretreatment with antagonist BIBP3226 abolished the cognitive effects of QRFP. The neuropeptide did not affect the anxiety level of rats. This study provides unique evidence regarding the role of QRFP in the consolidation of memory and gives the basis for further investigations of neuropeptide's cognitive effects.

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P6.19 Interactions between external and internal attention processes during working memory task

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A number of reviews have examined the interaction and similarities between the broad categories of attention and working memory. These literatures converge on the prospect that attention to internal representations and to external stimuli occurs via a shared resource, resulting in a competitive interaction between external and internal attention. However, it seems that all the studies that has been reviewed before modulated only limited number of factors that contribute to drive our attention internally versus externally. In this current study our aim was to develop a new working memory paradigm that investigates the interactions of more of several factors associated with external and internal attention.

We used a modified version of the acquired equivalence test. The main task was to recall color-shape associations between segmented circles with different colors and white shapes. The following parameters were manipulated during the task: number of segments of the circle, the switch between memory items from trial to trial, the switch of target location from trial to trial, and the number of associations that needed to be maintained (working memory load). As a pilot study we registered the data through online distribution of the task using EprimeGo. To obtain other measures than behavioural data, we also began with on-site experiments, where we registered eye-movement data as well.

In total, 64 healthy volunteers participated in the study. Part of the participants had also eye-movement data (n=14). Our results showed significant interactions between all the investigated factors during the task, indicating that external and internal attentional subprocesses share a common resource pool. Furthermore, the effect sizes for eye-movement data were larger than for the behavioural measures, indicating that eye-movement data can dissociate more sensitively the attentional subprocesses.

Our results indicate that the developed cognitive test is sensitive enough to dissociate different attentional processes, and we suggest to further use it in clinical studies.

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P6.20 Combined application of memantine and alpha7 nicotinic acetylcholine receptor agonist PHA-543613 improves novel object recognition memory in aged rats

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Aging is generally related to emerging neurocognitive disorders (NCDs), that pose serious public health problems worldwide. Since rodents show age-related cognitive impairment and certain pathological aspects of NCDs, they are intensively used for development of novel treatment strategies. Combination therapies using memantine and cholinesterase inhibitors have recently showed limited superiority over their monotreatments. However, combined treatments may reach better efficacy when memantine is combined with alpha7 nicotinic acetylcholine receptor (nAChR) agents.

Here our aim was to test the hypothesis that the cognitive enhancer effect of memantine in a naturally aging rat model can be potentiated through simultaneous modulation of alpha7 nAChRs. Effects of monotreatments as well as co-administration of the selective alpha7 nAChR agonist PHA-543613 and memantine were tested on declarative memory of rats using the novel object recognition (NOR) paradigm.

First, the dose-response relationship was determined for PHA-543613 and memantine. Effects of PHA-543613 were tested at 0.3 mg/kg, 1.0 mg/kg and 3.0 mg/kg doses, while memantine was administered in the doses of 0.1 mg/kg, 0.3 mg/kg and 1.0 mg/kg. Results show that PHA-543613 in 0.3 mg/kg dose improved the discrimination index of the aged animals as they explored the new objects more than the familiar ones. Memantine monotreatments were effective in the 0.1 mg/kg dose. Next, the effects of combination treatments were tested with very low memantine (0.01 mg/kg) and PHA-543613 (0.1 mg/kg) doses. We showed, that monotreatments with subeffective dose of memantine and PHA-543613 did not improve the declarative memory impairment induced by aging. However aged animals who received the combination treatments, performed above the level of subeffective monotreatments.

Results suggest the existence of a beneficial interaction between memantine and alpha7 nAChR ligands in terms of cognitive enhancement, and provide further evidence supporting the hypothesis on the prominent role of the alpha7 nAChRs in the procognitive effects of memantine.

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P6.21 Evidence for a general neural signature of face familiarity

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The emergence of neuronal representations of face familiarity has been widely investigated, and there is evidence for differential processing of familiar and unfamiliar faces. [1, 2] In this study, we investigated the existence of a neural signature of face familiarity using a cross-experiment decoding design. We analyzed event-related potentials evoked by unknown and experimentally familiarized faces from a recently published study by Ambrus et al., using a set of experiments involving different participants, stimuli, and modes of familiarization. [1] Participants were either familiarized perceptually (Perceptual: N = 42), via media exposure (Media: N = 24), or through personal interaction (Personal: N = 23), with no participant taking part in more than one experiment. The focus of our study was to investigate, whether there is a shared signal of face familiarity, that is detectable across these different methods of familiarization. Therefore, we decoded familiarity evoked response patterns from participants in one experiment, using classifiers trained on the aggregated data of the participants of another experiment, in which a different familiarization method was used. We detected significant familiarity decoding across all three experiments, which were most pronounced predominantly over posterior and central regions of the right hemisphere in the 270 – 630 ms time window. This effect was strongest involving the Media and Personal familiarization, as well as between the Perceptual and Personal conditions. We suggest that the neuronal representation of face familiarity contains a shared component, that is independent of familiarization context and stimuli. As we furthermore detected a sustained pattern of temporal generalization, this study also offers evidence, that face familiarity might be a general processing cascade, which is maintained over time.

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P6.22 Secretagogin marks amygdaloid PKCδ interneurons and modulates NMDA receptor availability

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The amygdala is a central hub for emotional processes and defensive behavior. It coordinates the avoidance response the dangerous stimuli, specifically upon conditioned fear. Central amygdaloid nuclei are pivotal in these processes through their local gamma-aminobutyric acid (GABA)-ergic interneurons. Protein kinase Cδ (PKCδ)-positive “fear-off” and somatostatin-positive “fear-on” neurons were identified as major regulators of danger-induced behavior. At the cellular level, glutamatergic neurotransmission through postsynaptic N-methyl D-aspartate (NMDA) receptors drives threat-induced changes in synaptic function. Calcium plays critical roles in synaptic neurotransmission by priming neurotransmitter release. Secretagogin is a calcium-sensor protein whose expression is activity dependent and specific to a hitherto undefined GABA interneuron subclass in the central amygdala. Even though a role of secretagogin in presynaptic integration is plausible, its specific contributions to modulating fear-responsive neurons remains unknown.

Here, we characterized secretagogin-containing interneurons in the centrolateral amygdala across mammals. We identify secretagogin+ neurons as a subpopulation of PKCδ+ cells, which were classified originally as “fear-off” neurons to determine the balance between conditioned flight and fright responses. We show that secretagogin-positive neurons modulate the avoidance response to conditioned danger through the regulation of the postsynaptic surface availability of NMDA receptor 2B subunits. Inactivation of secretagogin-expressing neurons, or ablation of secretagogin itself, provided causality for the role of calcium-dependent feedback regulation at the cellular level.

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Poster session - Topic 7
Behaviour

P7.01 Modifications of the gastrointestinal microbiome are intimately involved in the control of behavioural processes

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In recent years, our knowledge on the intestinal microbiota has greatly improved. Dysfunctions of the intestinal microbiome have been shown to produce modifications of peripheral and brain functions, including the organisation of behaviour. Therefore, aim of the present study was to assess and elucidate the effects of qualitative and quantitative disturbances of the gut microbiome on various behavioural responses in adulthood. In addition, we also intended to unravel the changes of short chain fatty acids along various phases of the experiment. The impact of the alterations on the behaviour were examined in adult male Wistar rats. Animals have been divided into four - 1. antibiotics treated; 2. antibiotics and probiotic treated; 3. probiotic treated; 4. control groups. As antibiotics treatment, rats were given broad spectrum antibiotic mixture dissolved in their drinking water for 4 weeks. Probiotic treated groups daily received our probiotic mixture (containing beneficial bacterial species) with their food for 14 days. Behavioural tests were conducted following the respective modifications of the microbiota. Fresh faecal samples were obtained in definite intervals to monitor the changes of the short chain fatty acids.

Our findings demonstrate significant group-differences in the behavioural tests and in the analysis of short chain fatty acids' as well. Abnormal behavioural phenomena and decreased short chain acids concentration were identified among the antibiotic treated animals, but these did not exist anymore after the probiotic treatment. The present findings well demonstrate that the gastrointestinal microbiome plays serious role in the organisation of behavioural processes.

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P7.02 Post-stress activity of calretinin positive cells in the paraventricular thalamic nucleus is required for long term, stress induced disturbance of sleep behavior

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Sleep disorders caused by stress affect millions of people around the world, but its neurobiological bases are still unclear. The calretinin-positive neurons of the paraventricular thalamus (PVT/CR+) are in a unique position to participate in stress induced sleep disturbances since their activity is significantly affected both by stress and by sleep-wake transitions. In this study we aimed to determine the activity of PVT/CR+ cells before and after the exposure to a natural stressor (fox odor, 2MT, 10 min) and to test causal relationship between post-stress PVT/CR+ activity and post-stress sleep behaviour using optogenetic inhibition (1 hour) of PVT/CR+ cells after the stress situation. Since sleep disturbances not only involve sleep but also the pre-sleep behaviour we separately analysed neuronal activity and behaviour in the nest before sleep. Recordings involved 3 hours sessions for five days before and after stress using movable tetrodes and optogenetic tagging of PVT/CR+ cells.

PVT/CR+ cells displayed strongly state dependent activity during pre-stress days. Wake activity in the nest was lower than outside the nest and firing further decreased at the onset of sleep. At the day of the exposure to 2MT both firing rate and synchrony among PVT/CR+ cells increased. Firing rate remained elevated for four days after the stress, with strongest change in the nest. Bursting activity of PVT/CR+ cells decreased during NREM sleep after the stress. These changes were accompanied by altered locomotor activity (EMG, displacement) and nesting behaviour, showing disturbed sleep. Optogenetic inhibition of PVT/CR+ neurons for one hour after 2MT presentation prevented altered locomotion before sleep, the normal nesting behavior were reinstated and the unit activity did not change either in firing rate or in auto and cross correlations on the poststress days. These data together strongly indicate that altered post-stress activity of PVT/CR+ cells is crucial to establish the neuronal network responsible for the emergence of stress induced sleep behaviour.

P7.03 Behavioural effects of intraamygdaloid oxytocin in valproate induced autism rat model

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Autism spectrum disorder (ASD) is a lifelong neurodevelopmental disorder affecting about 1.5 % of children and its prevalence is still increasing. Anxiety is one of the most common comorbid sign of ASD. Despite the increasing prevalence, the pathophysiology of ASD is still poorly understood and its proper treatment has not been solved yet. In order to develop new therapeutic approaches, the valproate (VPA) induced rodent model of autism can be an appropriate tool. Oxytocin (OT), as a prosocial hormone, may ameliorate some symptoms of ASD.

In the present study we investigated the possible anxiolytic effect of intraamygdaloid OT on VPA treated rats using elevated plus maze test. Our results show that male Wistar rats, prenatally exposed to VPA spent significantly less time in open arms of elevated plus maze apparatus and performed significantly less head dips from open arms. Bilateral OT microinjection to the central nucleus of amygdala increased the time spent in open arms and number of head dips, it reduced the anxiety to the healthy control level. OT receptor antagonist blocked the anxiolytic effects of OT. Antagonist in itself did not influence the time rats spent in the open arms. Therefore, our results show that intraamygdaloid OT has anxiolytic effects on autistic rats.

The project has been supported by the University of Pécs, Medical School, Pécs, Hungary (PTE ÁOK KA-2020-06) and New National Excellence Program of the Ministry for Innovation and Technology (ÚNKP-21-5-PTE-1333).

P7.04 Resilience to generalization of fear correlates with better spatial learning performance in Intellicage.

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A traumatic life event can have differential long-term effects. While the majority can proceed without long-term consequences, vulnerable individuals develop posttraumatic stress disorder (PTSD). Similar response diversity can be found in animal experiments. The phenotype of being vulnerable or resilient may correlate with specific pre-traumatic behavioural characteristics. Recently, individual variation in different aspects of cognitive function has been implicated as a risk factor in PTSD vulnerability, however, in human clinical studies, it is often unfeasible to untangle causative relationships between pre-trauma vulnerabilities and post-trauma consequences. Using IntelliCage, a high throughput, automatized homecage monitoring system, we aimed to identify pre-trauma individual variations in cognitive function of mice that can predict core symptoms of PTSD, i.e. fear memory generalization and extinction deficits following trauma exposure.

Our system, consisting of two cages, was used to explore the performance of 28 mice in various learning paradigms including spatial, behavioural sequencing, patrolling and operant learning, as well as other -not test related- individual parameters were also measured. Namely, learning performance, different types of mistakes, motivation, anxiety, and behavioural flexibility were analysed. After these tests, mice have been exposed to a single series of footshocks as a traumatic experience, and 28 days later their contextual fear memory was measured in an environment similar to the shock (context A), while fear generalization and extinction were assessed in a different/safe environment (context B). The correlations of learning characteristics have been analysed with freezing levels in context B as a proxy for fear generalization.

Accordingly, we conducted a correlation analysis of 34 variables to find the best predictors of freezing response in context B. Our analysis revealed 3 parameters showing a significant level of correlation, which were all related to the performance in spatial learning suggesting that this cognitive domain may play a crucial role in fear generalization and support previous human findings linking pre-trauma cognitive abilities and vulnerability to PTSD. Further studies will elucidate putative common neurobiological (patho)mechanisms underlying reduced spatial learning performance and the inability to differentiate between trauma-related and safe contexts.

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P7.05 Regulatory role of hemokinin-1 in chronic restraint stress model of mice

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The Tac1 gene-encoded Substance P (SP) acting at tachykinin NK1 receptor has been described to be involved in psychiatric disorders, but NK1 antagonists failed in clinical trials. The closely related Tac4 gene-derived hemokinin-1 (HK-1) also binds to the NK1 receptor and plays a role in acute stress reactions in mice. Here we investigated Tac4 mRNA expression in stress and pain-related regions, as well as its involvement in chronic restraint stress-evoked behavioural changes and pain using Tac4 gene-deleted ($Tac4^{-/-}$) mice compared to C57Bl/6 wildtypes (WT).

