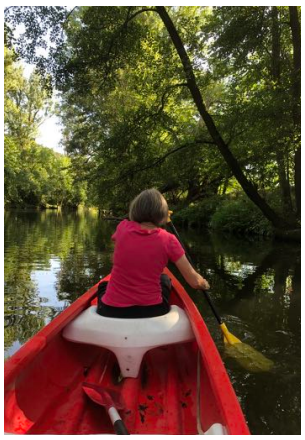
IZN Retreat 2022
Kloster Schöntal
July 17-19

IZN

Interdisziplinäres Zentrum für
Neurowissenschaften der
Ruprecht-Karls-Universität Heidelberg



Program

Sunday, July 17

Welcome Reception	**10:00**	Banquet hall	
Welcome Address	10:45	**Hilmar Bading** Managing Director of the IZN Heidelberg University	
Session 1 Chair: Emilio Isaias Camacho	11:15	**Dino Lüthi** University of Basel Basel, Switzerland	*Preclinical assessment of novel psychedelics*
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**		
Plenary Lecture Chair: Matthias Klumpp	19:30	**Franz Vollenweider** University of Zürich Zürich, Switzerland	*On the search for neuroscience-based therapeutic targets of psychedelics*
Posters & Drinks	20:30	Rooms 203, 204, 218, 219, and the hallway	

Monday, July 18

Breakfast	**7:00**		
Session 3 Chair: Bahar Dokht Tolou-Dabbaghian	9:00	**Natasha Mason** Maastricht University Maastricht, The Netherlands	*Mind-bending, heart-mending? The effect of psychedelics on flexible cognition and underlying neural mechanisms*
Session 4 Chair: Marion Friske	9:45	**Christian Klein** Heidelberg University Heidelberg, Germany	*Medicinal chemistry of psychedelics*
Posters & Coffee	**10:30**	Rooms 203, 204, 218, 219, and the hallway	
Session 5 Chair: Jonas Schimmer	11:15	**Katrin Preller** University of Zürich Zürich, Switzerland	*The effects of psychedelics on the human brain*
Lunch	**12:00**		
Session 6 Chair: Klara Rehder	13:30	**Malin Schmidt** Central Institute of Mental Health Mannheim, Germany **Tobias Buchborn** Central Institute of Mental Health Mannheim, Germany	*The psychedelic psilocin fosters neuroplasticity in iPSC-derived human cortical neurons* *Psychedelics in basic and preclinical research*
Session 7 Chair: Jing Yan	14:30	**"Poster Jam"** **Nadine Winkler** AG Schrenk-Siemens **Marco Siekmann** AG Ladewig **Ilknur Coban** AG Agarwal	 *Pluripotent stem cell-derived peripheral-to-central synapses: A microfluidic system to assess human nociceptive mechanisms in health and disease* *Introducing human raphe-type organoid to model the role of serotonin on cortical development* *Alpha2a adrenergic receptors on astrocytes regulate neuropathic pain*
Posters & Coffee	**15:15**	Rooms 203, 204, 218, 219, and the hallway	
Meetings	17:00	**Science/Pub Quiz** **IZN Investigator Meeting** group leaders only	Banquet hall Room 221
Dinner	**18:00**		
Music	**20:00**	**Jazz Pastry**	Courtyard or banquet hall (depending on the weather)

Tuesday, July 19

Breakfast	**7:00**		
Session 8 Chair: Irina Meyer	9:00	**Gerhard Gründer** Central Institute for Mental Health Mannheim, Germany	*Psychedelics and the future of psychopharmacology*
Session 9 Chair: Zvika Menahem	9:45	**Helena Aicher** University of Zürich Zürich, Switzerland	*Psychedelic-assisted psychotherapy in practice*
Coffee	**10:30**		
Panel Discussion Chairs: Lea Mertens & Peter Bengtson	11:00	**Panel Discussion: Psychedelics in Neuroscience and Medicine: Hype or Hope?** Participants: **Franz Vollenweider** **Helena Aicher** **Gerhard Gründer** **Dino Lüthi** **Alexander Moldavski** **Constantin Maier** **Thorsten Albrecht**	*Opening statements* *Is the subjective experience important?* *What are the benefits beyond standard treatment (pharmacotherapy, psychotherapy)?* *Role of the therapist / tripsitter* *Side effects and risks* *What is the status (acceptance / legality) of psychedelics in Germany?*
Group Picture	**12:30**	Location TBA	
Lunch	**12:45**		
Session 10 Chair: Juhyun Kang	14:00	**Nicolas Langlitz** The New School for Social Research New York, NY, USA	*Psychedelic innovations and the crisis of psychopharmacology*
Awards Ceremony	14:45	**IZN Students' Poster Prize** *Recipient TBA* Laudatio: Richard Fairless **Best Psychedelic Poster Design** *Recipient TBA* Laudatio: Anna M. H. Hertle **Foundation BrainAid IZN Master's Award** Stephanie Küppers (AG Grinevich) Laudatio: Christoph Schuster **Foundation Brain Aid IZN Dissertation Award** David Brito (AG Oliveira) Laudatio: Andreas Draguhn **IZN / Chica and Heinz Schaller Young Investigator Neuroscience Award** Vijayan Gangadharan (AG R. Kuner) Laudatio: Rüdiger Rudolf **Neuroscience Art Contest Winner** *Recipient TBA* Laudatio: Antje König	
Closing Remarks	16:15	**Hilmar Bading** Managing Director of the IZN Heidelberg University	

Poster Presentations

Nr.	Authors	Group	Title
1#	Mike M. Schmitgen, Florian Werler, Wolfgang Reith, Christian Wolf	Wolf	Functional correlates of neurological soft signs in heavy cannabis users
2	Iasmina Livia Hornoiu, Alycia M. Lee, Haoye Tan, Helmut Nakovics, Patrick Bach, Karl Mann, Falk Kiefer, Wolfgang Sommer, Sabine Vollstädt-Klein	Vollstädt-Klein	The role of unawareness, volition and neural hyperconnectivity in alcohol use disorder: A functional magnetic resonance imaging study
3	Merve Akan, Gunes Unal, Resit Canbeyli	Unal	The antidepressant activity of ketamine ointment in Wistar rats
4	Cedric Stahl, Christian Thiel	Thiel	Towards understanding the potential impact of ALG5 impairment on protein glycosylation and neurological abnormalities
5	Marion M. Friske, Francesco Giannone, Mona Senger, Robin Seitz, Anita C. Hansson, Rainer Spanagel	Spanagel	Chronic alcohol intake regulates expression of SARS-CoV2 infection-relevant genes in an organ-specific manner
6	Veronika Pohořalá, Thomas Enkel, Dusan Bartsch, Rainer Spanagel, Rick E. Bernardi	Spanagel	Sign- and goal-tracking score does not correlate with addiction-like behavior following prolonged cocaine self-administration
7	Nadine Winkler, Kritika Mittal, Joaquin Campos, Claudio Acuna, Katrin Schrenk-Siemens	Siemens	Pluripotent stem cell-derived peripheral-to-central synapses: A microfluidic system to assess human nociceptive mechanisms in health and disease
8	Sarah Janice Hörner, Nathalie Couturier, Roman Bruch, Daniele Caroline Gueiber, Mathias Hafner, Rüdiger Rudolf	Rudolf	Improved differentiation of hIPSC-derived Schwann cells for neuromuscular tricultures
9#	Nathalie Couturier, Sarah Janice Hörner, Daniele Caroline Gueiber, Mathias Hafner, Rüdiger Rudolf	Rudolf	Development of a pioneering isogenic 3D human model of NMJs *in vitro*
10	Daniele Caroline Gueiber, Sarah J. Hörner, Nathalie Couturier, Mathias Hafner, Rüdiger Rudolf	Rudolf	Machine learning-based prediction of cell viability for 3D-bioprints
11	Bahardokht Tolou-Dabbaghian, Jing Chen, Melanie Motsch, Norbert Weidner, Radhika Puttagunta	Puttagunta	The role of nociceptors and the α2-δ2 subunit of the voltage-gated calcium channel in spinal cord injury-induced neuropathic pain in mice
12	Danny Baltissen, Charlotte Bold, Marija Banicevic, Christian Buchholz, Ulrike Müller	Müller	Investigating the therapeutic potential of APPsα in a tau transgenic mouse model
13	Dominique Fäßler, Susanne Erdinger, Vicky Steubler, Michaela K Back, Susann Ludewig, Max Richter, Kang Han, Lutz Slomianka, Irmgard Amrein, Jakob von Engelhardt, David P Wolfer, Martin Korte, Ulrike Müller	Müller	Characterization of the APP gene family in the CNS
14	Lara Kilian, Susanne Erdinger	Müller	Effect of AAV-mediated CTα16 overexpression on spine density in wildtype mice
15	Lena Rehra, Verena Bengelsdorff, Danny Baltissen, Marija Banicevic, Ulrike Müller	Müller	Tamoxifen-inducible knockout mice of the APP gene family
16	Marija Banicevic, Lena Rehra, Danny Baltissen, Zvi Menahem, Laura Keppler, Ulrike Müller	Müller	Generation and *in vitro* evaluation of APP secretion-deficient APP variants in primary cortical neurons
17	Susanne Erdinger, Ulrike Müller	Müller	A psychedelic variation on Steubler, Erdinger *et al*. 2021
18	Zvi Menahem, Marija Banicevic, Meike Fellenz Lea Humbs, Carolin Stoffer, Gundula Braun, Christian J. Bucholz, Thomas Deller, and Ulrike Müller	Müller	Analysis of APP functional domains using APP/APLP2 deficient organotypic slice cultures
19	Debanjan Chowdhury, Beate Throm, Duncan MacLaren, Nina Beiber, Magdalena Schlesiger, Hannah Monyer	Monyer	Identifying mechanisms involved in acute alcohol-induced amnesia

