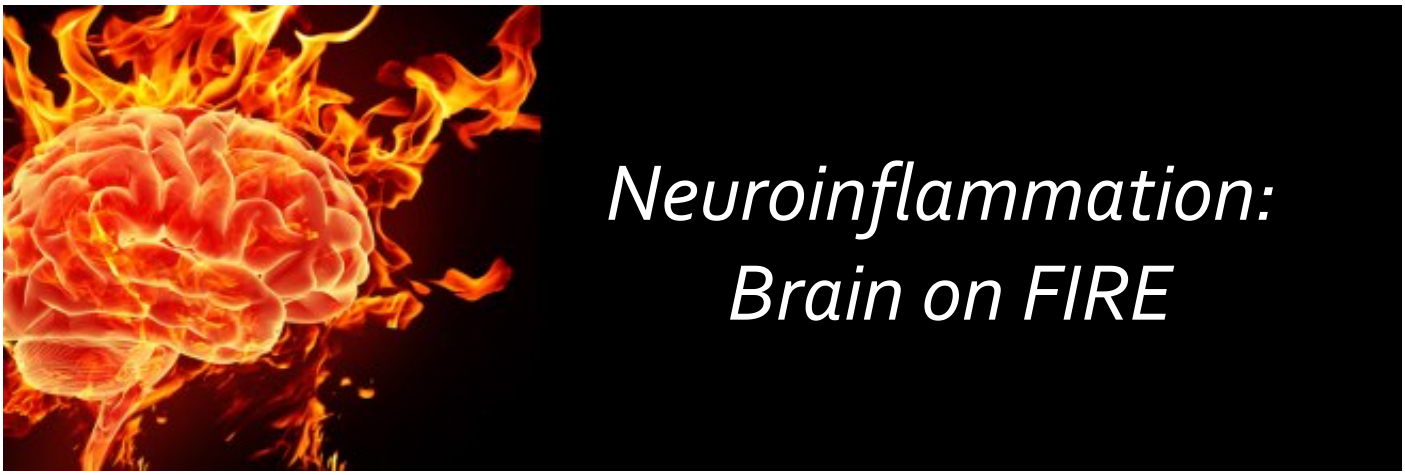
IZN Retreat 2024
Kloster Schöntal
July 14-15

IZN

Interdisziplinäres Zentrum für
Neurowissenschaften der
Ruprecht-Karls-Universität Heidelberg



Neuroinflammation:
Brain on FIRE

Retreat Program

Sunday, July 14

Welcome Reception	**10:00**	Banquet hall	
Welcome Address	10:30	**Hilmar Bading** Managing Director of the IZN, Heidelberg University Heidelberg, Germany	
Introduction	10:40	**Oliver Kann** Heidelberg University Heidelberg, Germany	
Session 1 Chair: Isabel Loss	10:55	**Katrin Kierdorf** University of Freiburg Freiburg, Germany	*Between the tides: ebbs and flows of microglia in health and disease*
Session 2 **"Poster Jam"** Chair: Anna Hertle	11:30	**Debanjan Chowdhury** Monyer Group	*Identifying mechanisms involved in acute alcohol-induced amnesia*
		Celia Lerma-Martin Schirmer Group	*Cell type mapping unravels tissue niches and interactions in subcortical multiple sclerosis lesions*
Lunch	**12:00**		
Free Time	**Canoeing** (meet at 13:15 at the parking lot next to the former train station) *or* **Walk 'n' Talk** *or* **Guided tour through the monastery** (meet at 14:00 at the baroque staircase)		
Dinner	**18:00**		
Session 3 **Plenary Lecture** Chair: Christy Yu	19:15	**Bart Eggen** University of Groningen Groningen, The Netherlands	*Human microglia diversity in development and disease*
Posters & Drinks	**20:15**	Rooms 203, 204, and the hallway	

Monday, July 15

Breakfast	**7:00**	*Please check out of your room prior to the first session, if possible.*	
Session 4 Chairs: Lennart Söder and Guelcan Demir	9:00	**Oliver Kann** Heidelberg Universtiy Heidelberg, Germany	*Activated microglia: a potent disruptor of neuronal network oscillations in neuroinflammation*
	9:35	**Colm Cunningham** Trinity College Dublin Dublin, Ireland	*The interaction of systemic inflammation with neuroinflammation disrupts neuronal integrity and behavior*
Posters & Coffee	**10:10**	Rooms 203, 204, and the hallway	
Session 5 Chair: Viktoria Greeck	11:15	**Richard Fairless** Heidelberg University Heidelberg, Germany	*Combating neuroinflammation-driven glutamate transporter dysfunction in autoimmune optic neuritis*
Meetings	11:50	Students' Meeting / Science Pub Quiz: Banquet Hall	
Lunch	**12:50**		
Session 6 Chairs: Natalie Ludwig and Tamara Pöpping	14:15	**Francesca Odoardi** University Medical Center Göttingen Göttingen, Germany	*Intravital imaging of T-cell mediated CNS autoimmunity*
	14:50	**Martina Absinta** Vita-Salute San Raffaele University Milan, Italy	*Multidimensional investigation of chronic inflammation in multiple sclerosis*
Group Picture	15:25	baroque staircase	
Coffee	**15:35**		
Awards Ceremony	16:05	**IZN Students' Poster Prize** *Recipient TBA* Laudatio: Rosanna Parlato **Foundation BrainAid/IZN Master Thesis Award** *Sofiya Zbaranska* (Monyer Group) Laudatio: Christoph Schuster **Foundation Brain Aid/IZN Dissertation Award** *Janina Kupke* (Oliveira Group) Laudatio: Christoph Schuster **IZN/Chica and Heinz Schaller Young Investigator Neuroscience Award** *Frédéric Fiore and Khaleel Alhalaseh* (Agarwal Group) Laudatio: Christoph Schuster **Neuroscience Art Contest Winner** *Recipient TBA* Laudatio: Antje König	
Closing Remarks	16:30	**Hilmar Bading** Managing Director of the IZN, Heidelberg University Heidelberg, Germany	
Bus Departure	17:00	from the parking lot next to the train station	

Poster Presentations

Nr.	Authors	Group	Title
1	**Frédéric Fiore**, Khaleel Alhalaseh, Felipe Bodaleo, Ram Dereddi, Amit Agarwal	Agarwal	Cortical oligodendrocyte precursor cells exhibit distinct calcium activity patterns during fate progression
2	**Pascal Klein**, Beate Throm, Kevin Allen	Allen	Coherence between proximal and distal reference frames modulate impact of proximal cues to grid cell stability.
3	**Felix Jose Kavarayil**, Kevin Allen	Allen	Grid cell anchoring to dynamic visual environmental cues during a visually guided navigation task
4	**Zihong Zhang**, Celia García Vilela, Anna M.H. Hertle, Jing Yan, Hilmar Bading	Bading	Unraveling the role of calpain and the NMDAR/TRPM4 complex in NMDA-induced neurotoxicity
5*	**C. Peter Bengtson**, Maite Börsig, Patricia Scharf, Christoph Trebesius, Rowena Groeneveld, Calvin Thommek, Hilmar Bading	Bading	An ex-vivo slice model to assess the impact of elevated extracellular glutamate and EAAT blockade on synaptic and extrasynaptic NMDA receptor function
6	**Dorothea Schall**, Chang Liu, Hatice Recaioglu, Simone Berkel	Berkel	KCNQ1 – a long underestimated potassium channel in the brain?
7	**Berin Boztepe**, Jonas Scheck, Lennart Heinz, Manuel Fischer, Rosa Eurich, Chenchen Pan, Frank Winkler, Sabine Heiland, Martin Bendszus, Michael Platten, Ina Weidenfeld, Michael O. Breckwoldt	Breckwoldt	Assessing the immune microenvironment in glioma models by correlative high field MRI and light sheet microscopy
8	**Johannes Ungermann**, Berin Boztepe, Michael Breckwoldt	Breckwoldt	Investigation of Meningioma Immune Cell Infiltration by Tissue Clearing in Freshly Resected Human Tumor Samples
9	**Lars Link**, Ashish Chouhan, Eva Kramer, Andreas Draguhn	Draguhn	Mapping the concepts of 'intelligence' in the neurosciences
10*	**Evangelia Pollali**, Andreas Draguhn	Draguhn	Neuropeptide Y effects on hippocampal network oscillations *in vitro*
11	**Max Ingo Thurm**, Georgia Koppe, Eleonora Russo, Florian Bähner, Daniel Durstewitz	Durstewitz	Non-stationary recurrent neural networks for reconstructing computational dynamics of rule learning
12	**Viktoria Greeck**, Sarah Williams, Jing Yan, Hilmar Bading, Richard Fairless	Fairless	Inhibition of NMDAR death complex signalling as a novel therapeutic approach to multiple sclerosis
13	**Ana Zovko**, Elena Munoz, Daniel Sierra Garcia, Sandra Horschitz, Quirin Krabichler, Philipp Koch, Valery Grinevich	Grinevich	Generation of iPSCs derived Oxytocin specific hypothalamic organoids and transplantation into rat brains
14	**Konstantinos Afordakos**, Alan Kania, Marina Eliava, Ana Zovko, Valery Grinevich	Grinevich	Anatomical investigation of the oxytocin sensitive interneuronal network across the hippocampal formation
15	**Huma Shaheen**, Ryan Patwell, Quirin Krabichler, Valery Grinevich	Grinevich	Probing oxytocin neuron activity in a rat making a choice between sucrose and conspecific
16*	**Babak Khodaie**, Lennart Söder, Andrea Lewen, Amr Elgez, Alexei V. Egorov, Oliver Kann	Kann	Effects of lactate utilization on sharp wave-ripple network activity in mouse hippocampal slices
17	**Amr Elgez**, Andrea Lewen, Babak Khodaie, Lennart Söder, Oliver Kann	Kann	Microglia-induced inflammatory neurodegeneration is partially reduced by blocking neurotransmission *in situ*
18	**Lennart Söder**, Andrea Lewen, Amr Elgez, Babak Khodaie, Oliver Kann	Kann	Neuronal network dysfunction and neurodegeneration mediated by TLR7/8-activated microglia depend on the immunological context
19	**Sreedevi Raghu**, Andrea Rosetti, Philipp Koch, Julia Ladewig	Koch	Generating hiPSC derived cortical organoids with enhanced neuronal maturation, improved functionality, and synchronized network activity
20	**Jessica Jung**, Sandra Horschitz, Philipp Koch	Koch	Development of a novel high-throughput screening method for drug target discovery in schizophrenia by means of utilizing co-cultured hiPSC-derived cortical neurons and microglia
21	**Ankita Kumar Bhamidipati**, Anne Hoffrichter, Malin Schmidt, Philipp Koch	Koch	Functional and molecular profiling of iPSC-derived neurons from patients in Spanish multiplex families with bipolar disorder
22	**Christy Yu**, Sandra Horschitz, Philipp Koch	Koch	High-throughput morphological characterization of iPSC-derived NGN2 neurons in schizophrenia
23	**Juhyun Kang**, Rohini Kuner	Kuner, R.	Bridging neural pathways: connectivity and plasticity between mediodorsal thalamus and nucleus accumbens in chronic pain