Tac4 mRNA was detected by the highly sensitive in situ hybridization RNAscope technique. Touch sensitivity was assessed by aesthesiometry, cold tolerance by paw withdrawal latency from 0°C water. Anxiety and spontaneous locomotor activity were evaluated in the light-dark box (LDB) and open field test (OFT) during 5 mins, depression-like behavior in the tail suspension test (TST) by immobility during 4 mins. Adrenal and thymus weights were measured at the end of the experiment.

Tac4 mRNA was detected in the hypothalamus, hippocampus, amygdala, somatosensory and piriform cortices, as well as dorsal root and trigeminal ganglia. We found abundant expression in the adenohypophysis, adrenal gland and thymus. In $Tac4^{-/-}$ mice stress-induced 20% mechanical hyperalgesia was significantly decreased, while the 75% cold hyperalgesia was not different compared to WTs. Non-stressed $Tac4^{-/-}$ mice spent significantly less time in the lit compartment of the LDB and in the central zone of the OF, longer immobility in TST than WTs. After stress they spent significantly more time in the light, made more transitions in LDB, and showed decreased immobility in TST. In WT mice thymus weight significantly decreased, while adrenal weight increased after stress. $Tac4^{-/-}$ mice had significantly greater adrenal and smaller thymus weights than WTs without stress, but significant changes were not observed in restrained animals.

We provided the first evidence for the anti-anxiety and potent nociceptive effects of HK-1 in chronic restraint stress paradigm. Identification of its targets might open new perspectives for therapy of stress-induced pain.

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P7.06 Median raphe region serotonergic neurons regulate depressive-like behaviour related changes in body temperature during forced swim test

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The midbrain median raphe region (MRR) plays a role in numerous different behaviours, but its effect on vegetative functions is debated. Additionally, it is unknown if there is a connection between the two. The MRR is mostly known for its serotonergic (SERT+) neurons, although they constitute a minor population in the nucleus. On the other hand, the classically stress-related corticotrophin releasing hormone (CRH) positive cells are also present in the MRR, but their role is unknown. Our aim was to investigatethe role of MRR, especially its SERT+ and CRH+ neurons in depressive-like behaviour in parallel with changes in core body temperature (BT) as a vegetative function.

Using pharmacogenetics control, excitatory and inhibitory designer receptors (DREADDs) were expressed in the MRR of Bl/6 mice. A biotelemetry system was implanted into the abdominal cavity to monitor changes in BT. Following the injection of clozapine-N-oxide (CNO), the ligand for DREADDs, behavioural tests were performed. Depressive-like behaviour was measured by forced swim test (FST). The same protocol was repeated in SERT-Cre and CRH-Cre mice, but only with control and excitatory groups.

As of the behaviour, excitation of the whole MRR increased floating, while marginally decreasing struggling. The test counts as a cold exposure, and thus, the BT decreased in all animals. However, the drop was smaller in the excitatory group, both during and after the test. The excitation of MRR SERT+ neurons marginally increased floating and significantly decreased struggling. Furthermore, the decrease in BT during and after the FST was reproduced in the SERT-Cre excitatory group. On the other hand, we could not detect any differences between the groups in the CRH-Cre mice.

Based on our results, the MRR effectively affects depressive-like behaviour as well as BT, which is primarily regulated by SERT+ neurons. This finding may have clinical relevance in human depressive disorders, as patients tend to have higher body temperature. CRH positive neurons do not seem to contribute to the regulation of depressive-like behaviour or body temperature.

P7.07 Median raphe region regulates stress and anxiety through CRHergic neurons

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Dysfunctions of median raphe region (MRR) were associated with stress-related psychiatric disorders, assumably due to its serotoninergic content. Recently we have found that corticotropin-releasing hormone (CRH), the main hypothalamic regulator of stress axis, is also abundant in this area. Therefore, we aimed to reveal the contribution of these peptidergic cells in stress adaptation.

Pharmacogenetic technique was used in male CRH-Cre mice. Control, stimulatory or inhibitory DREADD sequence was injected into the MRR using adeno-associated virus vector. Clozapine-N-oxide was used as ligand. Parallel with changes in stress-hormone concentration, anxiety- and depression-like behavior was also measured. Stress-induced activation of MRR-CRHergic cells were studied in CRH-Cre dtTomato crossbred animals. Accuracy of injections and c-Fos activation were investigated by immunohistochemistry.

Stimulation of CRH neurons in MRR increased corticosterone levels and relative spleen weight parallel with anxiety-like behavior in elevated plus maze, light-dark box, and fox odor test compared to controls. Inhibition of these cells had no effect on "normal" anxiety found in control virus injected animals. Difference was not found in tests measuring depression-like behavior either. Stress activated the MRR-CRH neurons measured by c-fos.

To summarize, stimulation of MRR-CRH neurons may induce stress-hormone elevation as well as anxiety-like, but not depression-like behavior. Thus, our results support the existence of a new acute stress regulatory brainstem CRH population, which play a key role in regulating stress adaptation both at hormonal and behavioral level.

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P7.09 Using appetitive motivation to train mice for spatial learning in the Barnes maze

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Barnes-maze, a well-known spatial learning paradigm, is based on the innate fear of rodents from large open spaces and their drive to hide. However, the apparatus itself is often not aversive enough to provoke the hiding response so additional factors (strong light, threatening sounds or odors) are often used for increased aversiveness, but these additional elements may render the method cumbersome.

The objective of this study was to establish a Barnes-maze learning paradigm with appetitive motivation in mice.

We used 12 C57BL6/J and 12 NMRI male mice in two experiments. The Barnes-maze was a circular metal table (1 m diameter) with twenty holes (5 cm ∅) evenly spaced along the perimeter. Under one of them we placed the escaping box where the mice could hide (target hole). Extra maze cues were placed in the room. We used chocolate cereal as reward. First, we habituated the mice to the chocolate cereal in their home-cage for 2 nights. Then the animals were put in the escaping box with a piece of reward and placed in the middle of the maze for 20 and 10 min on two consecutive days. After the habituation period the maze-learning started. At the beginning of a trial the mouse was placed in the middle of the maze and were allowed to move around and find the target hole for 5 minutes. There were 2 trials a day. The learning criterium was finding the target hole with less than 1 hole error. When the animal reached this criterium we changed the target hole location. Mice needed to re-learn the new position with the same criterium. We measured the latency to find the target hole (LT), number of visited holes until finding the target hole (NH), latency to reach the first visited hole (LF), initial error (distance of the first visited hole from the target hole, IE).

NMRI mice reached the criterium in trial 31, C57BL6/J mice in trial 21. LT showed a steep decrease until trial 5 (C57BL6/J) or trial 4 (NMRI) and a slow gradual decrease afterwards. During the trials LF remained less than 6 s. NH and IE decreased proportionally with time. After changing the box location LT, NH signficantly increased while LF remained the same. IE pointed to the original target hole. Mice learned the new target location within 12 trials (NMRI) or 18 trials (C57BL6/J).

In summary, appetitive motivation can be used to establish Barnes-maze learning. The contextual part of the task was learnt quickly, while the exact localization of the target hole required more time.

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P7.10 Sirt1 in AgRP neurons is necessary for exploratory behavior during calorie restriction

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Calorie restriction can prolong a healthy life span in mammals. The underlying mechanisms involved in this process are ill-defined. We show here that CR promotes the activity of NPY/AgRP neurons in the brain, and that disrupting Sirt1 (a deacetylase that senses NAD^+) in these cells leads to impaired behavioral but not metabolic responses to CR. These findings highlight the pivotal role of the NPY/AgRP neurons during CR in the regulation of complex behaviors.

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P7.11 Can locomotor impairments and anxiety-like behaviour alter the measurable memory-decline in the triple transgenic mouse model of Alzheimer's disease?

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Preclinical studies with animal models play crucial role in revealing the pathomechanism and identifying new treatment options for Alzheimer's disease (AD). In our study, the triple transgenic mouse model of AD (3xTg-AD) was used. During our previous observations decreased locomotor activity had been discovered, which might influence the outcome of other behavioural tests. In the attempt to better understand the 3xTg-AD mouse model different aspects of its motor skills and its anxiety-like behaviour was tested. Several behavioural tests were performed in order to measure the locomotor activity (open field (OF) test, rotarod test, grip test) and anxiety (fox odor (FO) test, elevated plus maze (EPM) test). Cognitive tests that are strongly based on motivation (social discrimination (SD), active avoidance, Morris Water Maze (MWM) test) were also performed. The experiments were carried out on six-month-old male 3xTg-AD animals in comparison with C57Bl/6 controls. In the OF test 3xTg-AD mice moved significantly less, while during the rotarod test, there was no difference between the genotypes. The performance of the 3xTg-AD animals was worse in the grip test. 3xTg-AD animals spent more time in immobile, 'freezing', posture during the FO test. In the EPM test, the transgenic mice stepped fewer times into the closed arm, without any genotype difference in the locomotion-independent anxiety measures. During SD and MWM the memory decline of the 3xTg-AD mice was confirmed. In contrast, during the active avoidance the strong stimulus of the electric foot-shock forced even the 3xTg-AD mice to learn the task as fast as their controls. We were able to conclude that the 3xTg-AD mice show decreased locomotor activity, decreased strength, and enhanced innate anxiety which might contribute to the differences observable during cognitive tests. In memory tests based on strong motivation (such as MWM test), the locomotor difference may disappear as 3xTg-AD mice might be more motivated to learn quickly. This phenomenon might influence the results of the mentioned memory tests. Thus, it is also important to take into account different motivational factors.

We are thankful to Balázs Hangya for providing the 3xTg-AD mice.

P7.12 Activation of the social decision-making network in valproate-treated, autism-model mice

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The Autism Spectrum Disorder (ASD) is a lifelong neurodevelopmental disease that is extensively prevalent, and males are multiple times more affected than females. ASD, at any degree of severity, is clinically characterized by social behaviour impairments, mainly involving difficulties in non-sexual social situations. Exposure to specific agents, such as valproic acid (VPA) during pregnancy, have been linked to ASD. In the current experiment we used the VPA-mouse model of ASD, investigating neuronal activation in the nuclei of the social decision-making network (SDMN) and nuclei that has regulator effect on the SDMN in juvenile male mice in various social settings.

The efficacy of VPA treatment was validated by three-chamber sociability test, commonly accepted for measuring the ASD-like behavioural phenotype.

c-Fos immunohistochemistry was performed in control and VPA-treated individuals in order to capture snapshots of the momentary activity of cells of the SDMN during two types of social situation: mice were kept with familiar companion (1); separated from familiar companion and kept isolated for one day (2); separated for one day and then reinstated to familiar cagemates (3). Certain brain regions of the SDMN showed marked differences according to social situations and embryonic treatments: e.g. the interfascicular nucleus (IF) of ventral tegmental area (VTA), which plays a major role in the mesolimbic reward system, showed increased activity after the reinstatement in VPA treated mice in contrast to the control individuals. Such a difference in the activity is most likely not independent from those we found also in the habenular nucleus (Hb). The Hb plays role in the regulation of the VTA through IF. Exposure to valproic acid most likely disrupts the SDMN and, therefore, affects the social interactions from early postnatal development, further hindering the acquisition of normal social behaviour.

P7.13 Pre-trauma behavioural risk factors of trauma vulnerability

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Experiencing a traumatic life event results in Posttraumatic Stress Disorder (PTSD) in vulnerable individuals (10-20% of trauma-exposed populations). PTSD can be characterized by extinction-resistant fear memories and generalization of fear to safe contexts. For better treatment options, it is essential to identify risk factors contributing to vulnerability for PTSD. In the literature it is well-documented that reduced cognitive abilities are associated with PTSD, however, their causal role, specific contribution, and neural background are not understood.

To identify specific emotional and cognitive domains, which predispose individuals to the development of fear generalization and impaired fear extinction, we exposed rats to a wide behavioural test battery prior to the trauma. Then subjects were exposed to a traumatic experience by means of inescapable footshocks. Four weeks later, freezing responses were quantified in an altered/safe context to assess generalization and extinction in order to differentiate vulnerable and resilient subpopulations (quartiles). We found that specific anxiety-like traits and operant learning-like characteristics are predictive factors of PTSD-like symptoms.

In a subsequent study, we contrasted these subpopulations by their gene expression profile in the prefrontal cortex (98 candidate genes using q-RT PCR).

We found major differences in the expression of interneuron markers such as CRH, VIP, with an additional decrease of neuronal activity in the vulnerable population. We confirmed these findings using immunohistochemical staining, particularly apparent in VIP/CRF-expressing interneurons. Interestingly, CRH knockdown in mPFC could decrease fear learning and expression, thereby recapitulating the resilient phenotype.

In conclusion, lower pre-trauma cognitive abilities are vulnerability factors in the development of fear generalization symptoms of PTSD. Moreover, mPFC CRH signaling seems to play a mediatory role in this process.

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P7.14 Median raphe region GABAergic neurons contribute to social interest in mouse

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Gamma-aminobutyric acid (GABA) is a well-known inhibitory neurotransmitter implicated in numerous physiological and pathological behavior including social interest. Dysregulation of the median raphe region (MRR), a main serotoninergic nucleus, is also characterized by increased social problems. As the majority of MRR cells are GABAergic, we aimed to reveal the social role of these cells.

Chemogenetic techniques was used in vesicular GABA transporter Cre mice and with the help of adeno-associated virus vectors artificial receptors (DREADDs, stimulatory, inhibitory or control, containing only a fluorophore) were expressed in MRR GABAergic cells confirmed by immunohistochemistry. Four weeks after viral injection a behavioral test battery (sociability; social interaction; resident-intruder) was conducted. The artificial ligand (clozapine-N-oxide, 1mg/10ml/kg) was administrated 30 min before the tests. As possible confounding factors, locomotion (open field/OF), anxiety-like behavior (elevated plus maze/EPM), and short-term memory (Y-maze) were also evaluated.

Stimulation of the GABAergic cells in MRR had no effect on locomotion nor working and social memory, however, it increased social interest during sociability and social interaction but not in resident-intruder tests. Accordingly, c-Fos elevation in MRR-GABAergic cells was detected after sociability, but not resident-intruder tests. In the EPM test, the inhibitory group entered into the open arms later, suggesting an anxiogenic-like tendency.