Nr.	Authors	Group	Title
20	Ivan Skorodumov, Merve Akan, Marcus Meinhardt	Meinhardt	Efficacy of R-ketamine in rat models of alcohol addiction
21	Marvin Urban, Tobias Buchborn	Meinhardt	Psilocybin increases impact of aversion conditioning on alcohol self-administration in rats
22	Ann-Kristin Kenkel, José Ricardo Vieira, Christian Litke1, Andromachi Karakatsani, Carmen Ruiz de Almodóvar, Daniela Mauceri	Mauceri	Role of the neurovascular unit and its molecular mediator LRG1 in persistent inflammatory pain
23	Bahar Aksan, Jing Yan, Javier Sanchez-Romero, Dimitris Missirlis, Daniela Mauceri	Mauceri	Molecular mechanisms of structural maintenance and plasticity in neurons
24	Irina Meyer, Clement Verkest, Francisco J. Taberner, Stefan G. Lechner	Lechner	PKA-dependent modulation of PIEZO2
25	Annasara Artioli, Fabio Marsoner, Anne Hoffrichter, Julia Ladewig, Philipp Koch	Ladewig	Deciphering alcohol addiction-associated gene regulation changes on a single cell level
26	Marco T. Siekmann, Raquel Pérez Fernández, Martin Kubitschke, Lutz Wallhorn, Ammar Jabali, Anne Hoffrichter, Olivia Andrea Masseck, Philipp Koch and Julia Ladewig	Ladewig	Introducing human raphe-type organoid to model the role of serotonin on cortical development
27	Raquel Pérez Fernández, Marco T. Siekmann, Annasara Artioli, Philipp Koch, Julia Ladewig	Ladewig	Modelling reward and addiction: development of an *in vitro* reward neurocircuitry
28	Amrita Das Gupta, Jennifer John, Livia Asan, Claudia Falfan-Melgoza, Carlo Beretta, Wolfgang Weber-Fahr, Thomas Kuner, Johannes Knabbe	Kuner, T	Multimodal analysis of structural plasticity of cortical grey matter volume in chronic pain
29	Marina Ruth Hesse, Maja Klevanski, Steffen Sass, Thomas Kuner	Kuner, T	Uncovering the molecular active zone nano-organization of the mammalian central synapse using multiplex 3D super resolution microscopy
30	Steffen Saß, Maja Klevanski, Thomas Kuner	Kuner, T	The calyx of Held is targeted by external, vGluT2-positive neurons that differ in active zone geometry and protein composition
31	Elena Muñoz Perez-Vico, Thorsten Lau, Sandra Horschitz, Julia Ladewig, Philipp Koch	Koch	Implications of the Val66Met polymorphism of the BDNF gene on neuronal morphology and function using human iPSC-derived neuronal cultures
32	Julia Wangemann, Anne Hoffrichter, Andrea C. Rossetti, Julia Ladewig, Philipp Koch	Koch	Human iPSC-derived microglia – towards modeling synaptic pruning-associated changes in schizophrenia
33	Klara Franziska Rehder, Anne Hoffrichter, Julia Ladewig, Philipp Koch	Koch	Deciphering the role of osteocrin in the pathogenesis of schizophrenia
34	Malin Schmidt, Anne Hoffrichter, Mahnaz Davoudi, Sandra Horschitz, Thorsten Lau, Marcus Meinhardt, Rainer Spanagel, Georg Köhr, Julia Ladewig, Philipp Koch	Koch	The psychedelic psilocin fosters neuroplasticity in iPSC-derived human cortical neurons
35	Marc Schulz, Bruno Chausse, Fadi Almouhanna, Andrea Lewen, Oliver Kann	Kann	Interferon-gamma induces lasting priming effects on microglia for several days
36	Pirathitha Ravichandran-Schmidt, Joachim Hass	Hass	Computational modeling time perception and its dopaminergic modulation
37	Francesco Giannone, Magdalena Chrószcz, Marion Friske, Arian Hach, Wolfgang Sommer, Anita Hansson	Hansson	Chronic alcohol exposure and posterior dorsomedial striatum inactivation induce increased habitual behavior in both operant conditioning and spatial navigation paradigms
38#	Christian Schmitz, Lea Mertens, Moritz Spangemacher, Gerhard Gründer	Gründer	Neural effects of psilocybin-treatment in patients with treatment-resistant depression
39#	Moritz Spangemacher, Manuela Brand, Laura Kaertner, Lea Mertens, Dennis Scharf, Christian Schmitz, Gerhard Gründer	Gründer	Three case reports from the EPIsoDE study: a comparison
40	Emilio U. Isaías-Camacho, Jesús M. Martín-Cortecero, Alexander Groh	Groh	A cortico-collicular pathway for defense suppression

Nr.	Authors	Group	Title
41	Filippo Heimburg, Josephine Timm, Nadin Saluti, Matthias Klumpp, Martin Both, Thomas Kuner, Alexander Groh	Groh	A tactile discrimination task to explore context-dependent sensory processing and perceptual salience in freely moving mice
42	Katharina Ziegler, Jan Burghardt, Ross Folkard, Antonio Gonzalez, Emilio Isaías-Camacho, Jesus Martin-Cortecero, Sailaja Antharvedi-Goda, Sanjeev Kaushalya, Linette Tan, Rohini Kuner, Rebecca Mease, Alexander Groh	Groh	Primary somatosensory cortex bidirectionally modulates nociceptive behavior in a layer-specific manner
43	Jonas Schimmer, Stephanie Küppers, Julia Lebedeva, Marina Eliava	Grinevich	Oxytocin facilitates sexual behavior in male rats acting at the ventral hippocampus
44	Stephanie Küppers, Jonas Schimmer, Valery Grinevich	Grinevich	Oxytocin improves positive emotional valence to painful stimuli via action in the anterior insular cortex
45	Francesco Scarlatti, Martin Löffler, Emanuel Schwarz, The IMAGEN Consortium, Herta Flor	Flor	A predictive neurosignature of the comorbidity between chronic pain and mood disorders
46	Maximilian Penzkofer, Susanne Becker, Christian Schmahl, Herta Flor	Flor	The relationship of ACE and violent video gaming: effects on pain perception, fear conditioning and pain-related empathy
47	Viktoria Greeck, S Williams, Ricarda Diem, Hilmar Bading, Richard Fairless	Fairless	Inhibition of NMDAR death complex signaling as a novel therapeutic approach to multiple sclerosis
48	Nadja Lehmann, Stefan Markovic, Christian Thome, Maren Engelhardt	Engelhardt	Development of AcD neurons in the murine hippocampus and primary somatosensory cortex
49	Isabella Boccuni, Andreas Draguhn, Claus Bruehl, Richard Fairless	Draguhn	Contribution of ambient glutamate and glutamate transporters to retinal ganglion cell vulnerability in experimental multiple sclerosis
50	Märt Rannap, Shinya Ohara, Menno P. Witter, Andreas Draguhn, Alexei V. Egorov	Draguhn	Structural and functional organization of the hippocampal-medial entorhinal output circuit
51	Nikolas Stevens, Andreas Draguhn, Martin Both, Christian Thome	Draguhn	Mouse CA1 pyramidal cells with dendritic axon origins receive specialized interhemispheric input at their basal dendrites
52	Christopher Koch, Katja Bauer, Francesca Ciccolini	Ciccolini	Investigating HES1 as a neural stem cell activator
53	Katja Baur, Carmen Carrillo García, Şeydanur Şan, Gabriele Hölzl-Wenig, Claudia Mandl, Francesca Ciccolini	Ciccolini	Growth/differentiation factor 15 controls apical niche homeostasis in the developing SVZ
54	Nadja Sharkov, Matthias Klumpp, Nikolas Stevens, Christian Thome, Janina Kupke, Andreas Draguhn, Ana Oliveira, Martin Both	Both	Different involvement of axon-carrying dendrite versus canonical neurons during learning processes
55	Dorothea Schall, Claudio Acuna, Gudrun A. Rappold, Simone Berkel	Berkel	Investigation of KCNQ1 function in human neurons with a focus on insulin signaling
56	Calvin Thommek, Peter Bengtson, Hilmar Bading	Bading	The role of excitatory amino acid transporters in excitotoxicity
57	Nikolaus Goessl, Anna M. H. Hertle, Kristina Battis, Wojciech Ambroziak, Sebastian Marty, Jan Siemens, Hilmar Bading	Bading	NPAS4 in chronic pain
58	Silvia Gleitze, Pedro Lobos, Andrea Paula-Lima, Cecilia Hidalgo	Bading	Iron chelation and ryanodine receptor inhibition offer protection against ferroptosis in primary hippocampal neurons
59	Zihong Zhang, Jing Yan, Celia Garcia Vilela, Anna M. H. Hertle, Hilmar Bading	Bading	The role of calpain in the degradation of the NMDA receptor and neurotoxicity
60	Rowena Groeneveld, Beate Throm, Kevin Allen	Allen	Object-vector cells in two mouse models of Alzheimer's disease
61	Ilknur Coban, Rangel Leal Silva, Annika Wenzel, Manuela Simonetti, Amit Agarwal	Agarwal	Alpha2a adrenergic receptors on astrocytes regulate neuropathic pain
62	Laura Kärtner, Moritz Spangemacher, Lea Mertens, David Erritzoe, Gerhard Gründer	Gründer	Psychedelisches Microdosing: mehr als „nur" Placebo?

Poster Abstracts

1 Mike M. Schmitgen, Florian Werler, Wolfgang Reith, Christian Wolf

Functional correlates of neurological soft signs in heavy cannabis users

Introduction: Neurological soft signs (NSS) reflect minor sensorimotor symptoms that are accessible via comprehensive clinical examination and are associated with mental disorders, particularly schizophrenia. First evidence suggest that cannabis-use disorders are also associated with NSS. The neural underpinnings of these symptoms are largely unknown at present. Here, we investigated associations between intrinsic brain activation (regional homogeneity, ReHo), heavy cannabis use, and NSS.
Methods: ReHo of 37 healthy controls and 29 heavy cannabis users (HCU) was compared between groups. Mean ReHo within regions showing differences were extracted. Further analyses were performed in a subsample of 26 controls and 21 HCU with complete NSS-data (Heidelberg NSS Scale).
Results: Between the groups, compared to controls, HCU had lower ReHo in bilateral precentral gyrus, left inferior occipital gyrus (lIOG), right triangular inferior frontal gyrus (rTrIFG), and higher ReHo in left postcentral gyrus (lPoG) ($p<0.005$ uncorrected, $k\geq17$). NSS levels were increased in HCU compared to controls across all NSS subdimensions ($p<0.05$ FDR-corrected). Multiple regression models revealed several significant associations between brain activity, cannabis-use behavior and NSS. Most notably, NSS-score of complex motor tasks was predicted best via ReHo in lIOG, lPoG, and rTrIFG ($p<0.001$, RStein_gleich_0.43).
Conclusions: Our findings indicate abnormal ReHo in HCU in regions associated with sensorimotor- and attentional control, response-inhibition, and visuomotor-integration. Importantly, we show associations between ReHo, cannabis using behavior, and execution of complex motor tasks. Given convergent findings in manifest psychotic disorders, the current data suggests an HCU-phenotype that may present with an increased risk for psychosis.