24	**Amrita Das Gupta**, Hongwei Zheng, Johannes Knabbe, Thomas Kuner	Kuner, T.	Chronic neuropathic pain induces neuronal loss in the secondary motor cortex of mouse models with spared nerve injury
25	**Catello Guida**, Anne Hoffrichter, Fabio Marsoner, Ammar Jabali, Julia Ladewig	Ladewig	Unraveling the functions of the transcription factor TBR2 in human neurodevelopment
26	**Selma Aghabashlou Saisan**, Jule Truberg, Laura Rueda Gensini, Moritz Mall	Mall	Unraveling the pathomechanism of neurodevelopmental disorders caused by MYT1L-mutations in patient-derived, prime-edited iPSCs and cerebral organoids
27	**Adriana Schneider,** Alexandra Merkel, Netta Ussyshkin, Lisa Ruff, Paula Zimmer, Daniela Mauceri	Mauceri	Atp8a2 controls phosphatidylserine externalisation, structural integrity and survival in neurons
28	**Debanjan Chowdhury**, Duncan MacLaren, Beate Throm, Nina Bieber, Magdalene Schlesiger, Hannah Monyer	Monyer	Identifying mechanisms involved in acute alcohol-induced amnesia
29	**Lara Kilian**, Marija Banicevic, Jennifer Just, Michaela Back, Irmgard Amrein, Jakob von Engelhardt, David P. Wolfer, Stefan Kins, Martin Korte, Ulrike C. Müller	Müller	Novel knockin mice lacking the alpha- and beta-secretase cleavage sites
30	**Zvi Menahem**, Meike Fellanz, Marija Banicevic, Lea Humbs, Carolin Stoffer, Gundula Braun, Dominique F äßler, Nadine Zahn, Christian J. Bucholz, Thomas Deller, Ulrike C. Müller	Müller	Analysis of APP functional domains using APP/APLP2 deficient organotypic slice cultures
31	**Verena Bengelsdorff**, Dominique Fäßler, Zvi Menahem, Lara Kilian, Gundula Braun, Christian Buchholz, Ulrike C. Müller	Müller	AAV-Cre mediated triple knockout of the amyloid precursor protein (APP) gene family in primary neurons: a novel system to investigate their physiological functions *in vitro*
32	**Tamara Pöpping**, Andre Rupp	Rupp	Variability and validity of phase lag estimations on MEG data
33	**Thomas Thäwel**, Christoph Körber, Hannah Kapell, Amel Zulji, Thomas Kuner, Lucas Schirmer	Schirmer	Decoding spatial and temporal cell-type specific vulnerability in neuroinflammation
34	**Celia Lerma-Martin**, Pau Badia-i-Mompel, Ricardo O. Ramirez Flores, Patricia Sekol, …, Lucas Schirmer	Schirmer	Cell type mapping unravels tissue niches and interactions in subcortical multiple sclerosis lesions
35	**Natalie Ludwig**, Lucas Schirmer	Schirmer	Iron scavenging and myeloid cell polarization
36	**Julia Dyckow**, Celine Geywitz, Natalie Ludwig, Klaus-Armin Nave, Wiebke Möbius, Lucas Schirmer	Schirmer	Role of oligodendrocyte-encoded Piezo2 during aging and demyelination
37	**Rangeet Manna**, Duncan MacLaren, Anushka Wakade, Kristoffer Damm, Magdalena Schlesiger	Schlesiger	Activation of VTA dopaminergic neurons modulates spatial coding in LEC and MEC
38	**Adeoye Ewedemi**, Marcus Meinhardt, Wolfgang Sommer, Rainer Spanagel	Spanagel	The effect of delay discounting on the medial prefrontal cortex
39	**Isabel Loss**, Rüstem Yilmaz, Francesca Tuorto, Elena Cairo, Philipp Koch, Jochen Weishaupt, Rosanna Parlato	Weishaupt	Morphological and transcriptomic signatures in patient-derived motor neurons carrying ALS causative KIF5A mutations.
40	**Ketrin Dimco**, Rüstem Yilmaz, Rosanna Parlato, Jochen Weishaupt	Weishaupt	Role of Myorg mutations in primary familial brain calcification (PFBC)
41	**Jahnavi Srinidhi**, David Brenner, Isabel Loss, Rüstem Yilmaz, Philipp Koch, Rosanna Parlato, Jochen Weishaupt	Weishaupt	Comparative analysis of different TBK1-ALS mutations in patient derived motor neurons
42	**Ivan Valkadinov**, Sophie Hebestreit, Patrick Kinz, Janine Schwahn, …, Jochen Weishaupt, Julian Conrad	Weishaupt	The primary familial brain calcification disconnectome
43	**Lena Eschholz**, Chantal Wissing, Maxime Maheu, Kathrin Sauter, Fabio Morellini, J. Simon Wiegert, Alexander Dieter	Wiegert	Targeting norepinephrine neurons of the locus coeruleus: a comparison of model systems and strategies
44	**Yilmaz Arda Ates**, J. Simon Wiegert	Wiegert	Reverse BiPOLES: expanding the toolkit for bidirectional optogenetic control of neuronal activity
45	**Marton Istvan Molnar**, J. Simon Wiegert, Andrey Formozov	Wiegert	G-protein mediated control of hippocampal interneurons in hippocampal network dynamics
46	**Avi Adlaka**, Thomas Kuner	Kuner, T.	Thalamic ensemble activity during distinct behaviors

*** Posters marked with an asterisk do not qualify for the IZN student poster prize.**

Poster Abstracts

1 **Frédéric Fiore**, Khaleel Alhalaseh, Felipe Bodaleo, Ram Dereddi, Amit Agarwal

Cortical oligodendrocyte precursor cells exhibit distinct calcium activity patterns during fate progression

Oligodendrocyte precursor cells (OPCs) serve as progenitors for oligodendrocytes (OLs) throughout their life, contributing to developmental and adaptive myelination, as well as myelin repair. OPCs are known to make synaptic and extra-synaptic contacts with axons, but the mechanisms by which they integrate the information relayed by the neuronal activity into Ca^{2+} signals, which in turn influence their fate and survival, are still unclear. Using newly developed transgenic mouse lines to express genetically encoded Ca^{2+} sensors in OPCs, we performed 2-photon microscopy in the somatosensory cortex of awake freely-moving mice while monitoring intracellular Ca^{2+} signals and cell-fate progression simultaneously. We found that OPCs exhibit distinct Ca^{2+} signatures during fate progression, and that their baseline Ca^{2+} activity is significantly reduced as OPCs transition from premyelinating to mature OLs. Additionally, we found that OPCs with generally low Ca^{2+} activity tend to proliferate, while OPCs with higher Ca^{2+} activity differentiated into OLs. In summary, our data suggests that different cell types within the oligodendrocyte lineage exhibit unique Ca^{2+} signatures, which they integrate to direct their fate decisions and lineage progression.

2 **Pascal Klein**, Beate Throm, Kevin Allen

Coherence between proximal and distal reference frames modulate impact of proximal cues to grid cell stability.

Grid cells are known to be the computational unit of spatial navigation skills and allows an animal to estimate its position in a given reference frame. However, it remains unclear to which proximal or distal cues these cells anchor to, especially in a scenario of conflicting input from either reference frames. This project aims at finding a distinguishing factor that modulates the impact of proximal cues to grid cell stability.

3 **Felix Jose Kavarayil**, Kevin Allen

Grid cell anchoring to dynamic visual environmental cues during a visually guided navigation task

Spatial navigation is a crucial cognitive process, with grid cells in the entorhinal cortex playing a key role. This study explores how grid cells anchor to novel visual landmarks. We developed a visually guided navigation task that allows precise control of visual stimuli using a custom-built, virtual reality setup.Our primary objective is to assess how quickly grid cells anchor to new visual landmarks and examine the link between this anchoring and behavioral performance. Using rodent electrophysiology, we measure grid cell integrity by focusing on orientation stability and the strength of the hexagonal firing pattern. The findings from this research provide valuable insights into the neural mechanisms underlying navigation. Moreover, the development of new behavioral tasks and instruments advances the methodology for studying the impact of visual landmarks on grid cell activity.

4 **Zihong Zhang**, Celia García Vilela, Anna M.H. Hertle, Jing Yan, Hilmar Bading

Unraveling the role of calpain and the NMDAR/TRPM4 complex in NMDA-induced neurotoxicity

N-methyl-D-aspartate receptors (NMDARs) are glutamate-gated calcium-permeable channels comprising two GluN1 subunits and two GluN2 subunits. NMDARs dependent excitotoxicity depends on the location of NMDARs: stimulation of synaptic NMDARs results in forming a neuroprotective ‘shield,’ whereas stimulation of extrasynaptic NMDARs promotes cell death. Previous study of our lab uncovers transient receptor potential melastatin 4 (TRPM4) expressed at the extrasynaptic site can bind to the NMDARs leading to cell death. We found extrasynaptic NMDARs activation leads to cleavage of GluN2A and GluN2B subunits, while GluN1 subunits and TRPM4 remain unaffected. Here, we propose the existence of a third protein that can lead to GluN2A and GluN2B cleavage when activated extrasynaptic NMDARs and focus on m-calpain as a candidate. Calpains, non-lysosomal cysteine proteases, play essential roles in brain function: activation of m-calpain downstream of extrasynaptic NMDARs induces neurotoxicity. Our findings demonstrate that inhibiting calpain activity via inhibitors or gene knockdown effectively prevents NMDARs cleavage. Additionally, disrupting the NMDAR/TRPM4 complex impedes NMDARs cleavage and reduces calpain-mediated breakdown product generation. Furthermore, knockdown of m-calpain, but not μ-calpain, protects hippocampal neurons from NMDA-induced loss of mitochondrial membrane potential. Intriguingly, in NMDA-induced cell death assay (24 hours), this neuroprotective effect is limited to the early 12-hour window, as its effectiveness diminishes thereafter. Furthermore, we found calpain knockdown decreases TRPM4 expression, suggesting its neuroprotective effect is through TRPM4 downregulation. These findings highlight the critical role played by the interplay between calpain and the NMDAR/TRPM4 complex in NMDA-induced neurotoxicity.

5* **C. Peter Bengtson**, Maite Börsig, Patricia Scharf, Christoph Trebesius, Rowena Groeneveld, Calvin Thommek, Hilmar Bading

An ex-vivo slice model to assess the impact of elevated extracellular glutamate and EAAT blockade on synaptic and extrasynaptic NMDA receptor function

The overload of glutamate transporters by excessive extracellular glutamate leads to excitotoxic cell death in multiple neurodegenerative diseases as well as stroke, hypoxia and traumatic brain injury. The sustained activation of extrasynaptic N-methyl-D-aspartate (NMDA) receptors by excess extracellular glutamate has been implicated in multiple disease models *in vitro* and *in vivo*. Pathological levels of extracellular glutamate arise from compromised or even reversed function of excitatory amino acid transports (EAATs) in a brain region and subtype dependent manner. We have used whole cell patch clamp of hippocampal CA1 pyramidal neurons in acute brain slices from adult mice (P70-91) to assess synaptic and extrasynaptic NMDA receptor function in the presence EAAT blockers and/or elevated glutamate levels (10-100 µM). Both DL-threo-β-Benzyloxyaspartic acid (DL-TBOA, 30 µM) and TFB-TBOA (3 µM) evoked large tonic currents mediated by NMDA receptors and occluded synaptic NMDA-receptor mediated excitatory postsynaptic currents (EPSCs) similar to the effects of bath perfusion with glutamate (10 µM). Such effects are likely due to reverse activation of the transporters. Lower concentrations of TFB-TBOA (100 nM) which do not block EAAT3, or the selective EAAT1 or EAAT2 blockers (UCPH101 and dihydrokainate respectively) had little effect. The lack of a commercial EAAT3 selective blocker makes its contribution difficult to assess. These results identify the importance of EAAT2 in buffering excess glutamate and maintaining synaptic and minimizing extrasynaptic NMDA receptor activation in a physiological ex-vivo slice model from mature mice.