We confirmed the role of MRR-GABAergic cells in promoting social interest. However, different subpopulations (e.g. long vs short projecting, various neuropeptide containing) might have divergent roles, which might remain hidden and requires further studies.

P7.15 Effectiveness, temporal considerations and brain mechanisms of extinction training in male rats.

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Fear extinction suppresses traumatic fear memories and is of upmost importance on avoiding the development of different mental disorders, such as Posttraumatic stress disorder (PTSD).

The present study examined the effectiveness of three different extinction protocols. The main difference between the protocols was the starting point of the extinction training after the electric foot shock (1 or 28 days later). We investigated the efficacy as well as the durability of the fear extinction concentrating on their temporal aspects and specific neuronal activation patterns. Male Wistar rats subjected to inescapable electric foot shocks were used as animal models. Behavior (freezing in trauma-context) and stress hormone levels (adrenocorticotropin and corticosterone in serum) were followed during extinction. Subsequent to the termination of the extinction training a c-Fos immunohistochemistry was performed to identify the neuronal activation patterns. We were focusing on three brain areas implicated in PTSD (prefrontal cortex (PFC), hippocampus (HC) and amygdala).

A spontaneous recovery of extinguished recent (starting 1 day after trauma) fear memory was detected three weeks after training. Opposite to that, the extinction of remote fear memory (extinction started 28 days after trauma) was significantly more long lasting, as no recovery was observed at the same period. Hormone measurements performed at the end of extinction trials suggest that adrenocorticotropin, but not corticosterone responses resembles behavioral extinction without any sign of relapse. Furthermore, after the extinction of recent fear memories, re-exposure to the conditioning cage increased the activity of the medial prefrontal cortex (mPFC), and decreased the activation of the central and medial amygdala as compared to rats that were not exposed to extinction training. In this specific situation, no differences were observed in the HC activity. However, in the case of remote conditioned fear, extinction decreased fear-induced neuronal activation in the HC and basolateral amygdala, but no major effects were observed in the mPFC .

These findings demonstrate that the extinction of remote fear memories is more effective than that of recent ones, possibly because of the temporal changes in the neuronal networks underlying fear expression.

P7.16 Stress prediction for field technical specialists

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A mobile application for real-time assessment of fatigue in field technical specialists

Background

Our society places increasing demands on employees, partly due to the nature of their work and partly due to their duty hours (ie, multi-shift work schedule, work at night or in the early morning hours). Therefore, managers may face growing expectations to understand and deal with the employee's increasing fatigue and its consequences. The better understanding of the causation of fatigue as well as more accurate assessments of fatigue may lead to better management of this symptom and may help reduce the number of accidents and the number of sick hours/days, as well as improve productivity and quality of life of the employees.

Objectives

The aim of our study was to develop a tool that allows real-time monitoring of fatigue and helps better understand which work-related factors have the most significant impact on fatigue in field technical specialists.

Methods and results

We developed a mobile application for Android devices using the platform of Mobilengine (https://mobilengine.com/). 15-item questionnaire was implemented into our application that was specifically designed to assess physical, cognitive and social aspects of fatigue in field technical specialists. A visual analogue scale (VAS) was also implemented to assess fatigue level. Two randomly selected questions from the above-mentioned 15-item questionnaire along with a VAS were assessed twice a day: upon initiation of the first and the last tasks of the day. (The last task was travelling home). The application was embedded in another Mobilengine application that schedules the users' daily tasks and provides users with task-specific instructions. Users were obligated to complete the questionnaires as part of their daily tasks. In addition, the application collected passively the following metadata: number and type of workorders, tasks within workorders, customer relationship, travel time, working hours, workorders' changes within the day etc. Data were collected for 5 months.

Conclusion

We developed a mobile application that

(1) allows multidimensional assessment of fatigue in a work environment

(2) records details of the daily tasks with time stamps,

(3) connect the collected active and passive data for every user which allows us to determine the reasons behind the actual fatigue level

P7.17 Computerized socio-behavioral analysis in color coded rodents

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Social behavior consists of different patterns conspecifics demonstrate when they are together. Experimental analysis of social behavior revealed that these patterns depend on the previous history of the animals. In the present study we analyzed the social interaction between 3 rats, of which one experimental animal has been treated differently. The male rats we used, freely interacted in an open-field arena during the 30 min sessions and were videorecorded throughout. We color coded the rats and adapted a previously developed custom-made software, which could recognize the differently color-coded rats using computer vision. The video recordings were analyzed with the software, which detected the trajectories of all 3 animals, and specifically calculated the time spent near and away from the wall as an indicator of the anxiety level of the animals. In addition, 2 other behavioral elements were calculated: approach-avoiding behavior, and nosepoking the mates. Initial experiments started with 2 days of baseline recordings. Statistical analysis was used to establish if the behavior of the animals changed in these 2 days. Then, on the following days, the experimental rats were manipulated. We addressed the effect of spending the previous night with a conspecific as opposed to being isolated. In this experiment, we found a significant reduction in the number of approach-avoidance behavior of the experimental animals when the animals were grouped together before the experimental session. In turn, the number of node pokes was not altered. In the other 2 experiments, the animals were fasted for a day before the experiment, or a cell group in the posterior thalamus was chemogenetically stimulated. In these cases, we did not observe any change in the approach-avoidance and nosepoking behavior of the animals. The data suggest that grouping vs. isolation is the strongest factor modulating the investigated behaviors of rats. The observed reduction in approach avoidance with unaltered frequency of nosepoking suggests that the animals touch each other with the same frequency but avoid contact less frequently if being together the preceding day. In turn, fasting or stimulation in the posterior intralaminar thalamus do not have significant effect on these behaviors implying the specific effect of isolation on the social interactions between the rats.

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P7.18 Chemogenetic study of posterior thalamic neurons related to anxiety-and depression-like behaviors

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The posterior intralaminar thalamic nucleus (PIL) has been suggested to play an important role in the regulation of different social behaviors: chemogenetic manipulation of PIL calbindin neurons resulted in changes in maternal behavior while stimulation of the PIL-medial preoptic area pathway increased direct social interactions between adult females. PIL neurons are activated in response to pup exposure in mothers but also upon adult social encounter. The objective of the present study was the investigation of social function of PIL calbindin neurons with parallel assessment of anxiety- and depression-like behaviors.

We injected stimulatory, control and inhibitory (rAAV5-hSyn-DIO-hM4D, Gq,-,Gi/-mCherry) adeno-associated viruses into the PIL of calbindin-Cre mice to induce Cre-dependent cell type specific expression of designer receptors exclusively activated by designer drugs (DREADDs). Clozapine-N-oxide (CNO) was used as the designer ligand of DREADDs. All injection sites were histologically validated. In turn, functioning of the stimulatory DREADD was verified by appearance of c-Fos following CNO injection. In the virally injected animals, we performed different social behavioral tests (direct social interaction, sociability and preference for social novelty) following CNO and also vehicle injections. In addition, we performed elevated plus maze and forced swim test to measure anxiety- and depression-like behavior of mice, respectively. The results demonstrated that stimulation of PIL neurons increased while their inhibition reduced anxiety-related behavior, while depression-like behavior was not altered.

We also mapped the projections of calbindin positive neurons from the PIL and found labelled fibers in a variety of different brain regions. In some of these brain regions, such as the medial preoptic area, lateral septum, periaqueductal gray, Fos activation appeared in response to CNO in animals injected with the stimulatory virus suggesting that PIL calbindin neurons activate them. Therefore, it is likely that these brain areas mediate the anxiety-like behavior driven by PIL calbindin neurons.

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P7.19 Sex-specific parenting and depression evoked by preoptic inhibitory neurons

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The role of preoptic GABAergic inhibitory neurons was addressed in parenting, anxiety and depression. Pup exposure and forced swimming resulted in similar *c-Fos* activation pattern in neurons expressing vesicular GABA transporter in the preoptic area with generally stronger labeling and different distributional pattern in females than in males. Chemogenetic stimulation of preoptic GABAergic cells resulted in elevated maternal motivation and caring behavior in females and mothers but aggression towards pups in males. Behavioral effects were the opposite following inhibition of preoptic GABAergic neurons suggesting their physiological relevance. In addition, increased anxiety- and depression-like behaviors were found following chemogenetic stimulation of the same neurons in females while previous pup exposure increased only anxiety-like behavior suggesting that not the pups, but overstimulation of the cells can lead to depression-like behavior. A sexually dimorphic projection pattern of preoptic GABAergic neurons was also identified, which could mediate sex-dependent parenting and associated emotional behaviors.

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P7.20 Automatically monitored home-cage behavior of female mice throughout the reproductive cycle

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Pregnancy and lactation brings a variety of drastic changes to a female's life. These physiological and behavioural changes serve the survival and well-being of the offspring. Both reproductive stages are associated with increased nutritional needs due to physiologic changes of the female and the metabolic demands of the offspring.

In this study, we characterized spontaneous behavior of female mice during reproductive stages using the fully automated monitoring system IntelliCage. Before the experiment we implanted radiofrequency transponders to each female mice under isoflurane anesthesia. The transponder-tagged animals were in turn individually recognized in any of the 4 corners. A correct corner belonged to each animal where they could opt to drink water or 1% sucrose solution both measured as lick number. First, females lived in the IntelliCage for 10 days, when males were added for mating. Some of the females got pregnant and gave birth to pups while other females remained in the IntelliCage as controls living together with the mothers for the entire 70 days experimental period.

Mothers had significantly increased correct/total corner visit ratio and sucrose/total lick ratio. Corner visits and total licks were higher at night, however, we did not detect any change in correct/total corner visit ratio and sucrose/total lick ratio. Furthermore, mothers visited correct corners significantly more frequently during late pregnancy and lactation periods and made significantly less incorrect visits during late pregnancy compared to other periods. Interestingly, we observed that females who got pregnant later on, drank more sucrose as virgins compared to virgins who never became pregnant.

The results are consistent with expected increase of fluid intake of mothers, increased nighttime activity of the animals, and reduced activity during late pregnancy. In addition, we revealed elevated sucrose preference of mothers suggesting selective craving for sucrose. We observed that increased nighttime activity proportionally increased correct and incorrect corner visits, suggesting that the exploratory drive increased as much as the drinking. In contrast, late pregnant animals selectively choose to visit only the correct corner.

In conclusion, IntelliCage system is suitable for observing long-term spontaneous behavior of mice and revealing differences between groups and different time periods which may remain hidden if using classical tests.

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P7.21 Chemogenetic evidence that posterior intralaminar thalamic neurons stimulate maternal behavior in rats

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In a previous study, we established the activation of the posterior intralaminar thalamic (PIL) neurons during social interactions between adult female rats. In this study we focused on the role of PIL in a special type of social interaction, maternal behavior, during which the animals display specific behavioral patterns such as suckling, anogenital licking, pup grooming and nest building which is accompanied with neuronal activation of certain brain areas.

For manipulation of PIL neurons, adeno-associated virus was injected into the PIL using stereotaxic apparatus. The virus expressed the fluorescent tag mCherry and a DREADD (Designer Receptors Exclusively Activated by Designer Drugs) in the infected cells. mCherry was used for tract-tracing and the DREADD for chemogenetic stimulation. We used excitatory (hM3D) DREADD, which was activated by clozapine-N-oxide (CNO). Behavioral tests were recorded during the chemogenetic stimulation. After perfusion of the animals, we performed histological analysis. We found mCherry positive fibers in multiple brain areas. The anterogradely labeled fibers were most abundant in the medial preoptic area, the lateral septal nucleus, the paraventricular hypothalamic nucleus and the infralimbic cortex. We also identified the brain areas activated by maternal care using the c-Fos method. We found neuronal activation in the PIL, and also in the medial preoptic area, and the lateral septal nucleus.

The behavioral tests were performed on the first week of the postpartum period, 2-7 days after parturition. On the first day of the experiment, vehicle was injected to the animals. Pup preference test, spontaneous maternal behavioral test and pup retrieval tests were recorded. On the second day, the same behavioral tests were repeated starting 1.5 hour after CNO administration. Chemogenetic stimulation significantly increased the pup preference index, the duration on pup related behavioral elements, such as suckling, pup grooming and anogenital licking. The activation of the PIL neurons also increased the duration of nest building and reduced the latency of the pup retrieval. We also performed elevated plus maze test and forced swim test to measure anxiety- and depression-like behaviors, on which the chemogenetic stimulation of the PIL had no effect.

Based on these results, PIL neurons may participate in the regulation of maternal behavior conveying sensory inputs from the pups to higher brain areas.

P7.22 A new brain mechanism promoting physical contact in social behaviour

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We previously identified the posterior intralaminar thalamic nucleus (PIL) as a relay station of socially relevant sensory information innervating and activating oxytocin-secreting neurons upon social encounter. Here, we addressed to characterize the exact role of the PIL neurons and their projections to the preoptic area of the hypothalamus in the control of the social behavior.

First, we determined the effect of chemogenetic stimulation of PIL neurons on social interactions between familiar adult female rats using the DREADD technique. The brain activation patterns were determined following direct social interaction, and also with the exclusion of physical interaction using the c-Fos technique. The projections of PIL neurons were analyzed using anterograde tract-tracing. The selective chemogenetic stimulation of the preoptic area-projecting PIL neurons was performed using double viral injections and also by using intracerebral cannula for CNO administration directly into the preoptic area.

PIL projects to several socially implicated brain regions, such as the medial amygdala, the medial preoptic area, the paraventricular and dorsomedial hypothalamic nuclei and the infralimbic cortex. Chemogenetic stimulation of the PIL resulted in the activation of previously anatomically identified target areas and also increased the duration of direct interactions during social behavior. Direct contact during social interaction caused the largest increase in the activity in the medial preoptic area. Specific chemogenetic stimulation of the PIL-preoptic pathway led to elevated direct social contact.