2 Iasmina Livia Hornoiu, Alycia M. Lee, Haoye Tan, Helmut Nakovics, Patrick Bach, Karl Mann, Falk Kiefer, Wolfgang Sommer, Sabine Vollstädt-Klein

The role of unawareness, volition and neural hyperconnectivity in alcohol use disorder: a functional magnetic resonance imaging study

Background: Automated alcohol craving and habitual alcohol consumption characterize the later stages of alcohol use disorder (AUD). This study re-analyses previously collected functional neuroimaging data in combination with the Craving Automated Scale for Alcohol (CAS-A) questionnaire, a retrospective measure of automated alcohol craving over the last drinking period.
Methods: We assessed 49 abstinent male AUD patients and 36 male healthy controls (HC) during an fMRI-based alcohol cue-reactivity task. We performed whole-brain analyses examining the associations between CAS-A and other clinical variables and neural activation patterns in the alcohol versus neutral contrast. Furthermore, we performed psychophysiological-interaction (PPI) analyses to assess the functional connectivity between predefined seed regions and other brain voxels.
Results: In AUD individuals, higher CAS-A scores correlated with greater activation in dorsal striatal, pallidal and prefrontal cortical regions, and with lower activation in visual- and motor-processing regions. We also identified correlations between nonvolition-related CAS-A sub-scores and the seed regions posterior insula and inferior frontal gyrus (IFG). Between-group PPI analyses showed extensive connectivity between the seed regions IFG and angular gyrus and several brain voxels in AUD versus HC.
Conclusions: This study applied a new lens to previously acquired alcohol cue-reactivity fMRI data by correlating neural activation patterns with clinical CAS-A scores to elucidate potential neural correlates of automated craving and habitual alcohol consumption. Our results support previous findings showing that alcohol addiction is associated with hyperactivation in habit-processing regions, with hypoactivation in areas mediating motor and attention processing, and with general hyperconnectivity.

3 Merve Akan, Gunes Unal, Resit Canbeyli

The antidepressant activity of ketamine ointment in Wistar rats

Rapid onset antidepressants launched a new era in depression treatment after 50 years of monoamine hypothesis. Ketamine has a fast-acting antidepressant effect by antagonizing N-methyl-D-aspartate receptor. Classical administration methods of ketamine have the disadvantages of bioavailability loss, gastrointestinal (GI) tract absorption or plasma level fluctuations. Nasal spray ketamine is the most recent alternative overcoming bioavailability loss and GI tract absorption while causing blood fluctuations and the problem of blood fluctuation and abuse potential. As ketamine is a physiochemically ideal for skin penetration and shea butter is a natural permeation enhancer and emulsifier, a transdermal ketamine-shea butter ointment is a worthy choice to be investigated in depression. To offer a sustained delivery of ketamine as an antidepressant, the present study utilized 5% ketamine-shea butter ointment on Wistar rats and assessed the immobility, locomotor activity, thigmotaxic behavior and arm preferences in the Forced Swim Test, Open Field Test (OFT) and Elevated Plus Maze (EPM), respectively. Ketamine group received %5 ketamine-shea butter

ointment and control group received the same total amount of vehicle ointment. The application was done on their trimmed dorsal back, twice daily for 2 days. Ketamine group showed twice less immobility in the FST while having no significant difference in the locomotor activity compared to the control group. The anxiety levels were similar for the groups as thigmotaxis and closed arm preference in the OFT and EPM, respectively. The results showed that 2-day application of 5% ketamine ointment ameliorates behavioral despair as a user-friendly and abuse deterrent method of sustained delivery.

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4 Cedric Stahl, Christian Thiel

Towards understanding the potential impact of ALG5 impairment on protein glycosylation and neurological abnormalities

Protein glycosylation refers to the attachment of sugar structures to proteins and lipids in various cellular pathways. It includes the addition of N-linked glycans, O-linked glycans, phosphorylated glycans, glycosaminoglycans, glycosylphosphatidylinositol (GPI) anchors and C-mannosylation to peptide backbones. Glycosylation of proteins occurs in the endoplasmic reticulum (ER) and the Golgi apparatus. In these organelles, glycosyltransferases and glycosidases form carbohydrate structures in a series of steps that are determined by factors such as substrate availability, enzyme activity, extent of gene transcription, and enzyme location within the organelles. Congenital defects affecting the synthesis or processing of glycan structures of glycoconjugates most commonly result in severe neurological defects termed "Congenital Disorders of Glycosylation" (CDG). ALG5 is a dolichylphosphate glucose synthase located in the ER and is essential for the activity of the glycosyltransferases ALG6, ALG8 and ALG10. To date, no ALG5 defects have been detected, but diverse ALG6 defects have been identified, all characterized by severe psychomotor retardation, seizures, speech disability, dysmorphic symptoms, among others. The aim of this work is to generate ALG5-deficient cells and to investigate the influence of ALG5 defects on protein glycosylation and metabolism.

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5 Marion M. Friske, Francesco Giannone, Mona Senger, Robin Seitz, Anita C. Hansson, Rainer Spanagel

Chronic alcohol intake regulates expression of SARS-CoV2 infection-relevant genes in an organ-specific manner

Chronic alcohol consumption and alcohol use disorder (AUD) have a tremendous impact on the patient's psychological and physiological health. There is some evidence that chronic alcohol consumption influences SARS-CoV2 infection risk, but the molecular mechanism is unknown. Here, we generated expression data of SARS-CoV2 infection relevant genes (Ace2, Tmprss2 and Mas) in different organs in rat models of chronic alcohol exposure and alcohol dependence. ACE2 and TMPRSS2 represent the virus entry point, whereas Mas is activating the anti-inflammatory response once the cells are infected. Across three different chronic alcohol test conditions, we found a consistent upregulation of Ace2 in the lung, which is the most affected organ in Covid-19 patients. Other organs such as liver, ileum, kidney, heart, and the brain showed also up-regulation of Ace2 and Mas but in a less consistent manner across the different animal models, while Tmprss2 was unaffected in all conditions. We suggest that alcohol-induced up-regulation of Ace2 can lead to an elevated stochastic probability of cellular virus entry and may thus confer a molecular risk factor for a SARS-CoV2 infection.

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6 Veronika Pohořalá, Thomas Enkel, Dusan Bartsch, Rainer Spanagel, Rick E. Bernardi

Sign- and goal-tracking score does not correlate with addiction-like behavior following prolonged cocaine self-administration

In classical conditioning, sign-tracking reflects an animals' interaction with a conditioned stimulus in expectation of a given reward (unconditioned stimulus, US), while goal-tracking describes an interaction directed toward the location of delivery of a US. As cues previously paired with drugs promote drug-seeking and taking behavior in animals and humans and thus contribute to the severity of substance abuse, sign-tracking may represent a maladaptive cue-focused behavior that may increase addiction vulnerability compared to goal-tracking. Recent studies support this possibility. Previous work in this area has focused primarily on paradigms using relatively limited exposure to drug. Here we used the DSM-IV-based 3-criteria (3-CRIT) model and examined whether a relationship exists between sign- or goal-tracking phenotypes and the prevalence of criteria associated with addiction-like behavior following extended cocaine self-administration as measured in this model. Forty-six male Sprague-Dawley rats underwent a Pavlovian conditioned approach (PCA) procedure and were characterized as goal-trackers (GTs), intermediates or sign-trackers (STs). The animals were subsequently trained to intravenous self-administer cocaine during 45 self-administration (SA) sessions and characterized for the 3 criteria outlined in the model: persistence of drug-seeking, motivation for cocaine taking and resistance to punishment. We performed correlational analyses on the traits measured, finding no relationships between PCA score and addiction-like characteristics measured using the 3-CRIT model. However, STs showed significantly greater resistance to punishment than GTs. Overall, PCA scores may not be a valid predictor for identifying addiction vulnerability in the 3-CRIT model, but may predict a single feature of the 3-CRIT model, resistance to punishment.

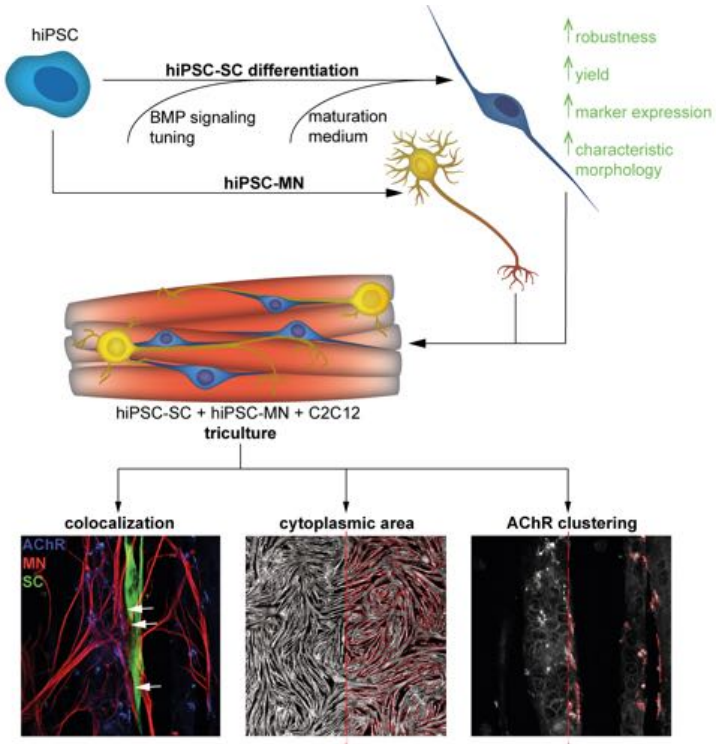
7 Nadine Winkler, Kritika Mittal, Joaquin Campos, Claudio Acuna, Katrin Schrenk-Siemens

Pluripotent stem cell-derived peripheral-to-central synapses: A microfluidic system to assess human nociceptive mechanisms in health and disease

Neurocircuits are involved in a multitude of body and brain functions such as motor behaviors and somatosensation. *In vivo* they are characterized by cell body compartmentalization and formation of defined synaptic connections between different neuronal subtypes. Synapses between peripheral nociceptors and central neurons play a pivotal role in conveying nociceptive information from the periphery to the central nervous system. To study the fundamental genetic, cellular, and synaptic mechanisms mediating physiological and pathological nociception in a human cell background, we derived peripheral nociceptors (iNocis), central glutamatergic neurons (iGluts) and GABAergic neurons (iGABAs) from human pluripotent stem cells using forced expression of specific combinations of transcription factors. We aim to reconstruct the formation of functional iNoci-to-iGlut synapses in a microfluidic device allowing spatial separation of neuronal subtypes. The development of neural circuitry is permitted by fine microchannels between the different compartments. We characterized the formation of peripheral-to-central synapses morphologically using advanced light microscopy, and physiologically using optogenetic tools for patch-clamp. We envision this system to be used as a tool to study cell-type specific neuromodulation under normal and genetically dysfunctional synaptic transmission.