6 **Dorothea Schall**, Chang Liu, Hatice Recaioglu, Simone Berkel

KCNQ1 – a long underestimated potassium channel in the brain?

KCNQ1 is the pore-forming alpha subunit of a voltage-dependent potassium channel complex. It is predominantly expressed in the heart and in the inner ear, however, it is also present in the brain. Genetic variants in KCNQ1 have been linked to several disorders, like Romano-Ward syndrome, Jervell and Lange Nielson syndrome and diabetes mellitus type 2. Moreover, KCNQ1 is also discussed to contribute to the pathogenesis of Alzheimer´s disease and neurodevelopmental disorders, even though it´s function in the human brain remains unknown. In this study we aim to elucidate the function of KCNQ1 in human neuronal cells. We generated homozygous and heterozygous KCNQ1 knockout (KO) iPSC lines which were differentiated into neuronal stem cells (NSCs) and into cortical neurons. iPSC-derived NSCs with loss of KCNQ1 show reduced neurite outgrowth compared to the isogenic controls, indicating a role of KCNQ1 already during early neuronal differentiation. This is in line with results from the expression analysis focusing on different neuronal marker genes in cortical neurons. Cortical neurons with loss of KCNQ1 reveal impairments in neuronal differentiation, with reduced expression of genes encoding for pre- and postsynaptic proteins and an increased expression of cortical layer 1 marker Reelin. In summary, we obtained first evidence for an important role of KCNQ1 during neuronal differentiation which we will follow-up with functional and “omics” analysis.

7 **Berin Boztepe**, Jonas Scheck, Lennart Heinz, Manuel Fischer, Rosa Eurich, Chenchen Pan, Frank Winkler, Sabine Heiland, Martin Bendszus, Michael Platten, Ina Weidenfeld, Michael O. Breckwoldt

Assessing the immune microenvironment in glioma models by correlative high field MRI and light sheet microscopy

Gliomas are malignant brain tumors with an immunosuppressive tumor microenvironment (TME). We used the Toll-like receptor 7 agonist CDNP-R848 to induce a proinflammatory shift in the TME, effectively treating preclinical GI261 glioma (Turco *et al*., Nat Commun. 2023). While MRI is the main clinical tool for monitoring glioma treatment, it cannot visualize key immunological TME components. Preclinical GI261 and SB28 gliomas were xenografted in mice, followed by MRI monitoring. Mice received three doses of intravenous CDNP-R848 or a vehicle control. To examine spatial patterns of immune cells in the TME, we performed light sheet microscopy (LSM) with whole-brain immunostaining of myeloid and T cells using iDISCO for CD3 and Iba1 to reveal immune cell influx, distribution, and therapy-induced changes. CDNP-R848 induced glioma regression in GI261 (ORR: 75%), primarily through macrophage activation during the effector phase (week 3), whereas SB28 was fully resistant. Cleared mouse brains of GI261 showed T cell accumulation around peritumoral microvessels and “non-classical” myeloid cell recruitment, with macrophage accumulation in the choroid plexus, corpus callosum, leptomeninges, and particularly increased Iba1 signal in the ipsilateral cortex. Our findings suggest that white matter tracts are potential immune cell infiltration routes. Additionally, we propose that tumor size correlates with the number of infiltrating immune cells. Our preliminary data shows that untreated mice have higher proportions of microglia, while widespread peritumoral myeloid activation appears therapy-independent, and that TME complexity develops before therapy induction. These findings indicate that the recruitment pathways of myeloid and T cells are crucial to understand the resistance mechanisms towards glioma immunotherapy.

8 **Johannes Ungermann**, Berin Boztepe, Michael Breckwoldtraguhn

Investigation of Meningioma Immune Cell Infiltration by Tissue Clearing in Freshly Resected Human Tumor Samples

Meningioma (MNG) is the most common intracranial primary tumor. As space is limited within the skull, clinical manifestation of MNG usually comprises unspecific and pressure-related symptoms such as headaches, seizures or dizziness/vertigo. Apart from neurofibromatosis and radio-damage, little is known about what causes MNGs to grow. In a recent study, analysis of copy number variations (CNVs) as well as methylation status allowed classification into the groups "merlin-intact", "immune-enriched" and "hypermitotic" MNGs, correlating with WHO grades I, II and III, respectively. The "immune-enriched" group displays an increased immune cell infiltration into the tumor, begging the question of the cell's origin, infiltration path and purpose within the tumor. Surgical resection is the standard treatment and is usually done en-bloc, leaving the tumor itself mostly intact. In this study, we aim to take advantage of this fact by using a tissue specific clearing protocol and light sheet microscopy to better understand the tumour's 3D architecture as well as its immunological microenvironment. We do so by using freshly resected human MNG tumor samples in collaboration with the Mannheim Neurosurgery Department and staining for macrophages, T-Cells and lymphatic/blood vessels. Ultimately, we hope to unravel the infiltration paths used by immune cells to shed light on their role in tumorigenesis and progression.

9 **Lars Link**, Ashish Chouhan, Eva Kramer, Andreas Draguhn

Mapping the concepts of 'intelligence' in the neurosciences

Intelligence' is a central term across various disciplines, including psychology, cognitive behavioral, and neural sciences. Recently, the increasing research dynamics and public visibility of Artificial Intelligence (AI) have further expanded the concept, with unknown impact on 'Biological Intelligence" (BI). Despite the term's interdisciplinary nature, no universal definition of intelligence encompasses all these disciplines. This study aims to clarify the concept of intelligence within the neurosciences by mapping out different explicit or implicit concepts of intelligence using a linguistic approach. The goal is to provide an overview of intelligence concepts and their associated terms and foster more precise and interdisciplinary communication. Our corpus- and computer-linguistic analysis utilized the entirety of available abstracts in the PubMed database (~18.313.500 abstracts published from 1881 to 2023). We extracted all abstracts containing the search term 'intellig*' and at least one term from a 'neuroscience glossary' composed from the index words of leading neuroscience textbooks. Co-occurrences of 'intellig*' and other terms and frequencies of relevant words were extracted. At present, we are applying further quantitative and semantic methods to unveil shared features, properties, and attributes of the different concepts of intelligence. The initial findings show that the term "intelligence" is complex and multifaceted in the field of neuroscience. Different linguistic clusters are associated with different concepts of intelligence, which are in part explained by the variety of underlying research focuses (e.g., cognitive processes, neural mechanisms, computational models). Our preliminary results underscore the complex and diverse nature of intelligence concepts within the neurosciences.

10* **Evangelia Pollali**, Andreas Draguhn

Neuropeptide Y effects on hippocampal network oscillations *in vitro*

Neuropeptide Y (NPY) is the most abundant neuropeptide in the brain, co-expressed with other neurotransmitters. It is expressed mainly by interneurons in different regions, including the hippocampus (HP). NPY binds to five G-protein-coupled receptors (R), from which Y1 and Y2 R are the most numerous in the rodent brain. NPY is implicated in the regulation of stress and can exert anxiolytic or anxiogenic effects, depending on the specific brain region and receptor subtype being activated. HP and specifically its ventral portion, contributes to this processing with an important role in emotional memory. At the same time, it exhibits robust oscillatory activity, associated with specific memory processes. Specifically, sharp wave-ripples (SWP-R) are considered to be connected with memory consolidation, while gamma oscillations support memory encoding. Therefore, we hypothesized that the effects of NPY on anxiety and fear memory might be associated with changes in hippocampal oscillations. We assessed the network oscillatory characteristics after administration of NPY or receptor-specific agonists and antagonists on mouse brain slices of the ventral-to-intermediate hippocampus. NPY strongly suppressed spontaneous SW-R in the hippocampal CA1 subregion through activation of Y2 R, but not Y1 R. Neither NPY nor selective activation of NPY receptors had any major effect on carbachol-induced gamma oscillations. These results show a specific effect of NPY at the network dynamics and are suggestive of modulation of memory consolidation mediated by Y2 R.

11 **Max Ingo Thurm**, Georgia Koppe, Eleonora Russo, Florian Bähner, Daniel Durstewitz

Non-stationary recurrent neural networks for reconstructing computational dynamics of rule learning

The update of behavioral policies in response to novel environments or shifting action-outcome contingencies is essential for survival. The medial prefrontal cortex (mPFC) is crucial for rule learning, cognitive flexibility, and uncertainty detection. During new behavioral paradigms or rule switches, animals show abrupt performance changes and corresponding sharp transitions in neuronal population dynamics. However, the precise computational mechanisms driving these phenomena are largely unknown. We aim to unravel these mechanisms through dynamical systems reconstruction using a non-stationary piecewise-linear recurrent neural network (PLRNN). This model captures neuronal adaptation to changing environmental contingencies with trial-specific connectivity matrices regularized by smoothness and continuity priors. We trained our model to reconstruct non-stationary neuronal dynamics from multiple single-unit (MSU) recordings in rodent mPFC during a probabilistic rule-shifting task. After training, the PLRNN accurately generates diverse single-unit firing rate profiles that match the geometrical and temporal characteristics of the original data. Task-related events can be decoded from the model-generated neural trajectories as effectively as from the original MSU activity. Change points identified in PLRNN-generated activity and original MSU recordings correlate tightly. Additionally, PLRNN-simulated trial-to-trial trajectories for both rules closely match those obtained directly from the data. Moreover, trial-specific connectivity matrices allow for highly accurate decoding of rule-type, unaffected by other task events, suggesting that behavioral changes are based on alterations in the underlying connectivity structure. In conclusion, the non-stationary PLRNN offers a novel framework for investigating time-variant neuro-dynamical phenomena during learning, and development providing insights into the computational mechanisms underlying flexible behavior and learning.