The results suggest that posterior thalamic PIL neurons convey socially relevant information to a variety of different forebrain centers, among which the preoptic area is involved in the processing of physical contact. Thus, we identified an important novel component of the social brain network, which may increase the motivation for positive direct interactions.

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P7.23 The role of PTH2 neuropeptide in social function – a study using PTH2 receptor KO mice

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Social behaviour is important for a variety of different species. In humans – one of the most sociable species – the impairment of social abilities can lead to neuropsychiatric disorders, which is an evidence of their significance. The role of parathormon 2 neuropeptide in social interactions was demonstrated in zebrafish (Anneser et al., 2020). Therefore, the role of the mammalian homologue of PTH2 neuropeptide, tuberoinfundibular peptide of 39 residues and its receptor, PTH2 receptor (PTH2R) in mammals is a relevant question.

Our objective was to examine the role of the TIP39-PTH2R neuromodulator system in social behaviour. We investigated the role of PTH2R in social tests, which were performed with PTH2R knock-out (KO) and wild type mice, in order to compare their behaviour. We carried out several supplementary tests, too, to examine the anxiety- and depression-like behaviour of the mice.

We found significant differences in the social novelty preference between wild-type (WT) and PTH2R KO mice. The latter spent more time with familiar (their previous cagemates) rather than with an unfamiliar mouse, which is in turn a characteristic of wild type mice. Another finding is that knockout mice had an increased latency of sniffing behaviours in social environment, which is an introductory behaviour between mice. In addition, knockout mice's general activity in open field was lower than the wild type mice's, which suggests the possibility of a generally increased stress level in mice lacking the PTH2 receptor. This is known to cause social stress in the animals.

In conclusion, the data suggest that different aspects of sociability are affected in the absence of the PTH2R suggesting a function of the TIP39-PTH2R system of the brain.

Reference:

Anneser, L., Alcantara, I. C., Gemmer, A., Mirkes, K., Ryu, S., & Schuman, E. M. (2020). The neuropeptide Pth2 dynamically senses others via mechanosensation. Nature, 588(7839), 653–657.

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P7.24 Effect of embrional valproinic acid and deltamethrin treatment on social behavior and in domestic chicks (Gallus gallus)

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Embryonic exposure to valproic acid (VPA) is known to produce sociability deficits, resembling human autistic phenotypes, in several vertebrate species. Animals living in groups prefer the proximity of peers and have the ability to perceive and to respond to social signals for modifying behaviour. Chicks of Galliform birds, known to display early preference behaviours, have been used extensively for adaptive learning studies. Our question was whether the investigated environmental contaminant, deltamethrin, can cause autism-specific behavioural abnormalities. The secondary goal was to create a potentially new animal model of pharmacological autism and compare it to the widely used model of valproic acid. Here, domestic chicken eggs were injected with sodium valproate (200 µl of 35 µmol/L solution) or with vehicle (distilled water) and deltamethrin (1.5mg/kg) on the 14th day of incubation. After hatching, the chicks were tested for sociability, and social memory before and after social isolation. Our findings confirm previous studies, reporting an adverse effect of VPA on embryonic development, including a tendency for aborted or delayed hatching and, occasionally, for locomotor disorders in a small percentage of birds. The most prominent finding was attenuation of sociability of VPA-exposed birds. Social memory of familiarized individuals is not yet formed in chicks at this age. Although deltamethrin treatment caused minor changes in behaviour, it did not cause a behaviour pattern similar to VPA, it was not associated with a decrease in sociability and vocalization. There is probably no direct association between deltamethrin and the incidence of autism. It seems that embryonic deltamethrin treatment is not an appropriate chemical model for autism.

P7.25 Memory consolidation is governed by signaling through gap junctions in the astrocytic network

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The involvement of astrocytes in oscillatory brain activity, both in physiological (e.g. slow wave activity) and pathophysiological processes (e.g. epilepsy) is supported by a growing body of evidence. By exploring various molecular interactions between neuronal and astrocyte networks, we have previously shown that blocking astrocytic gap junctions suppresses slow wave activity in rats in vivo (Szabó et al. 2017) and inhibits epileptiform activity in acute hippocampal slices (Vincze et al., 2019), suggesting a causal role of astrocytes in neuronal synchronization. Since slow wave sleep is associated with memory consolidation, perturbation of the astrocytic syncytium during this process may impact the working memory of rats. To this end, we activated astrocytic gap junctions using trimethylamine (TMA) or inhibited them with an astrocyte-specific connexin 43 (Cx43) antibody. Memory consolidation was evaluated using novel object recognition tests. Our results show that opening of gap junctions by TMA significantly enhances memory formation, while inhibition by Cx43 antibody impairs memory performance. Furthermore, we have found that TMA particularly improves memory of animals with weaker baseline performance. To explore the mechanistic background of memory enhancement of TMA, we also investigated whether TMA affects the slow-wave sleep ratio using electrophysiological measurements. We believe that large-scale synchronization in the astrocyte network through gap junctions plays a previously unrecognized, essential role in higher cognitive functions and may open up new avenues in the therapy of cognitive disorders.

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Poster session - Topic 8
Neuroendocrinology

P8.01 The role of microglia in the regulation of prolactin release

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Microglial cells may be involved not only in well established pathological responses but also in physiological functions of the nervous system. The best known function is the removal of unused synaptic connections. Since the maternal brain undergoes pronounced neuroplastic changes, we hypothesized that microglia may play a role in maternal adaptations.

In the present study, we examined the role of microglia in lactation as well as the formation of maternal behaviour. Microglial cells were eliminated from the brain by blocking colony stimulating factor 1 receptor (CSF1R) signalling for 3 weeks by a diet containing the CSF1R inhibitor Pexidartinib 3397 (PLX3397). Drug administration took place during pregnancy or only after parturition. As a result of treatment, 75-80 % of the microglial cells were eliminated from all areas of the brain verified with immunolabeling of the microglia marker IBA-1. The intensity of maternal behaviour was reduced when the treatment started before parturition but not when the drug was administered only in the postpartum period. Still, the pups, even foster pups, did not survive with the treated mothers suggesting deficiency in lactation. Indeed, the weight gain of pups during an hour long suckling bout (preceeded by 4 hours long pup separation) was reduced in treated mothers suggesting that lactation was affected by the reduced number of microglia, which prompted us to address prolactin levels in the animals. Activated prolactin-sensitive cells appeared in the arcuatus nucleus and other brain regions in the control but not in the PLX3397 treated mothers following suckling. To address if PLX3397 acted centrally, mother rats received continuous PLX3397 injection into their lateral ventricle via osmotic minipumps for 3 weeks, when suckling induced serum prolactin levels were measured in the blood obtained via jugular cannula. The level of prolactin in the treated mothers was markedly reduced as compared to control animals. The maternal behaviour of treated mothers was not changed in this experiment, either.

The results suggest that microglial cells participate in prolactin release. Thus, they are necessary for proper lactation, as well as the prepartum formation of maternal motivation but they are not required for behavioural changes in the postpartum period.

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P8.02 Comparison of endocrine disruptor-modulated nuclear receptor (ERs, TRs and PPARgamma) mRNA expression and simultaneous mitochondrial respiration rates in mouse hypothalamic tissue homogenates

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An increasing number of chemicals, either environmental pollutants of industrial origin, or mycotoxins from foodstuff or natural substances (like arsenic, phytoestrogens, etc.) are identified as factors with the ability to disrupt the normal regulatory processes of the neuroendocrine system. In the present study we assess the ability of three such known substances (endocrine disruptors, EDs), bisphenol A (BPA), arsenic (As) and zearalenone (ZEA), to modify the mRNA expression levels of estrogen- and thyroid hormone receptors (ERα,β; TRα,β) and peroxisome proliferator-activated receptor gamma (PPARγ) in hypothalamic tissue homogenates of mice. To assess whether such ED effect(s) also induce changes in the intensity of tissue metabolism, mitochondrial respiration was also measured, latter which is one of the best-known parameters of cellular metabolism. EDs were applied in three environmentally relevant concentrations as one single dose intraperitoneal injection. Results show that EDs used in the present study can modify mitochondrial respiration in a concentration dependent fashion, however, effects of distinct EDs differed from each other. Mitochondrial respiration rates were also dose-dependent; however, they did not mimic the dose-dependent pattern seen in the nuclear receptor mRNA expressions. These findings indicate that exposure to various EDs may not only alter a number of physiological regulatory pathways, but may also affect the intensity of the biological fuel-generator mitochondrial metabolism.

NKFI, OTKA 115613 (Attila Zsarnovszky)

P8.03 Investigating the effect of female hormone depletion on the progression of Alzheimer's disease

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Introduction: Alzheimer's disease is the most common type of cognitive dementia, affecting elderly women 1.6-3x more than similar aged man, or the younger generations. The advanced progression can be due to decreased hormone synthesis in post-menopause. Estradiol and progesterone both have neuroprotective potentials, and the lack of these hormones possibly aggravates cognitive decline.

Aim: The aim of our experiment is to investigate the relationship between female hormone depletion and the progression of dementia in a triple transgenic mice model of Alzheimer's disorder (3xTg-AD). The pathological hallmark is known to appear in 6-month-old animals; thus, we expect to see cognitive decline in the 4-month-old 3xTg-AD mice only after hormone depletion.

Material and methods: The experiments were performed on 3-month-old genetically modified female 3xTg-AD mice and their control equivalents**.** As a menopause model ovaries were removed (OVX), control groups received a sham surgery. After 1 month recovery the body composition of the animals were measured by an MRI scan. The cognitive capabilities were investigated with behavioral tests, like Y-Maze and Morris Water Maze (MWM). At the end of the experiment animals were decapitated and uterus was dissected.

Results: The uterus weight decreased, and the body weight increased significantly in the OVX animals. The MRI data showed that the body weight change can be due to fat accumulation. The 3xTg-AD genotype did not influence the somatic changes. In the Y-maze test 3xTg-AD mice moved significantly less, without any effect of OVX. In the MWM a difference between the learning capability of the 3xTg-AD SHAM and 3xTg-AD OVX group could have been detected.

Conclusions: Our experiment show, that the surgery was successful, the animals had menopausal symptoms. The OVX also tended to enhance cognitive decline. Thus, our data confirm that it can be one of the risk factors that aggravate dementia. Further morphological and behavioral tests are needed to understand the pathophysiology and the relationship behind.

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P8.04 Insulin-like growth factor binding protein 3 in the human hypothalamus

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Insulin-like growth factor-1 is a major hormone, e.g. mediating some of the actions of growth hormone. It is also synthetized in the brain and has been suggested to have some neuronal functions. In the blood, IGF-1 is bound to different binding proteins, the major one being insulin-like growth factor binding protein 3 (IGFBP3). Recently, we revealed the expression of IGFBP3 in different parts of the rat hypothalamus. Since its expression increased in the maternal hypothalamus, its role in maternal adaptation was suggested. Based on the involvement of the preoptic area in the control of maternal behaviours, preoptic IGBP3 may also be involved in this process. In turn, IGFBP3 was also induced in the arcuate nucleus of mother rats. Since dopaminergic neurons controlling lactation via prolactin secretion from the pituitary are located here, IBFBP3 was suggested to play a part in this process. Functional experiments supported this hypothesis as sequestering IBFBP3 from the brain reduced plasma prolactin levels. The existence of these regulatory pathways in human remained a question. Therefore, in the present study, we addressed the distribution of IGFBP3 in the human hypothalamus. Immunohistochemistry was performed in free floating sections of the human hypothalamus. To precisely identify the exact brain areas of IBFBP3 expression, consecutive sections were labelled with oxytocin and tyrosine hydroxylase. IGFBP3-positive neurons were identified in the infundibular (arcuate) nucleus. The distribution of these cells suggested that they are not the dopaminergic neurons of the arcuate nucleus but rather formed a cell cluster in the vicinity of the median eminence. Indeed, IGFBP3-positive fibers were abundant in the medial eminence suggesting that cell bodies in the ventral part of the arcuate nucleus project there. In addition to the IGFBP3 neurons in the arcuate nucleus, IGFBP-positive cell bodies were also found in some restricted parts of the preoptic area while some other parts of the preoptic area contained predominantly IGFBP3-positve fibers. These results suggest that IGFBP3 is expressed in the human hypothalamus, with a distributional pattern resembling to that in the rat. Consequently, a similar role of the hypothalamic IGF-1 system can be hypothesized in human as in rodents.

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P8.05 Expression of glucagon like peptide 1 receptor in neuropeptide Y neurons of the arcuate nucleus in mice

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Glucagon-like peptide 1 (GLP-1) and its agonists exert anorexigenic effect at least partly via acting on GLP-1 receptors (GLP-1R) in the arcuate nucleus (ARC). While the anorexigenic, proopiomelanocortin (POMC) neurons of the ARC were shown previously to express GLP-1R, the putative GLP-1R-content of the orexigenic, neuropeptide Y (NPY) neurons remained so far undetected. As GLP-1R is abundant in the ventromedial ARC, where NPY neurons are located; here, we address the possibility that GLP-1 can act directly on the orexigenic NPY system via GLP-1R. Double-labeling immunocytochemistry and in situ hybridization were performed on tissues of adult male mice to detect GLP-1R in NPY neurons. In double-immunolabeled preparations, GLP-1R immunoreactivity was observed in NPY neurons and in axons ensheathing the majority of NPY neurons. Ultrastructural studies confirmed that GLP-1R immunoreactivity is associated with the outer membrane of NPY perikarya as well as with axons forming symmetric type, inhibitory synapses on NPY-containing neurons. Double-labeling in situ hybridization experiments demonstrated the expression of GLP-1R mRNA in approximately 20% of NPY mRNA-containing neurons of the ARC. In summary, our data demonstrate the presence of GLP-1R protein and mRNA in NPY neurons of ARC and also reveal the innervation of NPY neurons by GLP-1R-containing inhibitory neurons. These observations suggest that GLP-1 signaling can influence NPY neurons both directly and indirectly. Furthermore, GLP-1 signaling on energy homeostasis appears to involve both direct and indirect effects of GLP-1 on the orexigenic NPY neurons, in addition to the previously known effects via the anorexigenic POMC neuronal system.