8 Sarah Janice Hörner, Nathalie Couturier, Roman Bruch, Daniele Caroline Gueiber, Mathias Hafner, Rüdiger Rudolf

Improved differentiation of hIPSC-derived Schwann cells for neuromuscular tricultures



Neuromuscular junctions (NMJs) are tripartite synapses in the peripheral nervous system formed by motoneurons, skeletal muscle fibers, and terminal Schwann cells. Since they are affected in numerous neuromuscular diseases, a variety of NMJ *in vitro* models have been developed for mechanistic and pharmacological studies. To advance these models closer to the *in vivo* situation, efforts are made to increase their complexity in a relevant way by using human cells, creating specific 3D culture environments and spatial arrangements, and adding further cell types to traditional motoneuron / muscle cell cocultures. So far, Schwann cells have not been represented in NMJ cell models primarily due to technical limitations. We have established protocols to differentiate Schwann cells from human induced pluripotent stem cells (hiPSC) in a robust manner by tuning of BMP signaling during the differentiation, further maturation of Schwann cells in medium which is specifically composed to be compatible with neuromuscular tricultures, and to set up tricultures of hiPSC-derived Schwann cells and hiPSC-derived motoneurons in combination with murine C2C12 muscle cells or hiPSC-derived muscles cells. In 2D tricultures, we demonstrate colocalization of all cell types at sites positive for post-synaptic muscle acetylcholine receptor, and effects of cocultured cell types on myotube growth and receptor plaques. Furthermore, 3D-bioprinting and culturing methods are explored to selectively integrate hiPSC-derived Schwann cells into complete 3D neuromuscular cultures.

9 Nathalie Couturier, Sarah Janice Hörner, Daniele Caroline Gueiber, Mathias Hafner, Rüdiger Rudolf

Development of a pioneering isogenic 3D human model of NMJs *in vitro*

Skeletal muscle mediates voluntary movements thanks to specialized synapses called neuromuscular junctions (NMJs). As these control principal processes such as breathing, their functioning is of eminent physiological importance. Schwann cells, motor neurons, sympathetic neurons, and myofibers contribute to NMJs and an efficient crosstalk between these various cell types is key to NMJ formation, maintenance, and functioning. A variety of *in vitro* and *in vivo* model systems have been developed to uncover the processes occurring at NMJs in physiological and diseased conditions. Yet, each of these suffers from specific limitations. Rodent *in vivo* models have been used but translation to humans often remains critical. Self-organizing NMJ organoids recapitulate several NMJ features, but addressing cell-autonomous functions is difficult. Models based on human induced Pluripotent Stem Cells (hiPSC) have emerged, but these have lacked one or the other main actor of NMJs. Thus, we are currently developing a novel hiPSC-based 3D human model of NMJs, that will enable the critical crosstalk between Schwann cells, motoneurons and myofibers. So

far, hiPSC-derived muscle cells are able to cluster AChRs in “semi-3D” co-culture conditions either with motoneurons or Schwann cells. 3D culture conditions of hiPSC-derived muscle cells have been defined and allowed the maturation of myofibers embedded in a self-secreted extracellular matrix. The focus is drawn to define optimal culture parameters to co-culture the aforementioned 3D-muscular model with hiPSC-derived Schwann cells and motoneurons. Future research will attempt to make this 3D-NMJ model ready for the study of cell-autonomous behavior using hiPSC of healthy and diseased donors.

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10 Daniele Caroline Gueiber, Sarah J. Hörner, Nathalie Couturier, Mathias Hafner, Rüdiger Rudolf

Machine learning-based prediction of cell viability for 3D-bioprints

3D-bioprinting is a layer-by-layer approach of biological material deposition and has become a principal technology for the preparation of engineered organoids. However, due to the optical features of most hydrogels employed in 3D-bioprinting, it is hard to visualize the three-dimensional cell growth during the culture periods, which often comes with the uncertainty for weeks, whether a print had been successful or not. Often, only endpoint measurements using biochemical or fluorescence-based readouts discriminate positive from negative experiments, leading to an unacceptably long period of uncertainty. To improve this experimental gap, we aimed at establishing a machine-learning based approach that allows to use simple brightfield microscopic pictures to gauge the cell viability in 3D-bioprints. Therefore, a machine-learning based neural network was trained with corresponding brightfield images and cell viability data obtained from immunofluorescence-stained 3D-bioprints containing fibroblast cells.

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11 Bahardokht Tolou-Dabbaghian, Jing Chen, Melanie Motsch, Norbert Weidner, Radhika Puttagunta

The role of nociceptors and the α2-δ2 subunit of the voltage-gated calcium channel in spinal cord injury-induced neuropathic pain in mice

Following rodent spinal cord injury (SCI), below-level mechanical allodynia arises along with increased peptidergic nociceptors (Calcitonin gene–related peptide, CGRP+) labeling in laminae III-IV of dorsal horn, both of which are reversed by sensorimotor training. We aim to understand the role of this aberrant circuitry in SCI-induced mechanical allodynia. Pregabalin (PGB) blocks α2-δ subunit of voltage-gated calcium channels (VGCCs) and is commonly prescribed after pain development post-SCI. We believe the efficacy of PGB may increase if the window of administration is prophylactic. Here, we systemically administer PGB shortly after T11 moderate contusion in mice before the development of mechanical allodynia to examine if prophylactic use permanently blocks neuropathic pain development and structural rearrangements. C57BL/6J SCI female mice were treated with PGB (46 mg/kg, i.p., 2x/day) followed by assessment of mechanical and thermal sensitivity (von Frey and Hargreaves’ methods). PGB prevented mechanical allodynia and CGRP+ fiber plasticity in the laminae III-IV of L4-L6. To clarify if the mechanism of prophylactic PGB treatment is through blocking VGCC in nociceptors explicitly, we have utilized sensory neuron specific (Nav1.8) SNS-Cacna2d2$^{-/+}$ mice in which VGCC α2-δ2 subunit is knocked down (heterozygous) primarily in nociceptors. Over a 21-day period, we examined male SCI SNS-Cacna2d2$^{-/+}$ and Cacna2d2$^{fl/fl}$ (control) mice. Pre-operatively, SNS-Cacna2d2$^{-/+}$ mice exhibited lowered nociceptive responses. Strikingly, SNS-Cacna2d2$^{-/+}$ mice did not develop thermal or mechanical pain post-SCI. Moreover, place escape/avoidance paradigm (PEAP) revealed that SNS-Cacna2d2$^{-/+}$ mice do not develop mechanical allodynia. These results demonstrate the importance of the nociceptor population in conveying neuropathic pain following SCI.

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12 Danny Baltissen, Charlotte Bold, Marija Banicevic, Christian Buchholz, Ulrike Müller

Investigating the therapeutic potential of APPsα in a tau transgenic mouse model

Alzheimer´s disease (AD) is the most common type of dementia affecting about 5 % of adults above 65 years. AD is characterized by two major pathological hallmarks: senile plaques, mainly composed of Aβ peptides, and neurofibrillary tangles, consisting of hyperphosphorylated aggregates of tau proteins. Within the non-amyloidogenic processing pathway of the amyloid-precursor-protein (APP), the ectodomain APPsα is secreted. There is clear evidence, including studies from our group, implicating a therapeutic potential and a role of APPsα in neuroprotection against excitotoxicity, proteasomal stress and neurodegeneration not only *in vitro* but also when expressed by AAV vectors *in vivo*. Furthermore, APPsα has been shown to regulate two major tau kinases, GSK3beta and CDK5, in a positive manner. Based on these insights, it remains an important open task, to assess the therapeutic potential of APPsα in a transgenic mouse model (Thy.Tau22), showing tau-induced pathology restricted to the brain. For this reason, our project addresses the beneficial effects of intracranially injected AAV-APPsα in the Tau22 mouse model and uses mechanistic studies to identify targets and pathways regulated by APPsα.

13 Dominique Fäßler, Susanne Erdinger, Vicky Steubler , Michaela K Back, Susann Ludewig, Max Richter, Kang Han, Lutz Slomianka, Irmgard Amrein, Jakob von Engelhardt, David P Wolfer, Martin Korte, Ulrike Müller

Characterization of the APP gene family in the CNS

Alzheimer`s disease (AD) is the most prevalent neurodegenerative disease in humans. It is characterized by two hallmarks, Tau tangles and amyloid plaques, made up of aggregates of the amyloid precursor protein (APP). The key role of APP in the pathogenesis of Alzheimer disease is well established. However, the physiological function of APP and its family members APLP1/2 are not jet fully understood. In order to analyze the physiological function of the APP family, conditional triple KO (cTKO) were generated. These animals are fully, viable but showed altered brain morphology with agenesis of the corpus callosum and impaired lamination of the hippocampus. Further, electrophysiological recordings in the hippocampus of adult cTKO mice indicated a strong synaptic phenotype with pronounced deficits in the induction and maintenance of hippocampal LTP and impairments in paired pulse facilitation, indicating a possible presynaptic deficit, as well as severe behavioral abnormalities including cognitive decline and stereotypical- and impulsive-behavior traits. Moreover, *in vivo* calcium imaging of cortical neurons revealed an increase in the abundance of silent neurons which may disturb the excitation/inhibition balance. Here we used scRNAseq as well as nCounter experiment to determine the genetic makeup of our cTKOs in comparison to their corresponding littermate animals. Furthermore, follow up analysis were carried out by means of western blot and fluorescent in situ hybridization studies.