12 **Viktoria Greeck**, Sarah Williams, Jing Yan, Hilmar Bading, Richard Fairless

Inhibition of NMDAR death complex signalling as a novel therapeutic approach to multiple sclerosis

Multiple Sclerosis (MS) is a chronic autoimmune disease of the central nervous system characterised by acute inflammation and primary and secondary neurodegeneration in the grey and white matter. Although a range of immunomodulatory treatments exist, neuroprotective therapies are still lacking. Research indicates glutamatergic excitotoxicity as a contributing factor to pathophysiological processes in MS and its animal model, experimental autoimmune encephalomyelitis (EAE). This project aims to assess the role of the novel therapeutic agent FP802, which has been shown to inhibit glutamatergic excitotoxicity. In a chronic EAE model, FP802 prophylactic treatment significantly delayed disease onset and decreased disease severity, while therapeutic treatment had little effect. Similarly, prophylactic treatment with FP802 delayed disease onset in a relapsing-remitting EAE model, and abolished relapse when administered therapeutically. Additionally, both prophylactic and therapeutic treatment significantly reduced retinal ganglion cell degeneration, a commonly observed early event in EAE. Immunohisotchemistry revealed that FP802 had no effect on immune cell infiltration and demyelination. Immunohisotchemical assessment of axonal injury and neurodegeneration are still ongoing. Furthermore, the role of excitotoxic glutamate signalling in non-neuronal cells of the immune system and blood-brain barrier is unclear, but preliminary results suggest that FP802 has no effect in those compartments. Overall, we conclude that FP802 treatment positively modulates EAE disease progression, particularly when administered prior to disease onset. We suggest that this effect is due to the neuroprotective effect of FP802, while immune and blood-brain barrier function remain unchanged.

13 **Ana Zovko**, Elena Munoz, Daniel Sierra Garcia, Sandra Horschitz, Quirin Krabichler, Philipp Koch, Valery Grinevich

Generation of iPSCs derived Oxytocin specific hypothalamic organoids and transplantation into rat brains

Oxytocin (OT), a neuropeptide produced in the hypothalamus, is a potent modulator of emotions and social behavior acting via numerous brain-wide projections. The OT system is known to be dysregulated in many psychiatric diseases, such as anxiety, depression, PTSD, schizophrenia and autism spectrum disorder. So far, no therapeutic approach exists to target the brain's OT system to rescue the healthy behavioral phenotype. In recent years, a promising tool for targeted treatment of neuropathologies has emerged from the field of stem cell research: By applying instructive signals to human-derived induced pluripotent stem cells (hiPSCs), structures resembling specific regions of the brain can be generated and could in the future be transplanted as xenografts into the brain of patients. Here, we show first results of a successful production of hiPSC-derived OT neurons in ventral hypothalamic organoids, using a "self-patterning" approach based on the natural propensity of hiPSC to develop towards anterior brain regions with the addition of ventralizing inductive signals. We observed the expression of region-specific progenitor markers Rax1, Nkx2.1. and transcription factors necessary for adopting the identity of OT progenitors – Otp, Sim1 and Brn2. Due to the high diversity of neuropeptidergic neurons present in this brain region, we are establishing a 2-step selection process to obtain higher yields of OT-producing neurons, based on CRISPR/Cas9-gene-edited transgenic cell lines and OT-specific viral vectors.

Our study will pave the way towards individualized cell-culture generation of neuroendocrine hypothalamic cells, which one day could be implanted into the brains of patients suffering from neurologically impaired hormone systems.

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14 **Konstantinos Afordakos**, Alan Kania, Marina Eliava, Ana Zovko, Valery Grinevich

Anatomical investigation of the oxytocin sensitive interneuronal network across the hippocampal formation

Oxytocin (OT), a neuropeptide synthesized in the hypothalamic paraventricular (PVN), supraoptic (SON), and accessory (AN) nuclei, plays both hormonal and neuromodulatory roles. OT has been postulated to act in the brain in several modes: volume transmission through cerebrospinal fluid, somatodendritic release, or axonal release from long-projecting OT neurons. OT acts via the widely expressed oxytocin receptor (OTR), whose presence in several brain regions is not always accompanied by OT axons. The hippocampal formation consists of the hippocampus proper, subiculum, dentate gyrus, and entorhinal cortex, and is crucial for memory formation and consolidation, spatial navigation, contextual processing, emotional regulation, and adaptive learning. This brain region harbors several types of inhibitory neurons, which maintain neural activity balance, refine sensory inputs, enhance cognitive functions, and ensure brain circuit stability and efficiency. Notably, despite OTR being expressed throughout the hippocampal formation in both excitatory and inhibitory neurons, some hippocampal areas are devoid of OT axons. This study aims to investigate the components of the OT signaling system in the hippocampal formation, focusing on OTR-expressing inhibitory neurons. This was achieved using the OTR-IRIS-Cre rat line with CRE-dependent and oxytocin promoter-carrying viral constructs, as well as multiplex fluorescent *in situ* hybridization. We described the hippocampal OT innervation pattern, analyzed the percentage of excitatory versus inhibitory neurons, and examined their projections to further brain regions to identify potential physiological processes and behaviors modulated by this circuit. The findings will enhance our understanding of the functional architecture of oxytocin-sensitive networks and their role in modulating hippocampal functions.

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15 **Huma Shaheen**, Ryan Patwell, Quirin Krabichler, Valery Grinevich

Probing oxytocin neuron activity in a rat making a choice between sucrose and conspecific

The hypothalamic neuropeptide oxytocin (OT) plays a crucial role in reward processing, social behavior, and the intake of rewarding substances, such as food. Previous findings indicate that OT is critical for modulation of both processes - food intake and social communication. However, it is still enigmatic whether OT neurons can differentiate between these types of rewarding stimuli. To tackle this question, we established an operant social choice paradigm where adult female rats were trained to press two alternating levers for self-administration of sucrose or for opening a door for interaction with familiar conspecific. During free-choice trials between these two levers, the rats (n = 17) always preferred sucrose over the social interactions. To monitor intrinsic activity of OT neurons occurred at the choice events, we virally expressed Ca2+ sensor GCAMP6s in the hypothalamic paraventricular nucleus and subsequently recorded Ca2+ signals by fiber photometry. In first two rats analyzed, we found a relative increase in OT population activity during choice for sucrose compared to social interaction. Given well known prevailing pro-social action of OT, our first observations are unexpected and thus require registration of OT neuron activity in a larger cohort of animals.

16* **Babak Khodaie**, Lennart Söder, Andrea Lewen, Amr Elgez, Alexei V. Egorov, Oliver Kann

Effects of lactate utilization on sharp wave-ripple network activity in mouse hippocampal slices

Lactate, once considered merely a metabolic waste byproduct, has emerged as a multifaceted player in neuronal function, serving both as an alternative energy source and a signaling molecule with diverse effects. Brain lactate levels can raise in various physiological and pathological contexts such as increased neuronal activity, physical exercise, ischemia, and neuroinflammation (e.g., multiple sclerosis and Alzheimer's disease). These changes can affect neuronal energy states, network excitability, and synaptic properties, as reflected in key hippocampal network patterns such as sharp wave-ripples (SPW-R) that are crucial for memory consolidation. Our study seeks to elucidate how lactate fuel affects network activity, synaptic transmission and intrinsic neuronal properties. To assess the impact of lactate on neuronal energy metabolism and network function, we used acute hippocampal slices of mice (4-6 weeks old), and varied the concentrations of glucose and lactate in the recording solution. Local field potential (LFP) recordings were performed in the CA3 and CA1 regions to investigate spontaneous SPW-Rs that associate with intermediate energy demand. Synaptic transmission was assessed by electrical stimulation of Schaffer collaterals or by monitoring baseline field activities in CA1. Simultaneously, sharp microelectrode recordings were used to evaluate the intrinsic properties of CA1 pyramidal cells. Our results revealed that 20 mM lactate was insufficient to substitute for 10 mM glucose as a sole energy source because it led to a reduction in both incidence and amplitude of SPW-Rs in CA3 and CA1, but it did not affect ripple frequency (>180 Hz). Interestingly, SPW-R incidence was already reduced by partial replacement of glucose with lactate (lactate/glucose ratio of >1:1). We also added the monocarboxylate transporter (MCT1/2) blocker AR-C155858 to 10 mM glucose, which led to a reduction in SPW-R incidence, without affecting SPW-R amplitude or ripple frequency. When AR-C155858 was added to 20 mM lactate, it strongly reduced SPW-R amplitude, frequency as well as incidence. Synaptic transmission at CA1 synapses showed a marked reduction when substituting 10 mM glucose

with 20 mM lactate. However, intracellular recordings revealed only moderate changes in intrinsic firing properties of CA1 pyramidal cells under these conditions. In summary, lactate as a sole energy substrate is insufficient for maintaining SPW-R network activity and synaptic transmission in mouse hippocampal slices. However, MCT1/2 expression is important for metabolic flexibility of neuronal networks.

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17 **Amr Elgez**, Andrea Lewen, Babak Khodaie, Lennart Söder, Oliver Kann

Microglia-induced inflammatory neurodegeneration is partially reduced by blocking neurotransmission *in situ*

Microglia are pivotal in the pathogenesis of multiple brain diseases, including stroke, encephalitis, multiple sclerosis, and Alzheimer's disease. However, the mechanisms governing inflammatory neuronal dysfunction mediated by activated microglia remain elusive. Recent investigations have delineated distinct reactive microglia phenotypes that associate with different levels of network dysfunction, including slowing of oscillations and neuronal bursting. We hypothesized that there is a potential link between neuronal excitation/inhibition imbalance and enhanced energetic and oxidative stress, thereby exacerbating neuronal dysfunction and death. To explore this, organotypic hippocampal slice cultures were exposed to interferon–gamma (IFN-γ) plus lipopolysaccharide (LPS) for 24 or 48 hours to induce massive microglia-mediated neuronal dysfunction and cell death. In addition, a drug cocktail comprising TTX, CNQX, and D-AP5 was added to block action potentials and ionotropic glutamate receptors. Following exposure, we performed electrophysiology (local field potential recordings) in the slices and collected the supernatant to quantify nitric oxide (NO) release (Griess reaction), cell death (lactate dehydrogenase activity assay, LDH), and release of the proinflammatory cytokines TNF-α and IL-6 (ELISA). Our results revealed significantly lower fractions of dead slices and slices showing neural bursting by about 50% when the drug cocktail was present during IFN-γ+LPS exposure. Despite increased NO release, LDH release decreased significantly in drug cocktail treatment, which was also reflected by well-preserved stress-sensitive parvalbumin (PV)-expressing interneurons (PV immunostaining). These findings suggest (i) the partial regulation of microglial NO release by neuronal activity, and (ii) a neuroprotective role of neuronal activity blockade during microglial activation associating with severe inflammatory neurodegeneration.

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18 **Lennart Söder**, Andrea Lewen, Amr Elgez, Babak Khodaie, Oliver Kann

Neuronal network dysfunction and neurodegeneration mediated by TLR7/8-activated microglia depend on the immunological context

Innate Toll-like receptors (TLRs) play a critical role in activating microglia in various diseases of the CNS. Our study aimed to examine the widely unknown consequences of stimulation of TLR7/8, which are known to recognize single-stranded RNA (ssRNA), e.g. from viruses. For this, we conducted electrophysiological local field potential recordings of neuronal gamma-oscillations (30-70 Hz), biochemical assays, and immunofluorescence in rat organotypic hippocampal slice cultures. Our findings reveal that single exposure (48 h) to the TLR7/8 agonist Resiquimod (R848) induces the release of pro-inflammatory cytokines, like TNF-α, without disturbing neuronal network function. Paired exposure with lipopolysaccharide (LPS), a known TLR4 agonist, leads to a more pronounced, yet still moderate phenotype, characterized by the sporadic occurrence of neural bursts. Paired exposure with interferon-gamma (IFN-γ) results in a severe phenotype characterized by loss of network function and neurodegeneration. Immunofluorescence analysis demonstrated that this neurodegeneration is accompanied by microglial proliferation. The central role of activated microglia in these detrimental processes was further substantiated by the successful prevention of neurodegeneration and recovery of network activity through microglial depletion using clodronate. Additionally, inhibiting the TLR7/8 pathway with Enpatoran (M5049) proved to be a second effective neuroprotective strategy. Our experimental outcomes describe potential implications of TLR7/8 stimulation in viral-associated brain disorders, as well as in other CNS diseases associated with neuroinflammation such as Alzheimer's disease and multiple sclerosis.