P8.06 Age-dependent dynamics in acute and chronic stress-induced FOSB/ΔFOSB content in the extended amygdala, hypothalamic paraventricular, habenular, centrally-projecting Edinger-Westphal and dorsal raphe nuclei in male rats

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FOS proteins are products of early responding genes contributing to form the activator protein-1 (AP-1). This transcription factor is activated by various types of acute and chronic stimuli. The expression of fos genes results in an increase of nuclear FOS protein content, such as FOSB/ΔFOSB. Divisions of the extended amygdala [central (CeA), medial (MeA), basolateral- (BLA)] and the bed nucleus of the stria terminalis (BNST) exhibit high basal FOSB/ΔFOSB content that does not change in response to acute restraint stress (ARS) or chronic variable mild stress (CVMS). In contrast to the extended amygdala, the FOSB/ΔFOSB content increases in CVMS in the lateral habenula (LHb) and hypothalamic paraventricular nucleus (PVN). The centrally-projecting Edinger-Westphal nucleus (cpEW) and dorsal raphe nucleus (DR) also present increased FOSB/ΔFOSB immunoreactivity (ir) upon CVMS. The magnitude of FOS response to ARS declines during senescence, but the age-dependent dynamics of FOSB/ΔFOSB response/change to stress has not been characterized yet.

We aimed to semi-quantify the FOSB/ΔFOSB content in 13 brain areas [CeA, MeA, BLA; dorsolateral- (BNSTdl), oval- (BNSTov), dorsomedial- (BNSTdm), ventral- (BNSTv) and fusiform (BNSTfu) divisions of BNST; medial- (MHb) and lateral habenula (LHb), PVN, cpEW and DR] in the course of aging. Eight age groups [1-month-old (M), 1.5M, 2M, 3M, 6M, 12M, 18M, 24M] of rats were exposed to a single ARS. Six age groups (2M, 3M, 6M, 12M, 18M, 24M) were subjected to CVMS.

Our immunolabelling indicated that the FOSB/ΔFOSB content of all examined nuclei, except the PVN, was a function of age in control, ARS- or CVMS-exposed rats. The ARS dominantly increased FOSB/ΔFOSB ir in CeA, BNSTdm, BNSTv, BNSTfu, LHb and PVN in 1.5M-3M age periods. CVMS increased the FOSB/ΔFOSB ir only in the BNSTfu, PVN, LHb. Our results indicated that FOSB/ΔFOSB ir moderately decrease with age in all nuclei except in the PVN and BNSTfu.

In line with our previous observations on FOS, the magnitude of FOSB/ΔFOSB response to stress decreased with age in most of the examined nuclei. In contrast earlier reports of FOS ir, the PVN exerted a sustained age-independent FOSB/ΔFOSB that may contribute to the long-lasting adaptation response and plasticity of these neurons. It also may help the maintenance of the hypothalamus-pituitary-adrenal axis response throughout the lifespan.

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P8.07 Role of nesfatin-1 neuropeptide in metabolic changes following intrauterine undernutrition

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Intrauterine growth retardation (IUGR) is an unfortunate and common complication of pregnancy, which increases the risk of late onset type 2 diabetes mellitus (2TDM). Fetal hypothalamic programming during IUGR is a major contributor to the development of this phenotype. Nesfatin-1 is an anorexigenic neuropeptide that plays a central role in the hypothalamic regulation of energy balance and blood glucose level. However, its role in the pathogenesis of IUGR-induced 2TDM is unclear. To investigate this, we used intrauterine protein-restricted rats (PR) as IUGR models. We have shown that 12-week-old PR animals have a greater preference for consuming a high-fat diet over a normal diet than age-matched controls. They also have reduced glucose tolerance and increased insulin resistance. No such differences are detectable at 6 weeks of age between the experimental groups yet. In parallel, the mRNA expression of pro-nesfatin-1 is upregulated in several hypothalamic nuclei associated with energy balance regulation in PR rats by 12 weeks of age. Although the number of nesfatin-1-positive cells in the hypothalamus is the same in the PR and control groups at 12 weeks of age, the rate of embryonic nesfatin-1 cell generation differs from normal in PR rats.

Acute intracerebroventricular (icv) nesfatin-1 treatment reduces food and water intake as well as fasting-induced cell activation in the arcuate nucleus in control, but not in PR animals. Chronic icv nesfatin-1 treatment increases glucose tolerance and insulin sensitivity in control rats only. Our data suggest that due to developmental abnormalities PR rats acquire nesfatin-1 resistance in the hypothalamus, and especially in the arcuate nucleus by adulthood. The acquired nesfatin-1 resistance is probably a key factor in the development of late onset 2TDM in the IUGR phenotype.

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P8.08 Abnormal hypothalamic–pituitary–thyroid axis might influence the outcome of food-motivated learning tests in the triple transgenic Alzheimer's disease model mice

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Alzheimer's disease (AD) is an age-related neurodegenerative disease with progressive memory decline, which could be aggravated by other factors such as changes in thyroid hormone levels. Its transgenic mouse models are promising tools in understanding the underlying mechanisms.

We investigated male, 6-8-month-old triple transgenic (3xTg-AD) mice, known to show some pathological hallmark of the disorder. First, the cognitive decline as well as disturbances of fine motoric - as first sign of AD - were studied using motivation-based pellet retrieval (PR) and staircase tests. Next, blood glucose and lipid parameters were checked, as well as their thyroid axis was studied using ELISA for measuring free thyroxine (FT4) level in serum and qPCR for detecting changes in their pituitary thyroid-stimulating hormone β (TSHβ) and thyroid receptor-β2 (TRβ2) mRNA levels.

Paradoxically, 3xTg-AD mice provided better learning performance compared to age-matched controls with some disturbances in their fine motoric. In these 3xTg-AD mice, the blood glucose level was increased without any differences in the cholesterol and triglyceride levels. Increased blood FT4 level and higher pituitary TSHβ mRNA expression was observed in 3xTg-AD animals, while their pituitary TRβ2 mRNA level was reduced.

In summary, we confirmed the presence of impaired motor skill as an early symptom in 3xTg-AD mice. The results of food-driven learning tests may be influenced by altered driving force for food intake in 3x-Tg-AD animal. The hyperactive hypothalamic-pituitary-thyroid axis of 3xTg-AD mice might be the background for this „food craving". Although hypothyroidism was more associated with dementia, but hyperthyreoidism observable in 3xTg-AD animals might also contribute to the cognitive decline.

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P8.09 Effects of interleukin-1b microinjection in the anterior cingulate cortex of the rat

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In our study, effect of interleukin-1b (IL-1b) on feeding and metabolic processes, body temperature and nutrition associated behavioral and learning mechanisms have been examined in the anterior cingulate cortex (ACC).

Surgery: Microcannulas were implanted above the ACC in stereotaxic operation. During the microinjection session, delivery cannulas were passed through these guide cannulas to administer substances directly into the ACC. Chronic intraoral cannulas made of polyethylene tubes were also implanted into the oral cavity of the animals taking part in the taste reactivity test.

Microinjections: Substances (IL-1b, PBS as control, and in some experiments paracetamol as pretreatment) were administered into the ACC as bilateral microinjections.

Experiments: Short-, medium- and long-term food and water intakes were measured after 24 h food deprivation. Body temperature was determined rectally 2 hours after the microinjections. Blood glucose levels of the animals were examined in a standardized glucose tolerance test after 12 hours of food deprivation. Relevant plasma metabolites (total cholesterol, HDL, LDH, triglycerides, uric acid) were determined by means of a cold chemistry photometer. Species specific facial expressions and postural-locomotor behavioral patterns of animals in response to taste stimuli were examined in taste reactivity test. The potential taste aversion eliciting capacity of IL-1b itself and the potential modifying effect of IL-1b on the acquisition process of LiCl induced taste aversion were examined in conditioned taste aversion paradigm. We studied the species specific motor patterns and locomotor activity of the animals in open field test. Positive or negative reinforcing effects of IL-1b was examined by conditioned place preference test.

Results: IL-1b caused significant elevation in the body temperature of the animals and paracetamol pretreatment was able to prevent this body temperature increase, that is, the role of prostaglandin-mediated processes in the mechanism of action of IL-1b has been proved. Significant decrease was shown in the plasma levels of HDL and total cholesterol due to the IL-1b treatment. Alterations in the taste responsiveness were found in taste reactivity test in case of the lower concentration quinine, the higher concentration MSG and sucrose. Exploratory activity (locomotion, rearing) of the IL-1b treated animals was found to remarkably increase in open field test.

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P8.10 Estrogen converted from testosterone by aromatase neurons in hypothalamic arcuate nucleus decreases firing rate of arcuate kisspeptin neurons in neonatal male mice

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Testosterone (T) produced by the testes in neonatal male mice is indispensable to masculinize their brain. Nevertheless, literature data report that an estrogenic effect is also needed for the defeminization of the male brain. One therefore can suppose that testosterone is converted to estradiol by aromatase neurons and this estrogen then acts on the neuroregulatory pathway modulating sexual development of neonatal male mice, the exact mechanism has however not been revealed, yet. Aromatase neurons in the hypothalamic arcuate nucleus are sexually dimorphic. In addition, kisspeptin (KP) neurons adjacent to these aromatase neurons do not express aromatase but possess estrogen receptor-alpha (ER-alpha). Therefore, an interaction between these two neuronal populations seems to be a good candidate for such a process. We hypothesized that male arcuate aromatase neurons convert testosterone to estrogen to regulate kisspeptin neuron activity. In order to provide evidence for this hypothesis, whole-cell patch clamp studies were carried out in acute brain slices of KP-zsGreen neonatal mice. Testosterone pipetted onto the brain slice reduced kisspeptin neuron firing in males but not in females. This action was entirely prevented by prior bath application of the aromatase inhibitor letrozole or the ER inhibitor ICI-182780, indicating that T conversion to estrogen and ERs play a role in the effect of T. Estrogen was not derived from the recorded kisspeptin neurons because letrozole in the electrode solution (intracellular letrozole) did not interfere with T actions. Furthermore, 17-beta estradiol (E2) reduced arcuate kisspeptin neuron firing in males mimicking the effect of T. These experiments highlight a novel mechanism whereby aromatase neurons regulate the activity of KP neuronal population in the arcuate nucleus of the hypothalamus in neonatal male mice.

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P8.11 Increased expression level of glucagon-like peptide-1 receptor in the human hypothalamic paraventricular nucleus in type 2 diabetic subjects

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Glucagon-like peptide-1 receptor (GLP-1R) agonists have recently been approved for the treatment of type 2 diabetes mellitus (T2DM), however, the site of action of these drugs in the brain are not properly established. GLP-1R is present in the paraventricular hypothalamic nucleus (PVN) of rodents as well as human. Since the PVN plays a role in food intake regulation, we hypothesized that GLP-1Rs in the nucleus is affected in type 2 diabetic patients. We used *post mortem* human hypothalamic samples from six type 2 diabetes mellitus patients and five control subjects. Parallel cryosections were prepared from the PVN. Furthermore, we developed in situ hybridization probes for human GLP-1 receptor, as well as for human for oxytocin and galanin for identification of the PVN. Radioactive *in situ* hybridization histochemistry revealed abundant labeling in the PVN, particularly in its magnocellular subdivision (PaM). Quantitative analysis of the labeling demonstrated increased GLP-1R expression in the PaM in *post mortem* hypothalamic samples from T2DM subjects as compared to controls, while there was no difference in the expression level of GLP-1R in the other subdivisions of the paraventricular nucleus, and the hypothalamic dorsomedial nucleus (DM). In addition we found significantly reduced GLP-1R level in the infundibular nucleus (INF) of the type 2 diabetic patients confirming previous literature data. Our results in the PVN were confirmed by PCR technique. Furthermore, we demonstrated using Western blotting that the GLP-1R protein level is also elevated in the PaM of type 2 diabetes mellitus patients. Our data suggest that the increased expression of GLP-1R in the PVN is related to the dysregulation of feeding behavior and glucose homeostasis in T2DM. Specifically, GLP-1R in the PaM may have special role in the regulation of elevated blood glucose concentration.

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Poster session - Topic 9
Modelling

P9.01 Impaired brain metabolism in schizophrenia-like Wisket rats

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Translational research depends on the relevance of animal models replicating the human disease. The etiology of schizophrenia involves the interaction of genetic, developmental and environmental factors, therefore, a multiple hit rat model, named Wisket, was developed in our laboratory by combining developmental (post-weaning social isolation for 4 weeks), pharmacological (NMDA receptor antagonist, ketamine, treatment) and genetic (selective breeding based on behavioral phenotype for more than 40 generations) manipulations.

A wide-range of behavioral, neurochemical and histological disturbances were observed in Wisket animals. The altered brain metabolism is also a well-known phenomenon in schizophrenic patients. To further characterize the possible cerebral disturbances in these model animals, the aim of this study was to determine their basal brain metabolism in different brain areas applying positron emission tomography (PET) after ^{18}Fluorodeoxyglucose (^{18}F-FDG) administration.

Control Wistar and Wisket rats (n=6 in both groups) were anaesthetized with 3% Forane and injected with 11.3±0.8 MBq of ^{18}F-FDG in 100 µL saline via the lateral tail vein. 30 min after that radiotracer injection PET scans (static 30 min) were performed using the preclinical *MiniPET-II* device (Debrecen, Hungary). After 3D OSEM-LOR image reconstruction, volumes of interest (VOIs) were drawn around the examined regions using the BrainCAD image analysis software and quantitative standardized uptake values (SUVs) were calculated using the following formula: SUV= [ROI activity (MBq/mL)]/[injected activity (MBq)/animal weight (g)].

The total glucose uptake did not show significant differences between the two groups. However, significant reduction in the brain metabolism was detected in the hippocampus, striatum and amygdala of the Wisket animals compared to controls. Furthermore, moderate decreases in the ^{18}F-FDG uptake were detected in all the other investigated areas (cingulate cortex, thalamus and hypothalamus).