14 Lara Kilian, Susanne Erdinger, Ulrike Müller

Effect of AAV-mediated CTα16 overexpression on spine density in wildtype mice

In Alzheimer's disease, extracellular plaques consisting of Aβ, a product from proteolytic cleavage of the amyloid precursor protein (APP), are a major characteristic of this disease. However, there is increasing evidence that not only the excessive production of Aβ, but also the concomitant reduction of the soluble fragment APPsα that is generated with the alternative non-amyloidogenic pathway may contribute to AD pathogenesis. Counteracting this imbalance by increasing APPsα levels in the brain could rescue deficits in cognition and spatial memory in AD mice. Interestingly, APPsα and its C-terminal 16 amino acids (CTα16) enhanced cognitive performance even beyond the level of their littermates. To further investigate this nootropic property, an AAV-based vector coding for CTα16 was applied by intracranial stereotactic injection in wildtype C57BL/6J mice. The expression and secretion of CTα16 was quantified in hippocampus lysates by IHC and ELISA. Analysis of the dendritic spine density showed that, AAV-mediated overexpression of CTα16 did not increase dendritic spine density in the hippocampus of wildtype mice. We hypothesized that the effective concentration of CTα16 peptide was not met by the injected vector titer and therefore conducted an *in vivo* titration of AAV-CTα16. Compared to the already established vector AAV-APPsα, higher Venus and peptide expression was found. Quantitative analysis of CTα16 peptide concentration in animals injected with increasing AAV-CTα16 titers indicated a linear relationship between AAV-CTα16 vector titer and peptide expression and that an AAV-CTα16 vector titer of 1x109 gc/µl is sufficient to achieve similar molar concentrations of CTα16 in comparison to APPsα.

15 Lena Rehra, Verena Bengelsdorff, Danny Baltissen, Marija Banicevic, Ulrike Müller

Tamoxifen-inducible knockout mice of the APP gene family

The development of senile plaques is a key feature of Alzheimer's disease. These plaques are extracellular protein aggregates mainly consisting of the β-amyloid peptide (Aβ), which arises by proteolytic cleavage of the amyloid precursor protein (APP). While the role of APP as a precursor of Aβ is well established, its physiological functions remain elusive. APP is a member of a bigger gene family that includes the closely related APP-like proteins 1 and 2 (APLP1, APLP2). The function of these APP-related proteins and their regulation in the organism are also still unknown. While single APP family mutants are viable but without a strong phenotype, $APLP2^{-/-}APP^{-/-}$ and $APLP2^{-/-}APLP1^{-/-}$ double knockouts die shortly after birth indicating that APP family proteins serve redundant functions that are essential for viability. To circumvent early lethality of double knockouts and to analyze the role of the APP family regarding morphology and function of the nervous system as well as changes in behavior in a time-dependent manner, we generated Tamoxifen-inducible conditional knockout mice. Animals with floxed alleles ($APP^{flox/flox}APLP2^{-/-}$) were bred with the transgenic Cre-line ROSA26-CreERT2, which ubiquitously express the Cre-recombinase coupled to a mutated hormone binding domain of the estradiol receptor (ERT2). Therefore, Cre is only activated by introducing the synthetic ligand Tamoxifen, allowing a temporally regulated ubiquitous double knockout of APP and APLP2.

16 Marija Banicevic, Lena Rehra, Danny Baltissen, Zvi Menahem, Laura Keppler, Ulrike Müller

Generation and *in vitro* evaluation of APP secretion-deficient APP variants in primary cortical neurons

Amyloid precursor protein (APP) is the key player in Alzheimer´s disease development. It is transmembrane protein that is subject to complex proteolytical cleavage by a set of secretases, which gives rise of numerous extracellularly released

fragments. Both transmembrane APP signaling and secreted APP ectodomain fragments and are required for normal PNS and CNS function. Full length surface localized APP-FL may function as a receptor for different ligands and/or as a synaptic adhesion molecule (SAM). Some of the extracellularly released APP fragments (APPsα) support structural and functional synaptic plasticity, while the other ones (Aβ) express neurotoxic properties. Interdependence and regulation of APP functioning as a soluble ligand and as a SAM at the synapse *in vitro* and *in vivo* needs to be elucidated. In this work, several APP variants were cloned and evaluated *in vitro*. APPΔE1 lacks a domain which is responsible for trans-dimerization and SAM functions (adhesion-deficient variant). APPd8 and APPΔS622 have deletions around α-secretase cleavage site and therefore APPsα should not be generated (secretion-deficient variants). Corresponding AAV9 viruses were generated. AAV9s coding for secretion-deficient variants were used for transduction of $APP^{-/-}$ primary cortical neurons. It was shown that secretion of APPsα in secretion-deficient variants was almost completely diminished in neuronal cultures. In the future, AVVs will be injected into hippocampus and dentate gyrus of NexCre cDKO mice to assess whether acute application of APP variants lacking some domains can rescue impaired synaptic function, reduced spine density and neurite length and deficient hippocampus-dependent memory and learning that exist in these mice.

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17 Susanne Erdinger, Ulrike Müller

A psychedelic variation on Steubler, Erdinger *et al*. 2021

For underlying scientific data see Steubler, Erdinger *et al*. 2021 (EMBO J): Loss of all three APP family members during development impairs synaptic function and plasticity, disrupts learning, and causes an autism-like phenotype.

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18 Zvi Menahem, Marija Banicevic, Meike Fellenz Lea Humbs, Carolin Stoffer, Gundula Braun, Christian J. Bucholz, Thomas Deller, Ulrike 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-dependent 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, will enable us, in the future, to perform a detailed structure function analysis. Reconstituted OTCs, will be characterized with regard to neuronal morphology, spine density and electrophysiological properties.

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19 Debanjan Chowdhury, Beate Throm, Duncan MacLaren, Nina Beiber, Magdalena Schlesiger, Hannah Monyer

Identifying mechanisms involved in acute alcohol-induced amnesia

Acute alcohol intoxication can lead to impairment in the formation of episodic memories (memories of everyday events) and spatial navigation (the ability to navigate effectively). Hippocampal-parahippocampal regions support these processes. The network activity of these regions is coordinated by distinct GABAergic cells in the medial septum (MS). The effects of alcohol on these systems are largely unexplored. In this project, we aim to identify the neural pathways and mechanisms responsible for alcohol-induced memory deficits by combining optogenetics with *in vivo* electrophysiological recordings from the MS, Medial Entorhinal Cortex (MEC), and hippocampus (HC) in behaving mice. Administering alcohol intraperitoneally at a dosage of 1.5 g/kg led to a reduction in the firing rate of fast-spiking cells in the MS, MEC, and HC, with the strongest effect observed in the MEC. The firing rate of grid cells in the MEC also consistently reduced following alcohol administration. In contrast, while the majority of place cells in the HC had reduced firing rates, a subset of place cells had increased firing rates. At the network level, the LFP theta frequency in the MEC and HC decreased following alcohol administration. Stimulation of PV+ septal cells reliably paced the theta frequency in MEC and HC and stimulating CB+ septal cells recovered the alcohol-induced reduction in the firing rate of fast-spiking cells in the MEC. Thus, the present study investigates the neural circuits involved in alcohol-induced memory deficits and a possible way to restore these deficits.

20 Ivan Skorodumov, Merve Akan, Marcus Meinhardt

Efficacy of R-ketamine in rat models of alcohol addiction

Alcohol addiction, characterized by a compulsive desire to drink alcohol despite knowledge or evidence of its harmful consequences, affects about 23 million Europeans and creates a large health burden worldwide. Although substantial research has been done into possible therapies, currently available pharmacological treatments including disulfiram, naltrexone and acamprosate demonstrate limited efficacy. Over the last two decades, the fast-acting antidepressant effects of racemic ketamine were confirmed in many studies, resulting in FDA approval of its S-stereoisomer for the treatment of depression. However, the other optical isomer, R-ketamine, has recently been shown to induce a more potent beneficial effect on decreased dendritic spine density, BDNF–TrkB signaling and synaptogenesis, with less psychotomimetic side effects when compared to S-ketamine. In this light, the aim of our study is to assess the efficacy and safety of R-ketamine in two DSM-5-based models of alcohol addiction. Alcohol deprivation effect (ADE) model produces relapse-like drinking through repeated alcohol deprivation and reintroduction phases in single-housed rats having free access to alcohol-containing solutions in their home cage. Postdependent (PD) model targets negative affective state by intermittently exposing rats to alcohol vapor, which leads to the development of dependence, rats undergo operant training to self-administer 10% ethanol as a reward. We will use R-ketamine to determine its impact on alcohol relapse behavior and cue-induced reinstatement in male and female Wistar rats in both models of alcohol addiction. The results of ongoing experiments will be presented at the conference.

21 Marvin Urban, Tobias Buchborn

Psilocybin increases impact of aversion conditioning on alcohol self-administration in rats

Previous findings from our work group indicate that the mere application of psychedelics is not sufficient to disrupt alcohol self-administration in rats. In this study, we investigated whether the psychedelic psilocybin could facilitate aversion learning in rats trained to self-administer alcohol. This is meant to mimic the approach of combining psychedelics with some form of therapeutic intervention to treat addictions in humans. For four weeks, rats were trained in operant chambers to self-administer alcohol or water via lever-presses. After that, two and a half further weeks of daily operant chamber sessions followed, whereby the alcohol was adulterated with the bitter-tasting substance quinine, on the first two days of each week. 2h after each quinine-session, rats were injected with either psilocybin or saline. All animals showed a strong decrease of lever-presses on the days of quinine-challenge, compared to the mean in the last week of training. On the quinine-days, the saline-treated alcohol group continued to press significantly more often than the control group, trained with water, which highlights the hedonic value of alcohol. The psilocybin-treated alcohol group, however, showed the least lever-presses, pressing significantly less than their saline-treated counterparts. Also, aversion in these animals seemed to increase over time. These results indicate that psilocybin potentially increases the impact of aversion conditioning on alcohol self-administration. In a further step, markers of 5HT2a-related plasticity in samples from these rats will be examined by qPCR. If the results are generated timely, they will be included in the poster to back up the behavioral data.