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19 **Sreedevi Raghu**, Andrea Rosetti, Philipp Koch, Julia Ladewig

Generating hiPSC derived cortical organoids with enhanced neuronal maturation, improved functionality, and synchronized network activity

Due to the protracted and delayed functional maturation of neurons in iPSC derived brain organoids, the symptoms associated with psychiatric disorders, like pathological hyperactivity and hyperexcitability, manifest themselves in organoids only after 6-8 months. This highly limits the potential of organoids to be used as a model system for many neurological disorders. The main aim of this project is to establish a protocol for generating human iPSC derived cortical organoids with enhanced neuronal maturation, improved functionality, and synchronized network activity at a much younger age. A recent study by Ciceri *et al*., 2022 revealed that an epigenetic barrier acts on the chromosomal regions responsible for neuronal maturation. Hergenreder *et al*., 2022 described a cocktail of 4 bioactive drugs called GENtoniK, which could effectively lift this epigenetic barrier and accelerate the maturation of cortical neurons in 2D. We investigated this further to identify the appropriate window-of-competence and tolerance to GENtoniK in cortical organoids generated by following an already established protocol in the lab (Jabali *et al*., 2022). A systematic functional and morphological

characterization of the organoids at different time points by Immunostaining, Ca2+ imaging, electrophysiological recordings using 3D-CMOS MEA chips was carried out. Once we have established a suitable protocol that produces a cortical organoid that is capable of undergoing accelerated functional maturation, we plan to use the system to do functional characterization of already established disease models in the lab like Rett syndrome, and potentially new disease models like epilepsy and bipolar disorder.

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20 **Jessica Jung**, Sandra Horschitz, Philipp Koch

Development of a novel high-throughput screening method for drug target discovery in schizophrenia by means of utilizing co-cultured hiPSC-derived cortical neurons and microglia

Schizophrenia (SCZ) is a heritable psychiatric disorder affecting approximately 1% of the global population (Messias *et al*., 2007). This brain disorder is characterized by psychotic symptoms such as delusions, hallucinations, and disorganized speech, which typically become prominent during late adolescence and early adulthood (Pagsberg, 2013; Petanjek *et al*., 2011). Research indicates that individuals with SCZ exhibit reduced grey matter thickness and functional connectivity impairments, extending to synapse loss (Cannon *et al*., 2015). Furthermore, over 100 genomic regions have been implicated in SCZ pathogenesis, including the complement component 4 (C4) gene (Working Group of the Psychiatric Genomics Consortium, 2014). Our laboratory has developed a pruning assay based on a co-culture model of human induced pluripotent stem cell (hiPSC)-derived cortical neurons and microglia. Experiments with this assay demonstrated that overexpression of the C4A gene results in enhanced microglial pruning activity, leading to a less dense synaptic network compared to the isogenic non-overexpressing counterpart. To investigate potential targets influencing *in vitro* synapse engulfment by microglia, the pruning assay is being adapted for high-throughput screening using the Revvity Opera Phenix Plus High-Content Screening system with the explorer G3 workstation. To reduce experimental time, initial efforts have focused on implementing a new guided differentiation protocol for producing homogeneous neuronal cultures of cortical identity. Additionally, a cryopreservation protocol has been investigated to establish a microglia cell bank, facilitating rapid accessibility for high-throughput applications. Preliminary steps in developing a reliable analysis protocol for the pruning assay within the Opera Phenix Plus system have been achieved.

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21 **Ankita Kumar Bhamidipati**, Anne Hoffrichter, Malin Schmidt, Philipp Koch

Functional and molecular profiling of iPSC-derived neurons from patients in Spanish multiplex families with bipolar disorder

Bipolar Disorder (BD) is a commonly occurring neuropsychiatric disorder, and is categorised into BD type 1 and BD type 2. This project focusses on BD type 1, which is characterised by manic and depressive episodes. The high heritability of BD (~70%) indicates a strong genetic basis. However, the influence of genetic determinants on gene expression, molecular pathways and neural function remains elusive. The aim of the project is to decipher molecular and functional alterations associated with BD based on iPSC-derived neuronal cultures by performing a comprehensive transcriptomic and morphometric study. The patient and control cohorts originate from Spain and are age- and sex-matched. The patient cohort consists of members from multiplex families and the control cohort is unrelated to the patients. Since the prefrontal cortex (PFC) is consistently affected in BD, we first established a protocol to generate PFC neurons for the comparison of the patient and control iPSC lines. This protocol employs the morphogen FGF8 during dual SMAD inhibition to pattern the progenitors towards the dorsal forebrain. Immunocytochemistry of cortical progenitors revealed high expression of SP8 (PFC progenitor marker), regardless of whether FGF8 was present during dual SMAD inhibition. However, RNA bulk sequencing of the progenitors with FGF8 resulted in significantly higher expression of SP8 and other dorsal forebrain markers (FOXG1, FEZF2, and EMX2). We identified a significant differentially expressed Gene Ontology (GO) term ‘forebrain development’, which supports the role of FGF8 in dorsal forebrain patterning. BD and control PFC neurons generated using this protocol will be investigated.

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22 **Christy Yu**, Sandra Horschitz, Philipp Koch

High-throughput morphological characterization of iPSC-derived NGN2 neurons in schizophrenia

Schizophrenia (SCZ) is a complex brain disorder with heterogeneity in its clinical presentation of positive and negative symptoms such as hallucinations and social withdrawal respectively (APA, DSM-5 Task Force, 2013). Moreover, a common aspect of psychiatric illnesses is the high polygenic nature. Multiple genetic variants, as well as environmental factors, contribute to SCZ. Therefore, the large variation in how the disease manifests among affected individuals makes it difficult to pinpoint one biological pathway to treat. As part of the “Biomarker evaluation supporting clinical translation in schizophrenia” (BEST) project, we aim to identify phenotypic differences not only between controls and patients but also among patients at the cellular level. NGN2 overexpression in human induced pluripotent stem cells generates upper cortical layer neurons, which is a cell population that has been shown to be affected in SCZ (Batiuk *et al*., 2022). After establishing experimental conditions suitable for high-throughput imaging, we performed morphological characterization in five control and five SCZ cell lines at two time points. Preliminary results showed little variation within a condition. The upward trend in synapse density suggests neuronal maturation over time, although no clear pattern was found between

control and SCZ. Unbiased analyses allow us to study additional features and identify phenotypes that may not be typically associated with the disease. In combination with clinical data from patients, we can then make inferences about target biological pathways of SCZ based on multi-scale data.

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23 **Juhyun Kang**, Rohini Kuner

Bridging neural pathways: connectivity and plasticity between mediodorsal thalamus and nucleus accumbens in chronic pain

Motor cortex stimulation (MCS) is a neuromodulation technique that uses electrical impulses to alleviate chronic neuropathic pain. While clinically effective, the mechanisms by which MCS modulates neural pathways and reduces pain remain unclear. Our study focuses on neuronal changes in the mediodorsal thalamus (MD) and nucleus accumbens (NAc) circuits, critical for integrating sensory, emotional, and motivational aspects of pain. We aimed to explore MD-NAc circuit plasticity and functionality in spared nerve injury (SNI) model mice under therapeutic interventions, including drug application, forced exercise, and M1 activation. Our findings demonstrate significant physiological and functional changes in the MD-NAc connection in chronic pain and post-therapy. Electrophysiological data revealed that MD activation enhances intrinsic excitability in both D1R and D2R neurons within the NAc exclusively in the SNI model, linked to faster presynaptic replenishment in D1R neurons. Behavioral assessments using the Von Frey test indicated that forced treadmill exercise and M1 stimulation significantly reduced chronic pain. However, fiber photometry results suggested that M1 activation does not alter neuronal dynamics in either the MD or NAc. This conclusion remains tentative due to low sample sizes, high variance, and the primary expression of M1 activation in layer 5, which is not our main area of interest. Our study enhances the understanding of neural circuits influenced by MCS and suggests potential therapeutic strategies for alleviating chronic neuropathic pain.

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24 **Amrita Das Gupta**, Hongwei Zheng, Johannes Knabbe, Thomas Kuner

Chronic neuropathic pain induces neuronal loss in the secondary motor cortex of mouse models with spared nerve injury

Chronic neuropathic pain is known to induce structural plasticity in the brain, but its effects on the mesoscopic scale of tissue composition remain poorly understood. This study investigates the cellular composition of cortical areas and the structural variability of neurons during chronic neuropathic pain development using longitudinal *in vivo* two-photon microscopy and behavioral assessments in mice. We monitored cell type composition in cortical volumes containing approximately 25,000 cells each and tracked individual neurons in response to spared-nerve injury (SNI). Our findings reveal significant neuronal loss in the secondary motor cortex (M2), located adjacent to the cingulate cortex, within two weeks post-surgery. This study identifies M2 as a novel site affected by neuropathic pain and highlights neuronal loss as a crucial and previously underappreciated mechanism in the development of chronic neuropathic pain states.

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25 **Catello Guida**, Anne Hoffrichter, Fabio Marsoner, Ammar Jabali, Julia Ladewig

Unraveling the functions of the transcription factor TBR2 in human neurodevelopment

Among the different organs, the human brain has undoubtedly been the one most affected by evolution, with both morphological and functional changes. The human neocortex, in particular, is greatly expanded and exhibits increased complexity. The genetic mechanisms underlying the evolutionary changes in our neurodevelopment are, however, poorly understood. With the advent of human pluripotent stem cells (PSC) in combination with the discovery of efficient gene editing technologies and the ability to generate organotypic PSC-derived brain organoids we are now technologically equipped to decipher the molecular basis of the changes between our brain and that of our ancestors. In this project we apply gene editing in PSC and thereof derived organoids to study the function of TBR2, a transcription factor selectively expressed in IPs and functionally required for SVZ neurogenesis, during early cortical development. Using CRISPR/Cas9 mediated gene editing we generated PSC-TBR2-knockout (KO) lines. Following validation we applied a forebrain- and a cortical hem-type organoid protocols. When analyzing the transgenic organoids and isogenic controls we found that TBR2 is impacting in the forebrain on the generation and proliferation of the different types of intermediate progenitor cells including those expressing PPP1R17 or NHLH2. We also found that TBR2 is acting on the generation of the pre and subplate including the differentiation into pioneering neurons positive for the markers TBR1 and BHLHB5. In the cortical-hem-type organoids we found alterations in the developmental timing of cells expressing markers for the choroid plexus. By that our data suggests a role of TBR2 in forebrain and cortical hem development and proves that transgenic organoids represent a powerful tool to map gene function in brain development, to correlate genetics to functional phenotypes and to complement the long tradition of KO-models in developmental biology and neuroscience.