This study highlights the significantly lower brain metabolism in this schizophrenia animal model in several brain structures, suggesting the high level of reliability of this model not only in behavioral, neurochemical and histological parameters, but in the brain metabolism, as well.

P9.02 Network Path Convergence Shapes Low-Level Processing in the Visual Cortex

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Cognition emerges by counterstream feedforward and feedback interactions in the large-scale hierarchical network of the cerebral cortex. The counterstream, as a topological feature of the network of cortical areas, is captured by the convergence and divergence of paths through directed links. So defined, the convergence degree (CD) reveals the reciprocal nature of forward and backward connections, and also hierarchically relevant integrative properties of areas through their inward and outward connections. Although cortical dynamics are rooted in the anatomical network, the relationship of structure and function is far from clear. To understand how topology shapes large-scale cortical functioning, we studied the role of CD in network resilience and Granger causal coupling in a model of hierarchical network dynamics. Our results indicate that topological synchronizability is highly vulnerable to attacking edges based on CD, while global network efficiency depends mostly on edge betweenness, another measure of the importance of network links. Furthermore, similar to anatomical hierarchy determined by the laminar distribution of connections, CD-based topological hierarchy showed high correlation with causal coupling in feedforward gamma and feedback alpha-beta band synchronizations in a subnetwork including low-level visual cortical areas. In contrast, causal coupling did not correlate with edge betweenness. Considering the entire network, the CD-based hierarchy correlated well with both the anatomical and functional hierarchy for low-level areas that are hierarchically far apart. Conversely, in a large part of the anatomical network where hierarchical distances are small, correlations were not significant. These findings indicate that at lower levels of cortical hierarchy interareal connectivity closely shapes large-scale oscillatory dynamics. However, at higher levels hierarchy is not strictly determined, allowing for flexibility in hierarchical interactions needed to cope with varying demands.

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P9.03 Triple ligand-targeted nanoparticles cross the blood-brain barrier in vitro and enter human midbrain organoids

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Nanoparticles targeting transporters of the blood-brain barrier (BBB) are promising candidates to increase the brain penetration of biopharmacons. Solute carrier (SLC) transporters show a specific pattern at the BBB and are highly expressed in brain endothelial cells, which makes them potential targets for drug delivery. Here, we show that the transporter ligands ascorbic acid, leucine and glutathione used as targeting molecules on the surface of nanoparticles can increase the uptake of a 67 kDa protein cargo in cultured brain endothelial cells. Moreover, we demonstrate the ability of our triple-targeted nanovesicles to deliver their cargo into human midbrain organoids after crossing the BBB model. Finally, using a combination of techniques, we reveal that the cellular uptake of nanoparticles is temperature- and energy-dependent, is mediated by both endocytosis and fusion with the plasma membrane and is dependent on brain endothelial surface charge. Our data indicate that labeling nanoparticles with ligands of multiple SLCs can potentially be exploited to deliver drugs across the BBB and into the brain.

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P9.04 Structural determinants of gap junction channel formation from hemichannels

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Astrocytic gap junction channels (GJC) significantly contribute to the emergence of synchronized neuronal activity. Deeper understanding of the role of GJCs, however, is hindered by the lack of specific GJC inhibitors, which is largely due to the insufficient knowledge about how GJCs are formed and how can they be targeted pharmacologically. GJC channels are built from two connexons, each comprising six identical connexin subunits. The connexin structures are shaped by three disulphide bonds at each subunit, altogether by eighteen disulphide bonds in each hemichannel, that connect the extracellular loops of connexins. The connexon hemichannels (HCs) from the two opposing membranes are then held together by H-bonds to form GJCs.

We have recently shown (Héja et al., 2022), that GJC formation is delineated by stabilization centers surrounding H-bonding residues at the HC-HC interface. Since the extensive disulphide bonding system is located near to these stabilization centers, we intended to reveal the role of disulphide bridges in GJC formation. To this end, molecular dynamics (MD) was performed for the astrocytic gap junction subtype Cx43 in explicit membrane with intact (closed) disulphide bonds and after disulphide bridges were opened. In addition, MD simulations were performed for Cx43 hemichannels, obtained from the 3D structure of a non-GJC forming Cx subtype. We found, that 1) sequential closing of disulphide bonds drives the HC to a GJC-like structure and 2) proline residues 191P and 193P (in the immediate vicinity of 192C) and 59P (in the vicinity of 61C) position these cysteines to form the 61C-192C disulphide bridge.

We propose, that disulphide bonds – although located farther from the gap – have an indirect effect on GJC formation. The potential disulphide exchange appearing in HC coupling into GJC could rearrange vicinal L58 and Q57 containing stabilization centers, as well. Based on the conservation of Pro-Cys-Pro sequences in connexin subtypes, these conclusions can be applied to all isoforms .

The findings described here deepen our understanding of GJC formation and may help to design GJC inhibitors in the future.

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Poster session - Topic 10
Novel techniques

P10.01 Sensitivity study of two-photon laser scanning in mouse retina samples ex vivo

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Two-photon microscopy (TPM) is an important fluorescence imaging technique in biological sciences which provides high penetration depth, inherent three-dimensional sectioning and good detection sensitivity. However two-photon laser scanning (TPLS) of the eye is not well studied up to now. Previous calculations have shown that the required optical resolution can be achieved in the eye in vivo, and the aberrations can be corrected by adaptive imaging techniques. Therefore TPM seems to be a very useful method to scan the different layers of retina, although the harmful effects of TPLS have remained elusive.

Using TPM, the whole mount retina samples of B6 wild type or Thy1-GCaMP6f transgenic mice were investigated ex vivo. The feasibility of visualizing cellular structures was tested, and the harmful effects of TPLS were specified.

According to our results the living, native retina samples of B6 mice contained only few autofluorescent molecules, which are excitable by TPM. In contrast the ganglion cell layer of Thy1-GCaMP6f transgenic animals represent green, clearly visible and functioning ganglion cells. The imaging of B6 mice retina can be facilitated by fluorescent dyes, such as DAPI, and ethidium bromide, however these dyes has strong negative effect on cell viability. TPLS alone can easily damage any retinal tissue. The extent of destruction robustly depends on the following scanning parameters: duration, intensity, and wavelength. Duration and intensity dependence of demolition seems to be linearly increasing and additive. Moreover increased intensity can affect the retina tissue more powerful compared to the TPLS duration. Over 80 mW scan intensity the destruction is visible to the naked eye in every cases. The wavelength of scanning laser can also act differently in the retina, which may due to the sensitivity and adsorption unconformity of biomolecules. 10 minutes 30mW or 1 minute 300mW line scanning at 880nm can destroy almost all layers of retina.

The eye is the only part of the body where optical examination of the nerve tissue can be directly performed. TPM will be a promising method for the three-dimensional sectioning of retina samples, if the limitations of TPLS are well characterized. Our results will facilitate the preparation of a functional two-photon ophthalmoscope that can be used for scanning the human retina. Thereby the research of such central nervous system diseases will be available as Alzheimer's and Parkinson's disease.

This work was supported by the National Bionics Program (NBP) of Hungary.

P10.02 Development and preclinical validation of a modular multimodal read-write neural interface

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Understanding brain function represents the foundation of new therapies that target diseases of the central nervous system. During the past century, multiple technologies have been developed to record neuronal activity. Each brain activity access technology offers complementary advantages for investigating neural circuits at the microscale, mesoscale, and macroscale with different spatial and temporal resolutions. Since no single method offers access across these scales, probing the same population of neurons using different techniques is necessary to understand the integrated function of brain networks. Here, we present a novel modular chronic neural interface for monitoring and manipulating neural activity. The interface allowed us to perform mesoscale measurements with functional ultrasound technology in freely behaving animals, at an order of magnitude better spatiotemporal resolution compared to classical fMRI methods. Thus, we establish a new method that merges macroscopic (MRI), mesoscopic (fUSI), and microscopic (optical imaging) modalities providing the opportunity to understand brain function in large animal preclinical species that was not possible before.

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P10.03 Development of machine learning tools for the reconstruction of muscle movements from electrophysiological data

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Of all currently available active prosthetic technologies, myoelectric prostheses have the greatest potential to improve quality of life in upper-limb amputees. These devices can restore a degree of manual dexterity to patients who have undergone transradial or transhumeral amputation by allowing complex grasping motions to be controlled using the individual's remaining muscles. In most state-of-the-art systems, this control paradigm is realized through pattern recognition in surface electromyography (sEMG) signals, often with the help of machine learning tools. These methods are highly data intensive however, which makes knowledge transfer an especially attractive prospect for the development of prosthesis control systems.

In order to evaluate whether transfer learning might be suitable for sEMG classification tasks, we recorded a dataset of 7 basic hand movements (with three repetitions each) from 6 volunteers. All data was acquired using the MindRove armband, recording muscle activity from the forearm at 500Hz using 8+2 monopolar electrodes. In addition to the electrophysiological data, two depth video streams were captured from an Intel Realsense D415 and D435 camera pair to serve as the ground truth during annotation. The recorded time series were then divided into 250ms long segments with 125ms overlaps, and a number of commonly used feature sets (such Hudgins's) were calculated for every channel. One repetition out of three for every gesture from the first 5 subjects was held out as a test set, along with the entirety of the 6th subject's data.

For transfer learning, we used the highly successful PutEMG database due to its high number of participants and comparatively simple movement set with many repetitions. We downsampled this dataset to match our own in terms of sampling frequency, then calculated the same features as mentioned above and trained a random forest classifier to differentiate between movements. After hyperparameter tuning, this system achieved a mean accuracy of 89.8% and 77.4% after being trained on PutEMG and our own dataset respectively. Following this trial, a random forest-based transfer learning algorithm developed by Noam Segev et al. was used to apply the PutEMG model to the custom dataset. This resulted in a notable improvement of almost 10%, as the classifier became able to correctly predict hand movements in 87.1% of cases on average.

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P10.04 Flexible polymer-based neural probes designed for human intracortical laminar recordings

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In this study, we developed and validated a single-shank polyimide-based neural probe designed for laminar recordings from the human neocortex. The flexible probe has a 3.9-mm-long, 75 to 300-µm-wide and 10-µm-thick tapered, implantable shank which contains 24 linearly placed gold microelectrodes with a diameter of 20 µm and center-to-center distance of 150 µm. Two probe variants (edge-site layout with the recording sites located at the edge of the probe shank, and center-site layout with the sites placed in the middle of the shank) with two different connector types (Omnetics and Zero Insertion Force (ZIF)) were developed to compare their electrophysiological performance as well as their usability under different experimental conditions. To test the probes, first we performed impedance spectroscopy (from 1 Hz to 7500 Hz) *in vitro*, in physiological saline solution (n = 6 probes; 144 sites). The impedance magnitude of recording sites was 221 ± 130 kΩ at 1 kHz (mean ± s.d. of 117 sites; defective sites with high impedance values were removed from the calculation). Electrophysiological performance of the probes was validated in *in vivo* experiments by acute implantations into neocortical and hippocampal areas of anesthetized rats. To aid the insertion of the flexible probes into the brain tissue, on the one hand, we removed the dura mater over the targeted brain area. On the other hand, the probe shank was either fixed to a silicon shuttle using a small amount of polyethylene glycol, or was inserted without a shuttle but after cutting a small opening into the pia mater. In acute experiments, we recorded good-quality local field potentials, single- and multi-unit activity from the investigated brain regions, even after reusing a probe multiple times. We were able to monitor cortical slow waves as well as hippocampal gamma activity with the implant. Furthermore, we regularly detected spikes with amplitudes exceeding 100 µV and we could record the activity of multiple well-isolated single units simultaneously. Our future plans are to chronically implant the polyimide probes in rodents to evaluate their long-term electrophysiological performance as well as the brain tissue response in the vicinity of the probe shank. Our long-term goal is to test the devices in human cortical tissue, for example, by implanting them into the brain of drug-resistant epileptic patients who are potential candidates for brain surgery.

The research leading to these results has received funding from the Hungarian National Research, Development and Innovation Office (PD134196 to R.F., TUDFO/51757-1/2019-ITM to R.F. and I.U.) and from the Deutsche Forschungsgemeinschaft (DFG, Grant EXC 1086 to P.R.).

P10.05 Autofluorescence reducing in cat brain slices

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Detection of fluorescent reporter protein expression is necessary to develop precisely targeted optogenetic manipulation strategies. In large animal model species and in humans, tissue autofluorescence can jeopardize correct localization and quantification of fluorescent protein expression.

To develop efficient and targeted optogenetics in large animal species, we injected cats with several different serotypes of adeno-associated viruses (AAV), delivering green fluorescent protein GCaMP6m driven by neuron-specific promoter. To evaluate the effect of endogenous autofluorescent molecules, we compared the efficiency of autofluorescence reducing protocols.

Fluorescent signals varied between very high and undetectable levels in unstained slices. The high autofluorescence of the adult cat brain aggravated the detection of the specific signal. Application of fluorescent immunostaining increased the GCaMP6m signal, but also caused a higher non-specific signal. The autofluorescence reducing techniques efficiently diminished the autofluorescent background, but had an unwanted effect: the GCaMP6m signal also decreased, particularly in the axons of infected cells. By combining different autofluorescence reducing techniques we aim to find an appropriate method that minimizes reduction of the specific signal while minimizing autofluorescence in order to achieve the best signal-to-noise ratio.

Our results make it clear that methods applied routinely for the analysis of fluorescent signals in rodents need to be adapted and improved for application in large, preclinical animal species.

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P10.06 Demonstration of safe operation of a sharp-tip multimodal optrode in infrared neuromodulation of the rat somatosensory cortex: findings of the histological and electrophysiological evaluation

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Infrared neuromodulation (INM) is an emerging heat stimulation technique that exploits the inherent thermal sensitivity of neurons to excite or inhibit cellular activity. It has great potential both in the peripheral and central nervous system (Fekete 2020). The key advantage of INM is that it does not need any genetic modification of the target cells and can be applied in a spatially confined region without causing electrical artifacts in electrophysiology (Horváth 2020). The hypotheses in the background of inhibitory effect of INM are on one hand the elevated baseline temperature (Walsh 2016) or on the other hand that an increase in temperature leads to a net increase in the hyperpolarizing currents actually overcoming depolarizing current (Ganguly 2019). Despite the growing number of in vitro demonstrations of INM, there is limited information on the physiological response of intracortical cell population due to the lack of in vivo investigations. Although, several studies demonstrated the efficacy of INM, only a few attempts have been made to prove the safety of this neuromodulation approach. Especially, for intracranial INM there is still a lack of histological data on the effect.