22 Ann-Kristin Kenkel, José Ricardo Vieira, Christian Litke, Andromachi Karakatsani, Carmen Ruiz de Almodóvar, Daniela Mauceri

Role of the neurovascular unit and its molecular mediator LRG1 in persistent inflammatory pain

Persistent pain is sustained by maladaptive changes in gene transcription resulting in altered function of the relevant circuits. A prominent epigenetic mediator of adaptive processes in the central nervous system owing to their capacity to translate incoming synaptic activity into long-lasting transcriptional responses are histone deacetylases (HDACs). Recently, we found that long-lasting inflammatory pain cause nuclear export and inactivation of HDAC4 in neurons of the dorsal horn spinal cord. Expression of a constitutively nuclear localized HDAC4 mutant impaired the development of mechanical hypersensitivity but left acute and basal sensitivity unaltered. Next generation RNA-sequencing analysis revealed an inflammatory pain-dependent HDAC4-regulated gene program comprising known and novel mediators of sensitization including the Leucine Rich Alpha-2-Glycoprotein 1 (Lrg1). Lrg1 is known for being involved in signal transduction and endothelial dysfunctions. Using pharmacological and molecular tools to modulate Lrg1 expression, we determined that Lrg1 regulates mechanical but not thermal hypersensitivity. Further, we carried out a thorough structural and functional analysis of the neurovascular unit (NVU) and detected a transient opening of the blood-spinal cord barrier (BSCB) in persistent inflammation. Surprisingly, we additionally found that increased levels of Lrg1 were sufficient to compromise the tightness of the BSCB. Taken together, our results indicated a possible role of the NVU and its molecular mediators in the regulation of chronic pain.

23 Bahar Aksan, Jing Yan, Javier Sanchez-Romero, Dimitris Missirlis, Daniela Mauceri

Molecular mechanisms of structural maintenance and plasticity in neurons

Pathological changes of the dendrite architecture are hallmarks of many neurological disorders. Despite the fact that dendrites are mostly stable in adult neurons, little is known on the molecular mechanisms of dendrite maintenance and even less on its relation to structural plasticity. Previously, we identified Vascular Endothelial Growth Factor D (VEGFD) – an angio- and lymphangiogenic factor- as a crucial factor for the maintenance of dendritic morphology and the ability to form long-term memories. In neurodegeneration, such as stroke or in the excitotoxic retina, VEGFD expression is reduced and nasal or intravitreal delivery of VEGFD protects against stroke-induced damage or retinal ganglion cell death, respectively. The mechanisms of VEGFD-mediated dendrite stabilization are, however, not known. We now found that VEGFD acts like a molecular brake on neuronal morphology: normal expression of VEGFD maintains the dendritic architecture while VEGFD downregulation allows dendritic remodeling. We revealed that VEGFD stabilizes both actin and microtubules by performing atomic force microscopy as well as time-lapse live imaging in hippocampal neurons. Moreover, a phosphoproteomic screen identified several potentially VEGFD-regulated cytoskeleton-associated proteins. We functionally characterized the identified targets using gain of function and loss of function approaches. In addition, we monitored the VEGFD-regulated dynamics of dendrite structure using a machine-learning based algorithm for automatic dendrite segmentation in time-lapse series. Our study revealed the mechanisms of VEGFD-mediated dendrite maintenance and thereby contributes to our understanding of pathological dendrite aberrations.

24 Irina Meyer, Clement Verkest, Francisco J. Taberner, Stefan G. Lechner

PKA-dependent Modulation of PIEZO2

The mechanically activated ion channels PIEZO1 and PIEZO2 are of crucial importance for the detection and conversion of mechanical stimuli into biochemical signals in a variety of tissues. PIEZO2 has been shown to be modulated by several G-protein-coupled receptors (GPCRs) such as the P2Y2-receptor, the Bradykinin B2 receptor and GABAB receptor, as well as by direct activation of the classical GPCR downstream effectors Protein Kinase A and C (PKA, PKC). Here, we examined the molecular mechanism underlying PKA-mediated modulation of PIEZO2. To this end, we generated PIEZO2 channel mutants that lack PKA phosphorylation sites that were recognized by two different PKA-site prediction algorithms and that are conserved across all mammalian species. Strikingly, none of these PKA-sites appeared to be required for the PKA-dependent modulation of PIEZO2. We thus next examined PKA-mediated modulation of PIEZO2 mutants that lack entire intracellular domains and hence all possible PKA sites. These experiments revealed that the intrinsically disordered region 5 (IDR5), but no other intracellular domain, is required for the PKA-dependent modulation PIEZO2. IDR5 does not contain any predicted PKA phosphorylation site and we have previously shown that IDR5 is required for the activation of PIEZO2 by cytoskeleton-transmitted mechanical forces. Hence, we propose that PKA-dependent potentiation of PIEZO2 does not require phosphorylation of the channel and is probably mediated by an indirect cytoskeleton-dependent mechanism.

25 Annasara Artioli, Fabio Marsoner, Anne Hoffrichter, Julia Ladewig, Philipp Koch

Deciphering alcohol addiction-associated gene regulation changes on a single cell level

The harmful use of alcohol is a global problem causing 2.5 million deaths per year and accounts to the world's third largest risk factor for premature mortality, disability and loss of health. The acute and chronic exposure to the drug as well as cycles of abstinence contribute to gene regulatory changes and neuroadaptation in the brain. Although alcohol addiction poses major challenges to public health care systems, the medical needs of the patients are largely unmet and underlying neurobiological causes only poorly understood. In this context, induced pluripotent stem cell (iPSC)-derived brain organoids represent an attractive and innovative tool to decipher adaptive changes during disease onset caused by genetic or environmental challenges (including noxious substances) in a human setting and an unbiased forward approach. In this project, we developed a protocol to generate forebrain-type organoids from healthy controls and alcohol addicts. Cutting the organoids into 400 µm tissue slices results in an improved nutritive support and allows long-term cultivation. Sliced organoids have been cultured for up to 100 days, resembling late maturation stages of corticogenesis, with the appearance of the typical cortical layer markers in a spatially organized manner. In this project, we will perform single nuclei RNA sequencing (snRNA-seq) and snATAC-seq epigenetic profiling to monitor alcohol-induced changes in gene regulation and gene expression in an isogenic forward approach (non-exposed vs. exposed, acute, chronic intermitting, acute withdrawal). We expect that these experiments will help to define critical contributors in the pathogenesis of alcohol addiction, eventually leading to new therapeutic paradigms.

26 Marco T. Siekmann, Raquel Pérez Fernández, Martin Kubitschke, Lutz Wallhorn, Ammar Jabali, Anne Hoffrichter, Olivia Andrea Masseck, Philipp Koch, Julia Ladewig

Introducing human raphe-type organoid to model the role of serotonin on cortical development

Serotonergic neurons are located in the raphe nuclei of the hindbrain from where they project throughout the brain. Despite the well-known role of serotonin as a neuro-behavior modulator, emerging evidence point to an early function in the developing brain. However, the molecular effects of serotonin during early human brain development are poorly understood. In this context, human induced pluripotent stem cells (iPSC) as well as -derived cerebral organoids, which faithfully recapitulate certain aspects of early human brain development *in vitro*, have emerged as an attractive tool. By exposing apical and basal radial glia cell cultures to serotonin, we observed an enhanced proliferative activity. Moreover, the application of specific serotonergic receptor agonists and antagonists, revealed the respective receptor subtypes as mediators of this effect. We achieved similar results when targeting the progenitor pools in cortical organoids. Furthermore, we stablished raphe-like organoids through small molecule-driven modulation of signaling pathways like Wnt and SHH. Through the course of the differentiation, the progenitors display characteristic regional markers including HOXB2, NKX2.2 and FOXA2. Upon terminal differentiation, matured organoids display increased amounts of serotonergic neurons expressing serotonin. By applying the genetical encoded serotonin sensor DARKEN, we were able to detect release of serotonin in sliced cultures. When fusing raphe-type with forebrain-type organoids, we developed a model to study the projections of serotonergic neurons towards the cortical areas as we see also here serotonergic innervation in the cortical areas. Our hiPSC-derived, three-dimensional fusion model enables us to further decipher the role of serotonin in cortical development on the molecular level and open the possibility to study associated diseases.

27 Raquel Pérez Fernández, Marco T. Siekmann, Annasara Artioli, Philipp Koch, Julia Ladewig

Modelling reward and addiction: development of an *in vitro* reward neurocircuitry

The reward system is involved in the performance of key functions for the individual's survival and the continuation of species. It is well-known that the mesocortical and mesolimbic dopaminergic (DA) pathways that arise in the ventral tegmental area (VTA) and project to several brain regions, such as the cortex and ventral striatum respectively, are responsible for reward processing. Moreover, alterations in these pathways have been associated with the onset of severe pathological conditions like substance use disorder (SUD). However, research into the development of this system and changes leading to pathophysiological alterations, is limited due to the lack of a suitable model resembling human specific aspects. To overcome this limitation, we are using human induced pluripotent stem cells (hiPSCs) and thereof derived cerebral organoids. These three-dimensional (3D) models have been shown to faithfully recapitulate certain aspects of human brain development and associated neurological disorders *in vitro*. We developed a protocol for the generation of human midbrain-like organoids (hMLOs) with an enriched population of mesencephalic DA neurons (mesDA), some of them characteristic of the VTA. Moreover, we have differentiated organoids resembling the ventral striatum area containing GABAergic medium spiny neurons. Subsequently, we have fused these organoids with human cortical organoids (hCOs) to observed cell migration and mimic the development of mesocorticolimbic interactions. Thus, our pioneer work might be applicable to study the development of the reward system, and to model associated disorders by generating organoids derived from SUD-patient cells, or exposing the samples to addictive substances.