26 **Selma Aghabashlou Saisan**, Jule Truberg, Laura Rueda Gensini, Moritz Mall

Unraveling the pathomechanism of neurodevelopmental disorders caused by MYT1L-mutations in patient-derived, prime-edited iPSCs and cerebral organoids

Neurodevelopmental disorders (NDDs) such as ASD, epilepsy, and schizophrenia are characterized by a disruption of timely and spatially tightly coordinated events underlying brain development. The etiology of NDDs is heterogeneous and comprises mutations in chromatin regulators, i.e. transcription factors (TFs). The TF MYT1L suppresses non-neuronal cell fates during development and throughout life safeguarding neuronal identity. Around 200 de novo MYT1L-mutations have been reported worldwide resulting in NDDs with a high penetrance. The underlying pathomechanisms of NDDs upon MYT1L-mutations are, however, not fully understood yet. So far, delayed neurogenesis and deregulation of cell fate decision have been observed in conditional MYT1L-KO-iPSCs and -mice. In order to understand how different MYT1L-mutations result in NDDs, iPSCs from patients with the R75*- and R567P-mutation were recruited as a preclinical model. To mimic and investigate human brain development *in vitro*, cerebral organoids are formed with the patient-derived iPSCs. Cerebral organoids of Day 31 and Day 61 are obtained to characterize early and late neurogenesis and cell fate decision upon MYT1L-mutations. Further, isogenic control cell lines are generated by correcting the MYT1L-mutations in the patient-derived iPSCs using the prime editing technology. Currently, iPSCs obtained from the healthy relatives of the patients act as a non-isogenic control in the experiments. The prime-edited, patient-derived iPSCs allow to trace back the phenotype observed in the patient-derived iPSCs to the respective MYT1L-mutation and exclude the aspect of genetic variance being a potential reason for the phenotype differences observed between the non-isogenic control and mutational cell line.

27 **Adriana Schneider**, Alexandra Merkel, Netta Ussyshkin, Lisa Ruff, Paula Zimmer, Daniela Mauceri

Atp8a2 controls phosphatidylserine externalisation, structural integrity and survival in neurons

The plasma membrane is characterized by an asymmetric distribution of phospholipids, maintained by three enzyme classes: flippases, floppases, and scramblases. Phosphatidylserine (PS) is confined to the inner leaflet of the plasma membrane under normal conditions, and its externalization has been linked to both apoptotic and non-apoptotic processes within the central nervous system (CNS). Further, neuronal PS exposure can be transient in stressed but viable cells. Thus, interference with PS-triggered phagocytosis of neurons has been suggested to be beneficial in CNS disorders. The mechanism of action, expression levels, and response to stimuli of PS-shuttling enzymes in neurons remain poorly characterized. Using different imaging approaches, we found that an excitotoxic insult to primary hippocampal neurons triggers PS exposure. The exposure could be identified in the form of hotspots, primarily localized near spine protrusions and/or dendritic branching points. Notably, this phenomenon was accompanied by a reduction in expression levels of Atp8a2; a flippase responsible for the inward translocation of PS. We demonstrated that silencing Atp8a2-activity results in an increased PS exposure, particularly at dendritic branching points, coupled with reduced dendritic complexity and neurite length. Moreover, diminished Atp8a2 activity intensifies neuronal vulnerability to toxic insults both in-vitro and in-vivo, while boosting Atp8a2 expression provided neuroprotection. As mutations in Atp8a2 in humans have been linked to mental retardation, motor deficits and optic atrophy in humans, our work provides mechanistic insights into these genetic disorders.

28 **Debanjan Chowdhury**, Duncan MacLaren, Beate Throm, Nina Bieber, Magdalene Schlesiger, Hannah Monyer

Identifying mechanisms involved in acute alcohol-induced amnesia

Alcohol intoxication can impair episodic (autobiographical) and spatial (navigational) memory. These memories are formed and maintained in different subareas of the hippocampal formation, including the hippocampus proper (HC) and the medial entorhinal cortex (MEC). The rhythmic activity within these regions is in turn orchestrated by GABAergic projection neurons of the medial septum (MS). The effects of alcohol on these systems are largely unexplored. In this project, we examine the effects of acute alcohol intoxication on functional cell types and network-level activity in and between these regions by performing *in vivo* electrophysiological recordings in behaving mice. Alcohol administration at a dosage of 1.7 g/kg intraperitoneally impairs the performance of a reference memory task on the 8-arm radial maze. In an open-field environment, the same dose of alcohol causes a reduction in the firing rate of fast-spiking cells in the MS, MEC, and HC, with the strongest effect in the MEC. The firing rate of grid cells in the MEC, but not of place cells in the HC, is reduced by alcohol. At the network level, alcohol causes a reduction of the LFP theta frequency in both the MEC and HC. Phase precession, a temporal coding property of spatially selective cells, is affected by alcohol in grid cells in the MEC, but not in place cells in the HC. Hence, we infer that the MEC is more susceptible to alcohol than the HC.

29 **Lara Kilian**, Marija Banicevic, Jennifer Just, Michaela Back, Irmgard Amrein, Jakob von Engelhardt, David P. Wolfer, Stefan Kins, Martin Korte, Ulrike C. Müller

Novel knockin mice lacking the alpha- and beta-secretase cleavage sites

The pivotal role of the amyloid precursor protein (APP) in the pathogenesis of Alzheimer's Disease is well established. However, the precise physiological role of APP is still not fully understood. Both, transmembrane APP signaling and secreted APP fragments are required for normal development of the nervous system. Previous studies indicated that cell surface APP and the APLPs mediate synaptic adhesion, which is important during development and in the adult for processes that require synaptic stabilization. However, proteolytic cleavage of APP leads to APPsα secretion, which has been shown to support both structural and functional synaptic plasticity. Our goal is to understand the interdependence and regulation of these processes at the synapse *in vivo*. Hence, we have generated novel APP knockin mice expressing solely a secretion-deficient APP variant by introducing a genomic deletion (APPΔS622) encompassing the α- and β-secretase cleavage sites. APPΔS622 mice proved fully viable. Western blot analysis of APP processing in brain lysates indicated an accumulation of uncleaved APP at the cell surface that was paralleled by a prominent reduction of secreted, soluble APPs fragments and APP-CTFs. Intriguingly, the levels of post-synaptic proteins PSD-95 and Homer1 were significantly reduced. Moreover, electrophysiological recordings conducted in the hippocampus of adult APPΔS622 mice revealed a synaptic phenotype characterized by pronounced deficits in the induction and maintenance of hippocampal Long-Term Potentiation (LTP). Collectively, our findings suggest that soluble APPs plays a fundamental role in modulating and regulating the dynamic processes underlying synaptic plasticity.

30 **Zvi Menahem**, Meike Fellanz, Marija Banicevic, Lea Humbs, Carolin Stoffer, Gundula Braun, Dominique F äßler, Nadine Zahn, Christian J. Bucholz, Thomas Deller, Ulrike C. Müller

Analysis of APP functional domains using APP/APLP2 deficient organotypic slice cultures

Amyloid precursor protein (APP) has a key role in Alzheimer's disease (AD). Amyloid-β (Aβ), one of APP's main proteolytic fragments is produced by sequential cleavage of β- and γ- secretase. Aβ accumulation and oligomer formation is thought to cause synaptic dysfunction, leading to memory loss and ultimately to dementia. In the competing, non-amyloidogenic pathway, α-secretase starts the cleaving process within the Aβ region of APP, thus preventing the formation of Aβ peptides, and liberating the large soluble neuroprotective ectodomain APPsα into the extracellular space. APP belongs to a small gene family, including the APP-like Proteins (APLPs) APLP1 and APLP2, which have partially overlapping functions, and important physiological roles in synaptic plasticity, learning and memory. In this work, we established a strategy to investigate the role of APP family proteins at either the pre- or postsynaptic side of CA3-CA1 Schaffer collateral synapses. Using local injections of adeno-associated virus (AAV) based vectors into organotypic hippocampal slices (OTCs), we can knockout the APP family proteins (with cre-recombinase) and re-express, locally, different Cre-dependet APP proteolytic fragments. pAAVs of cre-recombinase and cre-dependent APP variants were cloned and evaluated, and their corresponding adeno-associated virus (AAV) based vectors were produced. Expression of the different APP variants, enables us to perform a detailed structure function analysis. Reconstituted OTCs are characterized with regard to neuronal morphology, spine density and electrophysiological properties.

31 **Verena Bengelsdorff**, Dominique Fäßler, Zvi Menahem, Lara Kilian, Gundula Braun, Christian Buchholz, Ulrike C. Müller

AAV-Cre mediated triple knockout of the amyloid precursor protein (APP) gene family in primary neurons: a novel system to investigate their physiological functions *in vitro*

To unravel the mechanisms of Alzheimer's disease (AD), our lab investigates the physiological functions of the amyloid precursor protein (APP) and its homologs, APLP1 and APLP2. For this purpose, conditional triple knockout (cTKO) mice lacking all three APP family members in excitatory forebrain neurons have been generated. Interestingly, these mice display a pronounced autism-like phenotype, but each experiment necessitates *in vitro* fertilization (IVF), a process that is both time-consuming and costly. Thus, I recently established primary mouse neurons devoid of all three APP family members using AAV-Cre transduction in APPflx/flx, APLP1-/-, APLP2flx/flx neurons. Utilizing these triple knockout (tKO) neurons, which lack the functional redundancy within the APP family, allows us to more precisely delineate APP's physiological functions. To reproduce the deficits observed in cTKO animals regarding spine density and synaptic transmission, the neuronal morphology of the tKO neurons will be characterized. Additionally, analyzing the cell surface proteome will provide insights into how the APP family mediates the surface trafficking of secretases and glutamate receptors. Finally, the complete absence of all APP family members in the tKO neuron system permits a highly precise investigation into the molecular mechanisms by which specific APP protein domains function through re-expression of deletion constructs. Additionally, proteolytic fragments like APPsα, renowned for its neurotrophic effects, can be employed to rescue deficits in the surface proteome and neuronal morphology. This is particularly significant for AD research, as recent findings have highlighted APPsα's potential to mitigate Tau-induced pathology.

32 **Tamara Pöpping**, Andre Rupp

Variability and validity of phase lag estimations on MEG data

Functional connectivity (FC) can aid in understanding which spatially distinct brain areas labour together during a task. Hence, FC helps us understand the neuronal networks underlying stimulus processing. In the past two decades, a vast variety of different metrics and estimators of FC have been described. However, no agreement has been reached yet on which metric is the most appropriate to characterize FC. Here, the goal was to explore a subtype of FC metrics; namely, estimates of phase lags in time series data. Three phase lag estimators - phase lag index (PLI), weighted PLI (wPLI), and debiased wPLI - were tested using both real and simulated magnetoencephalography (MEG) data. The effects of time series length, number of time series, degree of phase lag, degree of source mixing as well as the influence of noise on the estimators' results were analysed to explore the variability and reliability of the phase lag estimators for human MEG data.