A symmetrical 30° sharp tip multimodal silicon probe was acutely implanted in the cortex of anaesthetized rats. The bulk silicon shaft, as a waveguide delivered the external infrared light intracranially and the same probe held 12 platinum electrophysiological recording sites. By this approach we avoided the parallel insertion of multiple probes which would inherently contribute to cell death along the probe tracks. Boros et al. found that this sharp tip shape can further improve the overall infrared performance of the silicon waveguide compared to a simple blunt tip ending (Boros 2019). Moreover, this sharp tip shape conduces to reduce the penetration force and dimpling during implantation (Fekete 2015), otherwise brain tissue capillary vessels can be squeezed to such an extent that may cause irreversible damages to the neurons. In our work we also present results of histological investigations. NeuN staining was applied to reveal the effect of continuous-wave infrared illumination at 8 mW power and to compare the implantation tracks in terms of cell survival. Multichannel extracellular electrophysiological recordings support histological findings.

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P10.07 Use of Expansion Microscopy to reveal sub-synaptic protein organization

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Fluorescence microscopy is a fundamental tool for neuroscience, but the diffraction limit of light prevents the investigation of sub-synaptic cellular structures with traditional fluorescence microscopy. Within the last decades, numerous superresolution technologies have emerged which allowed breaking the diffraction limit for optical microscopy, but their widespread use is hindered by the high cost of the specific instruments or by technical limitations including e.g. the type of fluorescent labels or photobleaching.

In contrast, expansion microscopy (ExM) is a physical form of magnification that increases the effective resolving power of any traditional widefield or confocal microscope. In ExM, a swellable gel is synthesized throughout the sample. During gelation, specific biomolecules such as proteins and RNA can be covalently linked into the hydrogel network. After gelation, the structure of the sample is disrupted by breaking crosslinks and bonds in proteins; however, the anchored molecules (e.g., antibody tags, RNA, fluorescent proteins) are retained and isotropically expanded by dialysis in water. Thus, ExM enables nanoscale-resolution imaging of fixed cells and tissues on conventional diffraction-limited microscopes.

In the present study we used murine hippocampal cell cultures to test expansion microscopy. The cells were transfected with EGFP to outline individual cells, followed by traditional fixation and immunolabelling for synaptic markers. Nuclear size before and after expansion was compared, as a unit of measurement for the scale of expansion. Our results confirmed a 4.5-5.5-fold increase in size in every axes. When the morphology of EGFP expressing cells was analysed, no evident distortions in the shape of dendrites or dendritic spines were observed upon expansion. Using immunolabelling against pre- and postsynaptic proteins we also confirmed that expanded samples are suitable for studying sub-synaptic structures with conventional confocal microscopy.

Taken together, we confirm that expansion microscopy is a feasible yet reliable method to increase spatial resolution in fluorescence confocal imaging of neurons.

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P10.08 Organ-specific tropism profiles of synthetic AAV capsids in preclinical species

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AAV-mediated gene delivery is widely used in basic research and is gaining importance for human therapy. Newly engineered AAV capsids offer increasing specificity to target cell types or organs. Here we aim to develop and validate non-invasive, efficient and targeted gene delivery strategies to the brain in large-animal preclinical species. We assessed capsid tropism in central and peripheral locations of the nervous system upon systemic or intrathecal delivery in cats. Expression strength was evaluated via immunohistochemistry and quantitative PCR for the delivered transgene. Our results lay the foundation for brain-wide functional testing via calcium imaging and optogenetic activation of select neuronal populations.

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P10.09 Realization of a wireless optogenetics brain stimulator

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There has been a fast development in wireless devices focused on optogenetic applications. These wireless devices eliminate the physical constraints derived from tethered experiment setups, allowing optogenetic stimulation in freely-moving animals. Here we present the development and validation of a low-cost wireless optogenetics brain stimulator. The device uses a strong and efficient light source aiming at the construction of minimally invasive optogenetics and to maximize the coupling efficiency into the optical fibers. Optical power measurements were gathered to test the coupling efficiency and examine the time stability of the light emission. Our results show the feasibility of opsin activation with the presented device.

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P10.10 Assessment of neutralizing factors against engineered serotypes of Adeno-Associated Virus in preclinical species

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Adeno-Associated Viruses (AAVs) are gaining increasing importance in gene therapy. AAVs are non-pathogenic, have the ability to infect dividing and non-dividing cells and remain primarily episomal. However, neutralising effects can reduce or completely inhibit the expression of the transgene. Delivering engineered variants of naturally-occurring AAV capsids in humans with pre-existing immunity against natural AAV variants may show improved transgene expression compared to repeated exposure to the same capsid. Here we set out to identify the relation of neutralization cross-reactivity between naturally occurring and engineered AAV capsids. We seek to identify variants that are less cross-reactive and exhibit decreased immune-response escaping proportional to the distance measured in number of amino-acid differences to wild-type AAV variants. For quantification of neutralizing response, we establish a generic assay for the quantification of blood serum neutralizing activity against AAVs.

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P10.11 Spatio-temporal membrane potential and resistive current reconstruction from parallel multielectrode array and intracellular measurements in single neurons

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Here we show, that based on parallel multichannel extracellular and single-channel intracellular potential recordings, it is possible the reconstruct the spatio-temporal distribution of membrane potential with the spatial resolution of the extracellular recordings in a single neuron. Moreover, we show, that reconstruction of intracellular membrane potential made possible the distinction between two components of the current source density (CSD): the resistive and the capacitive currents. This distinction would provide a clue to the proper interpretation of the CSD distribution, as the resistive component corresponds to the active channel currents, both synaptic and voltage-sensitive channel membrane currents, while capacitive current corresponds to the passive counter currents. The importance of this distinction is further emphasized by different features of the resistive membrane current distribution compared to the CSD. As the CSD is a net membrane current, the sum of the CSD along a whole intact cell should be zero at each time moment, according to the charge conservation law. In contrast to this, the sum of the resistive current should not be necessarily zero since it governs the membrane potential dynamics. Thus, estimation of the spatial distribution of the resistive membrane current makes possible the distinction between active and passive sinks and sources of the CSD map and localization of the synaptic input currents, which makes the neuron fire. We validate our reconstruction approach on simulations and demonstrate its application on simultaneous and co-localized extra- and intracellular *in vitro* recordings in the hippocampal CA1 region using *in vitro* rat brain slice preparations.

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P10.12 Alignment of functional and anatomical layout of cortical map

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The multitude of brain areas in mammals implement computing tasks via distributed neuronal networks. Within brain areas, specific anatomical, connectivity and gene expression properties underlie the physiological, morphological and functional identities of computing units. In contrast to the apparent simplicity of Brodmann's original descriptions on functions of brain areas, the availability of multidimensional datasets defining the identity and boundaries of functional brain units dissolve the meaning of classical areal categorizations. Here we present our work targeting the alignment of functional maps and cellular-level histology of large-animal preclinical species, cats. We develop a new labeling method that may provide spatial reference landmarks across in vivo and sliced tissue configurations at high precision. This method may enable linking mesoscale functional maps to specific cellular circuit arrangements in different cortical layers and across classical brain areas.

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P10.13 PharmacoSTORM nanoscale pharmacology reveals cariprazine binding on Islands of Calleja granule cells

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Immunolabeling and autoradiography have traditionally been applied as the methods-of-choice to visualize and collect molecular information about physiological and pathological processes. Here, we introduce PharmacoSTORM super-resolution imaging that combines the complementary advantages of these approaches and enables cell-type- and compartment-specific nanoscale molecular measurements. We exploited rational chemical design for fluorophore-tagged high-affinity receptor ligands and an enzyme inhibitor; and demonstrated broad PharmacoSTORM applicability for three protein classes and for cariprazine, a clinically approved antipsychotic and antidepressant drug. Because the neurobiological substrate of cariprazine has remained elusive, we took advantage of PharmacoSTORM to provide in vivo evidence that cariprazine predominantly binds to D3 dopamine receptors on Islands of Calleja granule cell axons but avoids dopaminergic terminals. These findings show that PharmacoSTORM helps to quantify drug-target interaction sites at the nanoscale level in a cell-type- and subcellular context-dependent manner and within complex tissue preparations. Moreover, the results highlight the underappreciated neuropsychiatric significance of the Islands of Calleja in the ventral forebrain.

P10.14 Segmentation of the human anterior thalamus based on excitatory inputs and neurochemical markers

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The connectivity between the anterior part of the human thalamus (ATh) and the frontal cortex is crucial for complex cognitive processes. The ATh is also a major target for deep brain neurosurgery in various neurological disorders such as epilepsy, Parkinson's disease or essential tremor. Functionally relevant nuclear segmentation is essential for understanding both healthy and pathological processes of the brain, however, a comprehensive division of the human ATh, based on morphological and biological properties, is still lacking. Our aim was to create a reliable nuclear segmentation of the ATh based on thalamic cell types and afferentation.

In this project we used vesicular glutamate transporter type 1 and 2 (vGluT1, vGluT2), calbindin, calretinin and parvalbumin immunostainings in consecutive sections of post-mortem human thalami to label cortical and subcortical excitatory inputs, basal ganglia afferents as well as distinct thalamic cell types.

The combination of these markers clearly outlined the major nuclear organisation of the human ATh, however, some areas show different characteristics compared to accepted schemes. Both the arousal-related midline nuclei and the anterior nuclear group, which is involved in memory circuits, occupied much larger proportions of the ATh than indicated in previous human atlases. The rostral part of the mediodorsal nucleus lacked giant $vGluT1^+$ inputs but was innervated by $vGluT2^+$ terminals. Surprisingly, afferents of the two large motor systems (cerebellar and basal ganglia) did not display complete segregation as expected.

Our method of using a combination of neurochemical markers produces a relevant and functionally informative nuclear segmentation which provides a novel understanding of the functions of the thalamic nuclei. The results indicate nucleus-specific combinations of afferent systems which display both evolutionary conserved and highly derived human features. Further findings, in particular the understanding of inter-individual variation, will provide a uniquely useful perspective for neurosurgical procedures.

P10.15 Thalamic nuclear segmentation based on quantitative, automated detection of excitatory afferents in the human thalamus

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Analyses of the excitatory afferents has not been performed in the thalamus so far. Here we performed a quantitative analysis of glutamatergic inputs with previously unprecedented high quality immunolabeling in human anterior thalamic serial sections. We visualized both cortical and subcortical afferents using vesicular glutamate transporter 1 and 2 (vGluT1 and 2, respectively) in postmortem human brain samples.

Besides the manual delineation we designed a workflow for automated selection of boutons. Our high throughput automated data collection method enabled reliable and large scale quantification and morphological analysis of the afferent boutons. Although we found quite remarkable inter-individual differences regarding size and proportions between nuclei, the bouton distribution and density of same nuclei in different patients were remarkably similar. The highest vGluT2 bouton density was observed in midline nuclei ($5.5*10^4$ 1/mm^2) followed by intralaminar ($3.8*10^4$ 1/mm^2), anteroventral ($2.7*10^4$ 1/mm^2) nuclei. The bouton density of other nuclei in anterior thalamus, e.g. ventrolateral, reticular, mediodorsal or reuniens was below $1*10^4$ 1/mm^2.

We used this information for the parcellation of thalamic nuclei and to compare these functional information with the previously existing thalamic maps. We found notable discrepancy in spatial orientation and size relative to traditional atlases (especially in mediodorsal(MD), ventralis anterior (VA)-ventralis lateralis(VL) nuclei). Furthermore analyses of excitatory inputs allowed us to draw functional predictions to human thalamus. We found that that in VA-VL, the cerebellar and basal ganglia recipient motor systems are not entirely separated (vGluT2+ and vGluT2-). In MD: both subcortical (vGluT2+) and cortical (vGluT1+) drivers are found. In case of vGluT2, the size of boutons indicate functional differences. The presence of large vGluT2+ terminals in small islands inside the MD suggests distinct functional sectors within this nucleus. These islands represent primary subocortical drive, whereas the rest of the nucleus can be considered higher order as previously assumed.

Our data form the basis of a rational segmentation of the human thalamus, which is necessary for invasive brain surgeries. Furthermore it allows quantitative characterization of excitatory afferents and its alteration in pathological cases.

P10.16 Viral capsid-like RNA transfer in the brain: structural biochemistry of molecular tools and functional perspectives

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Mammalian genomes contain several homologs of the capsid-forming Gag protein-encoding gene. One of them, the Activity-regulated cytoskeleton associated protein (Arc), is an immediate early gene product expressed in neurons after high frequency stimulation. Arc has three structural variants: open, closed, and oligomeric forms. Arc was shown to bind and encapsulate RNA, and it can form capsid-like particles. Arc capsid is also capable of intercellular transfer of its mRNA in extracellular vesicles, and these vesicles can be found in peripheral blood as well. Former studies revealed the involvement of Arc in long-term potentiation and depression (LTP and LTD), synapse development, and the early development of the brain. However, it is still unknown, what is the role of the mRNA encapsulating capsid form of Arc, and how the mRNA transfer from one cell to the other affects synaptic functions. Based on the literature, we synthesized capsid inhibitor peptides to hinder capsid formation, and we also prepared Arc capsid-containing fraction from rat brain tissue. We validated the quality of fractions, and the effectiveness of inhibitors using electron microscopy. The perspective of the project is to understand the functional role of the Arc capsids by developing molecular tools to study brain diseases accompanied by memory impairment. Also, Arc capsids might be potential diagnostic tools in the future due to their RNA binding properties and their presence in peripheral blood.