28 Amrita Das Gupta, Jennifer John, Livia Asan, Claudia Falfan-Melgoza, Carlo Beretta, Wolfgang Weber-Fahr, Thomas Kuner, Johannes Knabbe

Multimodal analysis of structural plasticity of cortical grey matter volume in chronic pain

Grey matter volume (GMV) changes due to chronic pain have been extensively studied in humans, yet the underlying neurobiological mechanisms are poorly understood. The goal of the project is to investigate the cellular underpinnings of GMV alterations in chronic pain and possibly provide a basis for novel strategies for prevention and therapy of this debilitating disease. In a longitudinal study design, a MRI-compatible chronic cranial window was implanted in mice expressing eGFP in all cell nuclei. *In vivo* imaging of nuclei allows inferences on tissue volume (distances between nuclei) and cell type composition (cell type-specific nuclear features) at different stages of chronic pain. Cell types were identified by a novel algorithm that includes PyRadiomics and deep learning. MRI imaging (voxel-based morphometry, diffusion tensor imaging) was performed in parallel to *in vivo* two-photon imaging of the anterior cingulate cortex (ACC) at different timepoints up to 12 weeks after the induction of chronic neuropathic pain with the spared nerve injury model (SNI). Additionally, behavioral paradigms were employed during acute pain and progression towards chronic pain. Our preliminary data show depressive and anxious behavior phenotypes in SNI mice. Furthermore, the imaging data revealed nuclei properties and tissue volume changes that significantly correlated with changes in behavior of SNI mice. In summary, the study established a novel multi-modal approach suitable to provide a more comprehensive understanding of the cellular mechanisms underlying changes in GMV caused by chronic pain.

29 Marina Ruth Hesse, Maja Klevanski, Steffen Sass, Thomas Kuner

Uncovering the molecular active zone nano-organization of the mammalian central synapse using multiplex 3D super resolution microscopy

Synapses transmit action potential-encoded information. The presynaptic active zone (AZ) mediates neurotransmitter release in four steps: Voltage-gated calcium channels at the AZ cause local calcium influx upon depolarization (1), Numerous AZ proteins are involved in docking and priming of synaptic vesicles (2), transsynaptic cell-adhesion molecules align pre- and postsynaptic membranes (3), presynaptic plasticity is mediated by the AZ (4). Membrane fusion of the lipid bilayer is mediated by v-SNARE Synaptobrevin and t-SNAREs SNAP-25 and Syntaxin1. Regulatory- and scaffolding proteins including Complexin, Muncs, RIMS, RIMBP, Liprins, Erc, NSF, α/β-SNAP, Piccolo and Bassoon recruit calcium channels and vesicles in a distinct spatial and temporal sequence to the plasma membrane of the AZ for mediating exocytosis. Precise information about the nano-architecture of these multiprotein complex forming the AZ and synaptic vesicle recruitment and recycling machinery is still unknown. However, to obtain a better understanding of synaptic communication, an extensive knowledge of protein nano-architecture is crucial. Direct stochastic optical reconstruction microscopy (dSTORM) is a super-resolution technique which achieves a lateral resolution of <25 nm and an axial resolution of ca. 50 nm. and can resolve the intracellular organization of AZ proteins. Combined with a multiplexing method, dSTORM allows the identification of more than 15 proteins within the same sample with a high resolution. Here, we performed multiplexed 3D dSTORM imaging of major AZ proteins in the calyx of Held to uncover the molecular nano-organization of proteins facilitating exocytosis in synapses.

30 Steffen Saß, Maja Klevanski, Thomas Kuner

The calyx of Held is targeted by external, vGluT 2-positive neurons that differ in active zone geometry and protein composition

The calyx of Held is a giant model synapse in the nucleus of the trapezoid body (MNTB), an essential component of the mammalian auditory pathway. It is located in the brain stem and relays the excitatory input of a single globular bushy cell from the contralateral ventricular cochlear nucleus (VCN) onto the glycinergic principal cell. Due to its important role in sound localization, it is equipped with hundreds of active zones (AZ) to ensure sustained, high-fidelity transmission. To achieve this robustness only a small subset of AZs is released in response to an action potential (AP) at any given time. The underlying release probability is dependent on multiple factors, e.g., AP propagation and the geometry of AZs or synaptic vesicle (SV) pools. At least three distinguishable SV pools have been suggested: (1) the readily releasable pool, which is immediately available for release upon stimulation, (2) the reserve pool, which is frequently recycled but not in direct contact with the AZ membrane yet, and (3) the resting pool, which is highly immobile and presumably not recruited under physiological conditions. SV pool identity is most likely attributed by molecular markers that still need to be uncovered. Using viral injections and super-resolution microscopy, we have identified additional non-calyceal projections targeting the principal cell that greatly differ from calyceal inputs in synapse geometry and protein composition (e.g., VAMPs and vGluTs). These findings suggest that release properties are linked to the abundance of distinct synaptic protein family members, potentially by influencing SV pool identity.

31 Elena Muñoz Perez-Vico, Thorsten Lau, Sandra Horschitz, Julia Ladewig and Philipp Koch

Implications of the Val66Met polymorphism of the BDNF gene on neuronal morphology and function using human iPSC-derived neuronal cultures

Brain-derived neurotrophic factor (BDNF) is involved in a multitude of processes that are important for brain development including neuronal survival, neurite outgrowth or synaptic plasticity. An estimated 30 - 50 % of the population is homozygous or heterozygous for a single nucleotide polymorphism (rs6265) in the BDNF gene which causes a substitution of valine (Val) to methionine (Met) at codon 66 in the pro-domain of BDNF (Val66Met). Animal overexpression models indicate that the modification of the BDNF protein impairs the intracellular trafficking, as well as activity-dependent release of BDNF. The polymorphism was also associated to psychiatric disorders such as schizophrenia or major depression. We set out to investigate the effects of the Val66Met polymorphism on neuronal network formation and function in human induced pluripotent stem cell (iPSC)-derived neuronal cultures generated from healthy donors homozygous for either the Val or Met variant of the gene. To account for the given genetic heterogeneity of humans, we additionally generated isogenic cells lines. IPSC were differentiated into homogeneous cultures of cortical neurons expressing cortical layer-specific transcription factors. When comparing axonal outgrowth from neuronal cultures we observed a significant reduction in neurite lengths in neurons derived from Met/Met carriers. This was accompanied by a significant reduction in the number of branching points. When investigating BDNF trafficking, we observed a decreased number of BDNF + vesicles on neurites of Met/Met carriers compared to Val/Val neurons. Additional experiments should decipher, in how far the polymorphism affects neuronal function and network activity. Together, our data give first experimental evidence that the exchange of the amino acid Val to Met in the human BDNF gene has measurable implications in human neuronal development.

32 Julia Wangemann, Anne Hoffrichter, Andrea C. Rossetti, Julia Ladewig, Philipp Koch

Human iPSC-derived microglia – towards modeling synaptic pruning-associated changes in schizophrenia

Schizophrenia is a heritable psychiatric disorder which affects approximately 1% of the world population. The disease is characterized by a range of symptoms such as hallucinations, lack of motivation and depression, as well as cognitive impairments. The most significantly associated genetic locus for schizophrenia identified in genome-wide association studies lies within the major histocompatibility complex region and involves structurally distinct alleles of the complement component 4 (C4) genes. Thereby, alleles leading to a higher expression of C4A were correlated to an increased risk for schizophrenia. The complement cascade has been shown to play an important role in microglia-mediated elimination of synapses during development. Given the reduced numbers of synapses in brains of individuals with schizophrenia, it has been suggested that excessive complement activity and synaptic pruning contributes to the development of the disease during late adolescence and early adulthood. In this project we set out to analyze synaptic pruning in an induced pluripotent stem cell (iPSC)-derived *in vitro* model. We established a differentiation protocol which allows the generation of human microglia-like cells in high purity. These cells express key microglia markers, they have a high phagocytic capacity and respond to pro-inflammatory stimuli. In order to investigate potential differences in synaptic pruning capacity and activity we generated a C4A overexpression cell line and established specific co-culture conditions with mature iPSC-derived cortical neurons. We expect that this experimental setting will allow to determine genotype-phenotype relationships with respect to synaptic pruning and C4A expression and will serve as a potential model for drug discovery.

33 Klara Franziska Rehder, Anne Hoffrichter, Julia Ladewig, Philipp Koch

Deciphering the role of osteocrin in the pathogenesis of schizophrenia

About 21 million people worldwide are suffering from schizophrenia (SCZ). This neuropsychiatric disease is a multifactorial disorder with high heritability that is characterized by hallucinations, lack of motivation and memory deficits. On the molecular level, an imbalance of neurotransmitters and changes in the content of synaptic proteins and receptors have been suggested to critically contribute to the pathogenesis of SCZ. However, the exact pathomechanisms underlying SCZ remain elusive. While osteocrin (OSTN) is mostly described for its role in bone growth as well as muscle endurance, a recent report showed that OSTN has been evolutionary repurposed to be expressed in the CNS of some primates including humans. Interestingly, OSTN expression is regulated by the transcription factor MEF2C in an activity-dependent manner and affects neuronal morphology, by reducing neuronal ramification. Whereas MEF2C has been associated with SCZ, a link between OSTN and schizophrenia has not been described. We identified a patient suffering from SCZ with a heterozygous deletion of chromosome 3q28 where the gene encoding for the peptide OSTN is localized. Neurons generated from human induced pluripotent stem cells (hiPSCs) from this patient are characterized by changes in neuronal morphology compared to the unaffected first-degree relative. Specifically, Sholl analysis of neurons differentiated for about 50 days *in vitro* show a significant higher level of neurite ramification compared to control neurons. In addition, we identified differences in the length and distance to the soma of the axon initial segment, indicating a different level of neuronal excitability. Our data link the expression of OSTN to neuronal morphology and function in schizophrenia patients' neurons and might qualify as a molecular target for therapy.

34 Malin Schmidt, Anne Hoffrichter, Mahnaz Davoudi, Sandra Horschitz, Thorsten Lau, Marcus Meinhardt, Rainer Spanagel, Georg Köhr, Julia Ladewig, Philipp Koch

The psychedelic psilocin fosters neuroplasticity in iPSC-derived human cortical neurons

The serotonergic plant-hallucinogen psilocybin is studied as innovative medication in anxiety, substance abuse and treatment-resistant depression. Animal studies show that psychedelics promote neuronal plasticity by strengthening synaptic responses and protein synthesis. However, the exact molecular and cellular changes induced in the patient's brain are not entirely understood. Here, we treated cortical neurons derived from human induced pluripotent stem cells with the psychoactive 5-HT2A receptor agonist psilocin. We analyzed pre - and postsynaptic markers, pathways related to neuroplasticity and 5-HT2A receptor localization. Acute exposure led to a decrease in axonal extracellular 5-HT2A receptor presentation which may indicate receptor complex formation or internalization. We further found the number of presynaptic BDNF, SV2A, Synaptophysin and postsynaptic PSD-95 puncta to be increased 24 hours after 10 µM psilocin exposure. Synaptophysin, BDNF, phosphorylated TrkB (activated BDNF receptor) and Akt (pro survival pathway) protein level was elevated as well. Modulation of the axon initial segment, reduction of resting state membrane potential and upregulation of activity-related immediate early genes, like cFOS were indicative for an altered excitability. PKC and vesicle invagination inhibition abrogate psilocin-induced BDNF increase suggesting a PKC - and endocytosis mediated process. Co-treatment with a selective 5-HT2A receptor antagonist blocked the BDNF puncta increase, indicating a receptor involvement. These data suggest that exposure of human neurons to psilocin might induce a state of enhanced neuronal plasticity. This neuroplasticity booster could explain why psilocin is beneficial in the treatment of neuropsychiatric disorders where synaptic dysfunctions are discussed.