33 **Thomas Thäwel**, Christoph Körber, Hannah Kapell, Amel Zulji, Thomas Kuner, Lucas Schirmer

Decoding spatial and temporal cell-type specific vulnerability in neuroinflammation

Technological advances with single-nucleus transcriptomics have greatly improved our understanding of selective neuronal damage. Previous postmortem studies of human multiple sclerosis brains have shown that upper cortical excitatory neurons exhibit increased vulnerability.However, investigating changes over time in these vulnerable cell types after distal neuroinflammation remains challenging in human studies. To address these challenges, we utilized a focal lesioning mouse model with controlled inflammatory demyelination to subcortical white matter. We then collected sensorimotor cortex samples for analysis using both spatial and single-nucleus transcriptomics at various time points post lesioning. We hypothesize that distant white matter lesions induce dynamic, cell type-specific vulnerability and cortical inflammation. Preliminary data shows a compositional loss of deep cortical layer projection neurons from an early inflammatory time point onwards, as well as reactive transcriptomic changes in specific neuron subtypes and surrounding glial cells across cortical layers. These results suggest a reactive cortical inflammation distant from white matter lesioning as well as a selective neuronal vulnerability with demise of deep cortical layer neurons. In summary, these results help improve our understanding of neuron cell type-specific vulnerability and cortical environmental changes due to focal neuroinflammation.

34 **Celia Lerma-Martin**, Pau Badia-i-Mompel, Ricardo O. Ramirez Flores, Patricia Sekol, Philipp S. L. Schäfer, Christian J. Riedl, Annika Hofmann, Thomas Thäwel, Florian Wünnemann, Miguel A. Ibarra-Arellano, Tim Trobisch, Philipp Eisele, Denis Schapiro, Maximilian Haeussler, Simon Hametner, Julio Saez-Rodriguez, Lucas Schirmer

Cell type mapping unravels tissue niches and interactions in subcortical multiple sclerosis lesions

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system. Inflammation is gradually compartmentalized and restricted to specific tissue niches like the lesion rim. However, the precise cell type composition of such niches, their interactions and changes between chronic active and inactive stages are incompletely understood. We used single-nucleus and spatial transcriptomics from subcortical MS and corresponding control tissues to map cell types and associated pathways to lesion and non-lesion areas. We identified niches such as perivascular spaces, the inflamed lesion rim, or the lesion core associated with the glial scar and a cilia-forming astrocyte subtype. Focusing on the inflamed rim of chronic active lesions, we uncovered cell-cell communication events between myeloid, endothelial and glial cell types. Our results provide insight into the cellular composition, multicellular programs, and intercellular communication in tissue niches along the conversion from a homeostatic to a dysfunctional state underlying lesion progression in MS.

35 **Natalie Ludwig**, Lucas Schirmer

Iron scavenging and myeloid cell polarization

Myeloid cells that populate all human organs and blood are a versatile class of innate immune cells. They are crucial for sensing and regulating processes as diverse as tissue homeostasis and inflammation and are frequently characterized by their roles in either regulating or promoting inflammation. Recent studies in cultured cells and mouse models have highlighted the role of iron in skewing the functional properties of myeloid cells in tissue damage and repair. Here, we review certain emerging concepts on how iron influences and determines myeloid cell polarization in the context of its uptake, storage, and metabolism, including in conditions such as multiple sclerosis, sickle cell disease and tumor biology.

36 **Julia Dyckow**, Celine Geywitz, Natalie Ludwig, Klaus-Armin Nave, Wiebke Möbius, Lucas Schirmer

Role of oligodendrocyte-encoded Piezo2 during aging and demyelination

Background: Proper myelin wrapping along axons is essential to a fast saltatory conduction of action potentials. In the central nervous system (CNS), myelin is built by oligodendrocytes (OLs) and shows pathological abnormalities not only in demyelinating diseases such as multiple sclerosis (MS) but also in neurodevelopmental disorders. Piezo proteins are mechanosensitive ion channels which convert mechanical impulses into electrical signals. So far, studies have focused on their role in pain and touch sensation in the peripheral nervous system.
Objective: This study aims at understanding the role of Piezo2 in the CNS during development and aging with a special focus on the anterior visual system.
Methods: We investigated dynamics in expression profiles of Piezo1 and Piezo2 in the OL lineage in mouse retinae and optic nerves (ONs) during aging as well as in human ONs from patients, who died in the course of MS. Further, we performed rotarod behavioral experiments and electron microscopy in two different Piezo2 loss-of-function mouse lines.
Results: We observed a switch in the expression profile of Piezo1 and Piezo2 during development, suggesting that Piezo1 plays key roles in OL progenitors, whereas Piezo2 is critical for OL function. Then, we found that Piezo2 loss-of-function mice developed motor deficits during adulthood and aging. Ultrastructural imaging of Piezo2 loss-of-function ONs revealed changes in g-ratios and axon diameters pointing towards disturbed myelin wrapping. Investigating retinal flat mounts, we observed reduced retinal ganglion cell counts in Piezo2 loss-of-function mice during adulthood. In MS ON tissues, we found that Piezo2 becomes downregulated in OL cells at lesion rims, suggesting maladaptive/reactive changes at tissues at risk under disease conditions.
Conclusion: Our findings suggest that Piezo2 is important for proper myelination of axons, myelin-axon signaling and physiological neuronal functioning during adulthood and aging. This study will help decipher Piezo2 channel functions in OL lineage cells during homeostatic and disease conditions such as in MS.

37 **Rangeet Manna**, Duncan MacLaren, Anushka Wakade, Kristoffer Damm, Magdalena Schlesiger

Activation of VTA dopaminergic neurons modulates spatial coding in LEC and MEC

The formation and processing of episodic memory relies on hippocampo-entorhinal regions (H-EC). It has been shown in behavioural experiments in rats and mice that lesions of the H-EC impair the ability to form memories that require the association of object, spatial, and contextual information. Neuronal firing patterns in the different subregions of the H-EC reflect this function: Neurons in the medial entorhinal cortex (MEC) are specialized in various computations that keep track of the animal's position in space. A subset of neurons in the lateral entorhinal cortex (LEC) code for the identity of objects. Hippocampal neurons display a mixture of spatial and object-coding properties, and all three regions are modulated by context. These “building blocks” of associative memory have been predominantly identified in simple behavioural tasks that require a mouse or rat to forage in open-field environments of different colours and shapes. These environments might also include interesting objects to explore. It is an emerging challenge to identify how these building blocks are used during more complex memory tasks. Here, using self-stimulation of VTA dopaminergic neurons and/or administration of addictive drugs, we developed conditioned place and object preference tasks that can be used for repeated electrophysiological recordings and allow the assessment of how neuronal codes change when mice learn that different parts of the environment are paired with a rewarding signal.

38 **Adeoye Ewedemi**, Marcus Meinhardt, Wolfgang Sommer, Rainer Spanagel

The effect of delay discounting on the medial prefrontal cortex

Given the choice, most of us prefer to receive rewards sooner rather than later, and in greater amounts. However, decisions become more complex and intriguing when these preferences conflict: opting for less now or more later. A short temporal perspective may increase the value of intense, reliable and brief reward, such as alcohol. At the same time, a short temporal perspective may lead to a devaluation of reward that are less intense, variable and accrue value over long timeframes, such as prosocial reinforces. Experimentally, this process is measured as delay discounting.
Delay discounting is the decline in the present value of a reward with delay to its receipt. Higher discounting of future rewards is recognised as a risk factor for several impulsive disorders and addictions including alcohol use disorders. Notably, discounting rates can be modified by training or pharmacology, opening up for the development of interventions for regaining control. In our research, we developed a novel behavioural paradigm on delay discounting. To validate our paradigm, we administered amphetamine treatment to rats, with our result confirming the sensitivity of our model to pharmacological manipulation, by increasing the discounting of rats in our paradigm. Furthermore, we conducted experiments with the use of chemogentics to highlight the role of the medial prefrontal cortex (mPFC), a brain region implicated in AUD, as a critical area involved in delay discounting. Our findings shows that inhibition of the PFC results in a slight increase in the delay discounting. These results offers a promising insight for understanding the neurobiological underpinnings of AUD and future targeted interventions. Our research shows the importance of the PFC in regulating decisions associated with delay discounting and potentially, alcohol use.

39 **Isabel Loss**, Rüstem Yilmaz, Francesca Tuorto, Elena Cairo, Philipp Koch, Jochen Weishaupt, Rosanna Parlato

Morphological and transcriptomic signatures in patient-derived motor neurons carrying ALS causative KIF5A mutations.

Our group has previously described heterozygous ALS-causing mutations in the Kinesin Family Member 5A (KIF5A). KIF5A is a kinesin responsible for anterograde transport of proteins, organelles, RNA, and neurofilaments along the neurites. ALS-linked mutations occur in the kinesin's C-terminal cargo-binding domain and are caused by the disruption of exon 27 splicing. Indeed, a mutation resulting in KIF5A exon 27 skipping (ΔExon27) has been shown to cause altered protein and RNA interactions. Moreover, we recently found that the conformational changes caused by ΔExon27 is inducing neurotoxicity by abolishing KIF5A autoinhibition. We generated hiPSCs from one pre-manifest carrier of a heterozygous ΔExon27 mutation (c.3020+2T>C, P1) and from two ALS patients of a family with a c.2993-1G>A heterozygous mutation (P2, P3). These mutations alter exon 27 splicing differently, yet both are predicted to lead to the production of a common C-terminal aberrant end. In addition, patient-derived motor neurons displayed a significant increase in KIF5A inclusions in comparison to control. Our bulk RNA sequencing of MNs at D20 and D35 from three healthy individuals and three patients highlighted maturation-dependent alterations in pathways associated with RNA processing and translation. Along this line, mutant KIF5A displays reduced interaction with several ribosomal proteins as shown by proximity ligation assay. Here, we characterize changes in ribosomal proteins expression as an initial compensatory mechanism for impaired ribosomal synthesis and global translation dysregulation in patient derived motor neurons. Taken together, these findings will expand the mechanistic understanding of KIF5A/ALS pathology and will help identifying therapeutic targets for modulating ALS pathogenesis.