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P10.17 Electromyography-based Application Development For Stroke Rehabilitation

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Electromyography is a technique for recording the electrical activity of skeletal muscles, it informs us about pathology affecting the anterior horn cell, nerve roots, peripherial nerve, neuromuscular junction and the muscle. The recording of sensory and motor responses is performed using surface electrodes, measuring conduction velocity, amplitude and duration.

This project incorporates the MindRove armband, which has 8 channels, a sampling rate of 500 Hz and a resolution of 24 bits. The electrodes are arranged in a circle on the forearm. The inertial measurement unit using a combination of a 3-axis gyroscope and a 3-axis accelerometer.

Electromyography can be very useful in detecting various diseases affecting the muscles – disorders of muscles, nerves or neuromuscular junction – and more importantly in this case, it can be used in helping to recover from muscle paralysis. In the latter case electromyography functions as a biofeedback device, because even weak signals can be measured using an appropriate instrument, so even if a specific muscle contraction is not visible, the patient can still receive positive confirmation.

Diseases associated with muscle paralysis are common, with stroke accounting for the most cases, according to a 2015 survey conducted by Stroke Alliance for Europe coalition, affecting 763 out of 100000 habitants in Hungary, 60% of whom have paralysis of one arm.

We are creating a user friendly, easily applicable computer software that would help these people recover. The application is designed to include various training programs, during which users receive feedback on the functioning and development of their muscles, using real-time and historic data measured by the wirelessly connected armband.

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P10.18 Acousto-optics based simultaneous 3D imaging and photostimulation with temporal laser intensity modulation for precise temporal control of activity patterns at the level of individual neurons

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Investigating the network dynamics of neuronal populations in the cortical areas involved in vision with photostimulation is one of the hot topics of the past few years. However, measurement protocols, visual paradigms, and experimental scenarios have all been restricted by the technological constraints of conventional microscope systems. Here we present a novel, 3D acousto-optical scanning-based photostimulation equipment which enables ms resolution precise temporal control of activity patterns at the level of individual neurons. To demonstrate the efficacy of the system we used it to replay activity on a set of neurons found to be responsible for coding certain visual stimulation. For performing this first we used fast multilayer acousto-optical frame scanning, which enables recording the activity of arbitrary cortical layers sequentially with high speed (40Hz with 512x512 pixel resolution, in a 500x500 µm areas). By this, we can follow the activity of hundreds of cells in multiple (3-11) areas of the cortex. Next, we developed novel analytic software modules to automatically detect responding neurons and to define among the acquired cell-level activity patterns those that are involved in the perception of specific visual stimuli (i.e., representing a latent task variable of a task). Optical stimulation is presented with a custom virtual reality system that provides binocular depth-perception and a fully immersive experience for mice. Finally, we photostimulate hundreds of somata simultaneously with 3D imaging. For precise temporal control of the activity on the cells selected to be stimulated we developed novel driver electronics of the AO system allowing us to arbitrarily modulate the stimulating laser intensity with a granularity of 30 µs. The achievable temporal resolution of the activity playback was found to be 100 ms, however, note that this is not limited by the stimulation method itself, but by the precision of the activity detection which was measured with GCaMP6f. Using this system, we showed that we can effectively stimulate and measure up to 250 cells simultaneously or can replay precise temporal dynamics on up to 20 cells with 100 ms temporal resolution.

P10.19 Automated patch-clamp with automated analysis: Extracting compound-specific, concentration-independent biophysical properties of inhibition for sodium channel inhibitors.

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When targeting voltage-gated ion channels of excitable cells, one needs to deal with a “moving target”. Ion channels keep changing their conformation, and drugs keep dynamically associating and dissociating, depending on the conformation. It is not affinity in itself, that is important, but how the dynamics of cellular activity relates to the dynamics of drug access/egress and binding/unbinding.

Automated patch clamp experiments most often ask simple questions, and get simple answers. It is not because APC instruments could not perform experiments at the same level of sophistication as manual patch-clamp, but simply because it is difficult to perform a detailed analysis at a tolerably high throughput.

We have created a complex protocol for the IonFlux Mercury instrument in order to study sodium channel inhibitor compounds. The protocol can assess different aspects of their mechanism of action, including state-dependence, binding dynamics, modulation of gating, cooperativity, and aqueous phase-membrane partitioning. Instead of concentration-dependent measures (such as the shift of half-inactivation voltage or the delay in recovery from inactivation), we focus on properties that are independent of concentration and are true properties of individual compounds. We have developed a rapid, semi-automatic analysis method for obtaining compound-specific properties. These properties enable us to perform a classification of sodium channel inhibitor compounds based on their mechanism of action, and to correlate mechanisms of action with chemical properties. Our goal in this approach is to be able to predict specific mechanisms of action from chemical structure.

P10.20 Integrated Data Analysis of LFP and Two-Photon Imaging Recordings

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Hippocampal sharp wave ripples (SPW-Rs) are very important biomarkers for memory function in animals. Using simultaneous two-photon imaging and local field potential (LFP) recordings they can be investigated both on the cellular and network level. Manual detection of SPW-Rs along with the correlated cellular activity in recordings is unnecessarily tedious and it carries an inherent level of subjectivity. Our solution for overcoming this limitation was developing an integrated and automated data analysis software package. Along with LFP and two-photon imaging data, the package can also handle additional experimental information like running speed and licking times. We have assembled a set of graphical user interfaces (GUIs) and signal processing algorithms using the MATLAB programming language. For processing our recordings we have developed filters, artifact suppression and event detection methods. We have created three GUIs, which are used to efficiently interact with the algorithms. The first GUI handles importing the raw experimental data, running processing algorithms on it and detecting events of interest, the second can interact with saved detection files and finally the third was designed to interact with the database of events. The event database will serve as a crucial tool in assessing the information content of our data sets.

P10.21 Validation of the Dimensional Causality analysis method on evoked epileptic activity in vitro

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In recent years, we have developed a new causality analysis method named Dimensional Causality (DC), which is capable to detect and distinguish all basic forms of causal connections between two dynamical systems, based on observed time series: DC can detect directed causal connections, circular or bidirectional connection and can distinguish them from the two unconnected systems driven by a hidden common cause, or confounder. While the efficacy of the method has been demonstrated on time series from simulated dynamical systems here we validate our method on in vitro measurements, where the form of the causal connection is more or less well known. LFP and Intrinsic Optical Signal (IOS) were recorded during evoked epileptic activity in vitro, evoked by magnesium-free Ringer-solution. In our earlier study, we have shown that Sugihara's cross-convergent mapping (CCM) method detects directed causal relationship from the LFP towards the IOS activity without detectable signs of a feedback effect. However, CCM can not distinguish the directed connection from the effect of a confounder. Furthermore, the outcome of the CCM was ambiguous, one had to consider the time delay of the effect to get to a clear conclusion.

DC analysis showed a clear unidirectional causal connection from the LFP to the IOS in most of the cases, especially in the first period of the evoked seizure-like activity. However, as the epileptic activity decreases in the latter phase of the experiments the hidden existence of a hidden common cause becomes more probable. Note, that the direction of the causal connection was inferred without considering any observable time delay of the effect.

We have concluded, that in case of a weak and unreliable connection at the end of the seizure-like activity, the electric activity can be decomposed into a hidden common cause affecting both systems and an unconnected degree of freedom.

These results showed the applicability of the DC method for real-world experimental data and provided useful instructions for future applications.

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P10.22 Imaginary movement classification for brain computer interface systems using 3D and 2D convolutional neural networks

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When designing a Brain-Computer Interface system, the classification of EEG signals is an essential task. In this work, we designed two architectures to recognize EEG patterns corresponding to the imagined motoric movements, using neural networks based on 3-dimensional and 2-dimensional convolutions. In the preprocessing step, we have reorganized the 2D EEG channel matrix related to their spatial arrangement, resulting in a tensor with 3 dimensions: *SX x SY x Time*. As signals have high variance between certain people, we applied Transfer Learning (TL) in the way of pretraining deep-learning models over 50 subjects, and fine-tune weights with the data of the subject we want to test on. The networks were tested on the EEG recordings of the Physionet database; 2-way and 4-way classification of motor imaginary movements were performed. Without TL, with 2D convolution, we gained higher accuracy – 2 classes: 75.7%, 4 classes: 46.5%, but after the application of it the 3D network performed better – 2 classes: 80.5%, 4 classes: 63.8%.

We have tested if the application of the FASTER artifact-rejection algorithm improves the accuracy or not, it has turned out that the effect highly depends on the subject we test on: in some cases, we have gained around 10% performance improvement, however, in some other cases the algorithm declined results by the same amount.

Finally, we examined how much does the result depend on the shift of the signal in time. The models were trained and tested with fixed time offsets, using 2D and 3D convolutional networks and Transfer Learning. The results of both networks showed that when the first 1 second of the original 4-second-long epoch is included, the accuracy is higher (around 60%), but when that part is not presented it declines to 30%. An interesting observation is that if the first second after the epoch is involved, accuracy starts increasing again, implying the recognizable information is presented not just at the beginning, but even at the stopping of the imagination.

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P10.23 Surface Laplacian based motor imagery images classification using deep learning

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Motor-Imagery based brain-computer interface (BCI) systems are highly dependent on the classification of the EEG signals. These signals are noisy and differ from one subject to another and even for the same subject among different trials, and this is why designing a general classification model is not an easy mission. Convolutional Neural Networks (CNN) approach is dominant in computer vision and image classification, so we followed a trend in EEG signals classification in which these signals are transformed into images, and thus classifying such signals become an image classification problem. Surface Laplacian is used to improve the spatial resolution of the raw EEG signals before being transformed into images. The motor imagery EEG activity is mainly in the Mu [8-13 Hz] and Beta [13-30 Hz] bands, so the Surface Laplacian and the raw EEG signals were bandpass filtered accordingly. We used the Physionet dataset for EEG motor movement/imagery tasks which consists of 109 subjects (107 are used). The motor imagery EEG trials were transformed into 2-D images (with 2 channels, one for Mu band and the other for the Beta band) using the azimuthal projection and Clough-Tocher algorithm for interpolation, and these input images are fed to a CNN model to classify 4 different motor imagery classes. The average classification accuracy for the Surface Laplacian based images is 57%, while for the raw EEG images is 56.5%, and both are significantly better than the results of the Support Vector Machine (SVM) over the same dataset which has 54% average accuracy. This suggests that the Surface Laplacian potentials approach is promising when used with the signal to image transformation.

P10.24 Shape memory polymer based transparent electrode array for long-term multimodal neuroimaging

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Multimodal neuroimaging methods are efficient tools to map the brain functionalities with high spatial and temporal resolution. Combining two-photon microscopy with electrophysiological signal recording is feasible with using transparent electrode materials. The shape memory polymer (SMP) thiol-ene acrylate is an excellent substrate material due to its transparent nature. The stability and biocompatibility of intracortical SMP probes have been shown and the tunable elastic characteristics are beneficial to achieve long-term stability. In our work, we demonstrate a multimodal neuroimaging scheme using a thiol-ene acrylate based cortical implant. The micro-electrocorticography (µECoG) device's feasibility of measuring intracranial EEG and fluorescent GCaMP6 signals using two-photon excitation through the device is presented in mice. The stability of electrode yield was presented with in vivo impedance measurement over 75 days. During this period no sign of delamination or material degradation appeared. The recording quality was seen also by the high signal-to-noise ratio (1.04 to 5.74) throughout the course of the experiment and by the identifiable theta oscillations. The chronic immune response was characterized by Glial Fibrillary Acidic Protein (GFAP) staining of astrocytes and fluorescent Nissl (NeuroTrace) staining of neurons. After 80 days of implantation, the histological analysis revealed only a modest foreign body response. The result of cortical thickness measurement confirms the advantage of thiol-ene acrylate as a substrate as no significant difference was shown between implanted and control cortices. To determine the effect of the device on optical distortion and resolution, the sizes of fluorescent beads, neuronal cell bodies, and dendrites were determined without and under the transparent device placed in the light path of the two-photon microscope. The captured sizes of the detected object on the in vitro images showed a small difference between the presence and the absence of the device. In addition, the change in the relative intensity of fluorescent signals was determined in in vivo images under the long-term implanted device. During the 22 weeks in vivo measurements, the fluorescent activity remained and Ca^{2+} signals were captured. Based on the results we can say our device is suitable for multimodal imaging.

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P10.25 Use of glucose oxidase-based electrode (amperometric biosensor) in animal experiments

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The optimal functioning of all the cells in our body requires an adequate supply of energy, the main source of which is glucose. A good indicator of tissue activity is a decrease in their glucose levels. Our main goal is to develop biosensors that can monitor changes in glucose concentration *in vivo*. As this issue also has clinical implications for the monitoring of diabetics, appropriate methods have been developed in humans to monitor peripheral tissue glucose levels. However, there is currently no microelectrode available to monitor changes in glucose concentration in brain tissue. Furthermore, adapting the electrodes set to human measurements to the mouse dimension is not solved either.

The most widely used group of biosensors are amperometric enzyme electrodes based on oxidoreductase enzymes. To measure sugar levels, the oxidoreductive effects of the enzyme glucose oxidase can be exploited. Principle of measurement: the enzyme glucose oxidase is applied to the surface of the platinum electrode, where it forms hydrogen peroxide from glucose and oxygen. It diffuses to the electrode surface through an H_2O_2-selective, size-exclusion layer covering the electrode surface. The electrons formed during the electrocatalytic decomposition on the surface of the electrode give a detectable current signal, the magnitude of which is proportional to the glucose level of the solution to be measured. For biosensors with amperometric detection of H_2O_2, a size exclusion membrane is used on the platinum electrode surface to ensure selectivity. It is also important to consider the optimal layer thickness of the biosensors. As a first step in in vitro measurements, it is necessary to record a calibration curve in the measurement range that would be expected in *in vivo* experiments.

Overall, the developed sensor should be small (microelectrode) but stable enough, sensitive, and selective to collect only specific information about a small area of the mouse brain, informing us of changes in activity in that brain area with good temporal resolution.

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Registered participants

List of registered participants was prepared according to data available on 15 January, 2022.
Presenters' poster or lecture numbers are in bold.

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