40 **Ketrin Dimco**, Rüstem Yilmaz, Rosanna Parlato, Jochen Weishaupt

Role of Myorg mutations in primary familial brain calcification (PFBC)

Primary Familial Brain Calcification (PFBC), formerly known as Fahr's disease, is a clinically and genetically heterogeneous neurological disorder characterized by progressive bilateral intra- and perivascular calcifications in various brain regions, primarily the basal ganglia, but also including the cerebellum, thalamus, and brain stem. The clinical manifestation of PFBC is both incomplete and heterogeneous, with patients exhibiting symptoms ranging from being asymptomatic to experiencing severe movement disorders with cerebellar and extrapyramidal syndromes, along with cognitive and neuropsychiatric manifestations. Currently, no causal therapy exists for PFBC. We have recently identified the first gene associated with autosomal-recessively inherited PFBC, known as myogenesis regulating glycosidase (MYORG). MYORG is a putative glycosidase enriched in the endoplasmic reticulum (ER) and is potentially involved in post-translational modifications such as N-glycosylation. However, the specific function of this enzyme and its substrate remain unknown, as do the pathogenic mechanisms by which MYORG mutations lead to PFBC. Our research has demonstrated that overexpression of various mutant MYORG transgenes results in decreased expression levels of MYORG due to reduced protein stability, as determined by cycloheximide chase assays. Given MYORG's ER localization, we also analyzed the impact of these mutations on the ER stress response. As a second study model, we utilized primary astrocytic cultures isolated from MYORG mutant pups (mouse models already generated and available in our group) from differentially vulnerable regions (cortex and cerebellum). These cultures were analyzed to delineate morphological and molecular changes induced by the mutation, as well as to identify pathways and cell-autonomous pathomechanisms underlying PFBC through comparative bulk transcriptomics.
In summary, our findings will provide novel insights into the role of MYORG mutations in astrocytes within the context of PFBC, enhancing our understanding of the disease and potentially guiding future therapeutic strategies.

41 **Jahnavi Srinidhi**, David Brenner, Isabel Loss, Rüstem Yilmaz, Philipp Koch, Rosanna Parlato, Jochen Weishaupt

Comparative analysis of different TBK1-ALS mutations in patient derived motor neurons

Heterozygous mutations in the TANK-binding kinase 1 (TBK1) gene lead to ALS. TBK1 is a pleiotropic kinase controlling, among others, the autophagic process. TBK1 contains four domains: a serine/threonine kinase domain (KD) located at its N-terminal, a ubiquitin-like domain (ULD) and two coiled-coil domains, CCD1 and CCD2. The TBK1 loss-of-function variants cause an early truncation of the protein resulting in a decrease of TBK1 kinase activity and/or prevention of substrate-binding, for example autophagy adaptor proteins. This study has two major aims: 1) to characterize the cell-autonomous cellular and molecular effects of different TBK1 mutations in an ALS vulnerable neuronal population, and 2) to identify specific phenotypic markers associated with them. To this end, we re-programmed human iPSCs from primary blood mononuclear cells (PBMCs) from one ALS pre-manifest carrier of a heterozygous TBK1 mutation c.1760+1G>C (splice site mutation) and from two ALS affected members of a family with a TBK1 frameshift heterozygous mutation (c.78_79delAA/p.27Thrfs*2), and differentiated them into motor neurons. Additionally, we included a heterozygous and a homozygous line carrying the TBK1 missense mutation p.E696K and their isogenic control. First, we asked whether this TBK1 missense mutation leads to defective autophagy. Immunofluorescence analysis in the homozygous motor neurons showed a significant increase in the accumulation of p62 positive

autophagosomes, LAMP2 positive lysosomes and an increase in Galectin8 positive puncta, as a marker of damaged lysosomes, suggesting impaired lysosomal degradation. We characterised these puncta based on their size, number, and intensity, providing a possible readout to establish a high throughput screening platform for therapeutic compounds and to develop mutation-specific therapies.

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Ivan Valkadinov, Sophie Hebestreit, Patrick Kinz, Janine Schwahn, Olivia Kosche, Isabel Winzer, Max Brauner, Antje Knehr, Holger Wenz, Máté Maros, Vesile Sandikci, Anne Ebert, Lukas Eckrich, Rüstem Yilmaz, Rosanna Parlato, David Brenner, Jochen Weishaupt, Julian Conrad

The primary familial brain calcification disconnectome

Background: Primary (familial) brain calcification (PBC) is a condition characterized by bilateral “calcifications” mainly in the basal ganglia, cerebellum and thalamus. People with PBC can be asymptomatic, but can also develop severe progressive symptoms. These range from parkinsonism and ataxia to neuropsychiatric symptoms and epileptic seizures. Symptoms of the disease are likely due to brain mineralization, which is believed to be mainly comprised of calcium. Besides sporadic cases of PFBC, genetic causes have also been described and the majority of genes with autosomal-dominant (*SLC20A2*, *PDGFRB*, *PDGFB* and *XPR1) or* -recessive inheritance *(MYORG)*. With current imaging approaches, there is only a poor lesion-symptom association.
Methods: Here we used advanced structural imaging and sophisticated lesion-network mapping to thoroughly characterize the lesion networks associated with specific symptoms in a cohort of 43 persons with PBC.
All participants received high resolution structural imaging (1mm^3 T1MPRAGE) and dedicated imaging of the mineralizations (computed tomography (CT), susceptibility weighted imaging (SWI) and quantitative susceptibility mapping (QSM)). Mineralization maps were created from the different imaging modalities and normalized to MNI152 space for group comparisons in SPM12. The normalized mineralization maps were then used to establish specific disconnection maps for each participant and imaging modality using BCB toolkit.
Results: Multimodal structural imaging revealed differing mineralization map volumes for each imaging modality. The calcium lesions showed the most specific and convincing clinico-anatomical pattern. Using disconnectome mapping, we could identify distinct networks associated with affective and motor symptoms. These include mainly the frontostraiatal circuitry, anterior commissure, cerebello-rubro-thalamic networks and further aspects of the brainstem pyramidal and extrapyramidal circuitry.
Conclusion: The current analysis improved the understanding of mineralization related lesion-networks that are associated with specific symptoms in PBC. Establishing symptom-specific lesion networks can be the starting point for the development of non-invasive neuromodulation therapies for people with PBC.

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Lena Eschholz, Chantal Wissing, Maxime Maheu, Kathrin Sauter, Fabio Morellini, J. Simon Wiegert, Alexander Dieter

Targeting norepinephrine neurons of the locus coeruleus: a comparison of model systems and strategies

The locus coeruleus (LC) noradrenergic (NE) system is involved in a plethora of physiological and pathophysiological processes. Refining our understanding of LC function largely relies on selective transgene expression in molecularly defined cells, allowing targeted manipulation and readout of noradrenergic neurons. Here, we performed a side-by-side comparison of the most commonly used strategies to genetically access the LC, including different cre driver lines and promoter-mediated transgene expression. We report differences between these strategies in terms of transgene expression efficacy and molecular specificity. Notably, we found no behavioral alterations performing anxiety tests and memory tasks in cre-expressing mice of any mouse line as compared to wild-type littermates. Finally, to further ease the investigation of LC-NE function, we created a suite of constructs, including reporter proteins, calcium indicators, and optogenetic actuators whose expression is mediated by the previously described PRSx8 promoter. These constructs allow for monitoring and manipulation of LC-NE activity either in wild-type mice, or in combination with tissue-specific manipulations of different cre driver lines. The results of our study are crucial for the interpretation of results from previous experiments using the respective targeting strategies, as well as for the design of future studies.

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Yilmaz Arda Ates, J. Simon Wiegert

Reverse BiPOLES: expanding the toolkit for bidirectional optogenetic control of neuronal activity

In the field of neuroscience, the ability to manipulate neuronal activity with precision is crucial for understanding complex neural networks. The development of optogenetic tools has revolutionized this field. Modern tools have been optimized to allow multimodal manipulations, such as bidirectional dual-color control of neuronal excitation and inhibition with BiPOLES, which is composed of a red-light sensitive cation channel and a blue-light sensitive anion channel. Building on this foundation, we introduce Reverse BiPOLES, an optogenetic construct designed for bidirectional control of neurons using spectrally opposite action spectra compared to the original BiPOLES. Reverse BiPOLES employs the blue-light sensitive cation channel Chrome2S for neuronal excitation and the red-shifted anion channel raACR for neuronal inhibition. When used in conjunction with the original BiPOLES, Reverse BiPOLES expands the capabilities for neuronal manipulations. It enables mutually exclusive excitation and inhibition of two neuronal populations, providing

a versatile tool for complex experimental designs testing the function of one neuronal population of interest independently of a second, distinct population of neurons. In the future, these tools may be useful to disentangle the contribution of small, molecularly defined nuclei that are in close proximity to each other, such as neuromodulatory centers of the brain stem, to various physiological functions of the brain. Future development of bidirectional optogenetic actuators with action spectra compatible with optical voltage or calcium indicators and other optogenetic tools opens new avenues for multi-dimensional control of neuronal circuits.

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45 **Marton Istvan Molnar**, J. Simon Wiegert, Andrey Formozov

G-protein mediated control of hippocampal interneurons in hippocampal network dynamics

Human Neuropsin (hOPN5), constituting a phylogenetically conserved and separate opsin superfamily, is an endogenous bistable UV-sensitive and non-visual melanopsin variant. In humans it is found expressed in brain-, corneal-, skin tissues among others, with one possible role in melanin production in skin melanocytes. Being a bistable opsin, it has absorption maxima at 380nm (near-UV light), and deactivation maxima around 560nm. Recent work has suggested, it couples to G14 subtype of the Gαq-domain within the G-protein coupled receptor, preferentially activating PLCß-IP3-Ca2+ pathway. For Gq stimulation, available tools such as hM3Dq (chemogenetics) lack spatiotemporal precision. Also, modeling the downstream effects of Gq/11-coupled dopamine-, noradrenergic α1-, and 5-HT2A receptors in the brain is not yet well established. Here, we generated an expression vector to characterize hOPN5 activation via optogenetic means in hippocampal organotypical slice cultures, a reliable ex-vivo model. S5E2-promoter was employed within the construct, targeting parvalbumin-positive interneurons, a pathologically relevant cell type that influences oscillatory behavior of principle cells of the hippocampus. We streamlined a project in which after characterization promoter specificity; one applies single-cell electroporation, patch clamp and 2-photon calcium imaging with optogenetics to elucidate the effects rooting from hOPN5 activation. A recombinant adeno-associated virus construct is also being designed, opening up opportunities for mouse *in vivo* experiments as well. Manipulation of oscillatory phenomena from parvalbumin-interneuronal modulation will be parallelly investigated. To conclude, this project aims to develop a method for reliable, locally and timing-wise restricted Gq/11 stimulation in *ex vivo* and *in vivo* tissue.

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46 **Avi Adlaka**, Thomas Kuner

Thalamic ensemble activity during distinct behaviors

The posterior medial nucleus (POm) of the thalamus represents a higher-order thalamic nucleus characterized by its reciprocal connections with the primary somatosensory cortex (S1). It exhibits intricate projections to and from motor, premotor, association cortices, and the brainstem. Owing to its distinct firing characteristics and multifaced connectivity profiles, various functions ranging from maintaining arousal to higher somatosensory processing have been attributed to it. To address functional features of the mouse POm, we image relay neuron activity in freely moving mice while they perform different behaviors. We hypothesize that ensemble activity coinciding with distinct behavioral episodes will reveal functional contributions of the POm to these behaviors. Deep imaging is achieved by implanting GRIN lenses at GCaMP-expressing POm in conjunction with UCLA miniscopes. We reliably record robust signals emanating from ensembles of POm relay neurons. We correlate activity patterns and the identity of active neurons in diverse behavioral paradigms encompassing locomotion, sensory stimulation, and salience detection. Distinct neural ensembles were delineated and categorized according to their patterns of firing activity observed across various phases of behavioral experiments conducted across multiple sessions and tasks.

*** Posters marked with an asterisk do not qualify for the IZN student poster prize.**