35 Marc Schulz, Bruno Chausse, Fadi Almouhanna, Andrea Lewen, Oliver kann

Interferon-gamma induces lasting priming effects on microglia for several days

Priming of microglia (tissue-resident macrophages) with type II Interferon (Interferon-gamma, IFN-γ) is a complex immunological process leading to an exaggerated neurotoxic response upon a secondary inflammatory stimulus. The overreaction includes a high release of nitric oxide, loss of activity in the neuronal network, such as gamma oscillations, and neuronal death. However, the duration of microglial priming after removal of IFN-γ and effective strategies to alleviate the priming effects are widely unknown. Employing hippocampal slice cultures of the rat and serial exposure to IFN-γ (100 ng/ml, 72 h) and the bacterial cell wall component lipopolysaccharide (LPS, 100 ng/ml, 24 h), we report that the priming effects of IFN-γ on microglia resolve after around 10 days of IFN-γ removal. This is reflected by attenuation of nitric oxide release, lactate dehydrogenase activity (general marker of cell death) and tissue destruction. Our next steps in experimental planning include investigations of neuronal network functions after applying inhibitors of the IFN-γ-related signaling pathways leading to microglial priming.

36 Pirathitha Ravichandran-Schmidt, Joachim Hass

Computational modeling time perception and its dopaminergic modulation

Coordinated movements, speech and other actions are impossible without precise timing. Realistic computational models of interval timing are expected to provide key insights into the underlying mechanisms. Existing computational models of time perception have only been partially replicating experimental observations, such as the linear increase of time, the dopaminergic modulation of this increase, and the scalar property. In this work, we incorporate computational timing models, namely the state-dependent model (Buonomano, 2000) and the ramping activity model (Durstewitz, 2003) into a biologically plausible prefrontal cortex (PFC) model based on *in vivo* and *in vitro* recordings of rodents (Hass *et al*. 2016). We test whether any of these models can reproduce established experimental findings of time perception. For the state-dependent PFC model, we find all three timing properties faithfully reproduced. We show that the naturally occurring heterogeneity in cellular and synaptic parameters in the PFC is sufficient to encode time over several hundreds of milliseconds. Furthermore, we provide a mechanistic explanation for the origin of the scalar property as well as deviations from this law. The study of timing properties in the ramping activity PFC model is ongoing work. So far, linear timing works up to 2 seconds only when including the after-depolarizing current, which is dependent on calcium influx. We are currently in the process of testing the remaining properties and evaluating the underlying mechanisms.

37 Francesco Giannone, Magdalena Chrószcz, Marion Friske, Arian Hach, Wolfgang Sommer, Anita Hansson

Chronic alcohol exposure and posterior Dorsomedial Striatum inactivation induce increased habitual behavior in both operant conditioning and spatial navigation paradigms

Learning to automatize certain actions is essential for survival. On the other hand, maladaptive habitual responses have been hypothesized to underlie several psychiatric disorders including addictions. In habit formation, two principal steps can be distinguished: acquisition and consolidation of the behavior. Rodent studies have shown that these two phases are differentially controlled by two striatal brain regions: the posterior Dorsomedial Striatum (pDMS), mostly involved in goal-directed behavior, and the anterior Dorsolateral Striatum (aDLS), mostly involved in habitual behavior. Here we used two different approaches for studying habitual behavior in different learning paradigms: operant conditioning and spatial navigation. We tested such approaches on a well-established and validated animal model of Alcohol Use Disorder obtained via chronic intermittent alcohol vapor exposure and on controls. We finally analyzed the effects of a DREADD-mediated inactivation of the pDMS on the same paradigms. Our results show that rats with a history of chronic intermittent alcohol vapor exposure display generally higher habitual responses toward a natural sweet reward in both tested paradigms and that a similar effect is observed after DREADD-mediated inactivation of the pDMS.

38 Christian Schmitz, Lea Mertens, Moritz Spangemacher, Gerhard Gründer

Neural effects of psilocybin-treatment in patients with treatment-resistant depression

Background: Symptom remission is the desired goal of treatment for depression, albeit over 30% of the patients diagnosed with major depression show a treatment-resistant depression (TRD) (Rush *et al*., 2006). Given the paucity of innovative therapeutic approaches, psychedelic drugs produce interest in the scientific community. Recent clinical studies provide preliminary evidence for the efficacy and safety of psilocybin in the treatment of major depression (Carhart-Harris *et al*., 2021, Carhart-Harris *et al*., 2018, Davis *et al*., 2021). However, the neurobiological effects of psilocybin-treatment in TRD patients are insufficiently understood (reviewed by (Aday *et al*., 2020)). With this neuroimaging study, we want to investigate the neurobiological effects of psilocybin-treatment in TRD patients based on the EPIsoDE trial in order to fill this knowledge gap.
Methods: In order to investigate psilocybin-induced neural effects in TRD, we will perform functional magnetic resonance imaging at baseline (before intervention), 1 week and 6 weeks after treatment on 144 TRD patients of the clinical

EPIsoDE trial and 60 healthy subjects. The patients will be divided across three treatment arms treated with 25 mg psilocybin, 5 mg psilocybin or 100 mg nicotinamide. Our experimental approach will cover resting state analysis, but also important psychological concepts that potentially underlie the antidepressant effect of psilocybin. For this reason, we want to analyze how emotional processing (Hariri *et al*., 2002), reinforcement learning (Boehme *et al*., 2015, Deserno *et al*., 2020, Katthagen *et al*., 2020) and self-referential processing (Pankow *et al*., 2016) are affected under the treatment with psilocybin operationalized by task-based fMRI approaches.
Outline: With this controlled, randomized, double blind study design, we want to investigate neurobiological effects of psilocybin-treatment in TRD patients. The longitudinal placebo-controlled design and the comparison to healthy subjects allows the comparison of the remitted state with the healthy state.

39 Moritz Spangemacher, Manuela Brand, Laura Kaertner, Lea Mertens, Dennis Scharf, Christian Schmitz, Gerhard Gründer

Three case reports from the EPIsoDE study: A comparison

Background: In recent times, modern clinical trials of psilocybin have shown preliminary evidence for efficacy in treatment resistant depression (Carhart-Harris *et al*., 2016). The EPIsoDE study is a German phase II, randomized, bicentric, double-blind, active placebo-controlled parallel group trial to examine the efficacy and safety of psilocybin in treatment-resistant major depression. There are two dosing sessions and all patients receive at least one high/potentially therapeutic (25 mg) psilocybin dose (Mertens *et al*., 2022). Since the beginning of the study in July 2021, 50 patients have already been recruited and conducted in the trial. In this following draft we will present three different patients with very individual histories and vastly different outcomes. We will discuss learnings and implications for the treatment of future patients with psilocybin.
Methods: Three different case studies with individual patient history and their course over the study will be discussed. We compare their different outcomes by showing their HAM-D rating scores over the course of 12 weeks during the study. We will also compare their results in the follow-up six months after the end of the study.
Discussion: We will discuss different hypotheses, e.g., about the fast-acting antidepressant effects of psilocybin (Gründer *et al*., 2022), the importance of the acute experience (Roseman *et al*., 2018), the suspected mechanisms underlying the antidepressant effects of the treatment (Mertens & Preller, 2021) and resulting implications for the individual patient.

40 Emilio U. Isaías-Camacho, Jesús M. Martín-Cortecero, Alexander Groh

A cortico-collicular pathway for defense suppression

Animals and humans can quickly react to sudden events in their environment, i.e., orienting towards a friend or escaping from a threat. The superior colliculus (SC) is part of an evolutionary old brain structure producing orientation and defense movements to achieve these tasks. The central aim of this project was to test whether top-down cortical pathways can augment or suppress SC-mediated behaviors. To answer this question, we first looked at SC connectivity using an intersectional viral approach and observed extensive cortical innervation (cortico-collicular pathways) from the barrel field of S1 (BC) and the motor cortex (MC), as well as direct peripheral input coming from the brainstem (Bs). Within SC, general recipient neurons (RNs) and inhibitory (iRNs) were located laterally in the intermediate layers. More than 33% of all RNs were inhibitory, significantly greater than the general SC inhibitory/excitatory proportion of ~20%. By tracing axons from both RN types, we observed varicosities in thalamus, diencephalon, and brainstem. To investigate the functional role of specific RNs populations, I developed a behavioral paradigm to quantify SC-dependent defense behavior in mice, evoked by tactile stimulation of the mouse's whiskers and studied the ability of specific cortex-recipient SC neurons to modulate defense behavior. Mice displayed a significantly reduced defense behavior upon MC-iRNs activation. MC-RNs, on the other hand, tended to increase the animal's puff reaction, while BC- and Bs-iRNs and RNs did not modulate the defense behavior. Our study reveals that MC targets specific subpopulations in SC, which can bidirectionally modulate innate behavior.

41 Filippo Heimburg, Josephine Timm, Nadin Saluti, Matthias Klumpp, Martin Both, Thomas Kuner, Alexander Groh

A tactile discrimination task to explore context-dependent sensory processing and perceptual salience in freely moving mice

Functional neuronal ensembles in the posterior medial nucleus (POm) in the rodent whisker system (higher-order thalamus) are likely generated by motor, sensory and context-dependent variables such as whisking, locomotion or salience. Preliminary work suggest that POm specifically responds to tactile stimuli that are unpredicted and/or behaviorally relevant. Our goal is to understand the function of these "higher-order ensembles" in behavior. They could play a role in detecting salient stimuli and preparing an appropriate motor response. Therefore, we established a tactile discrimination task. Mice run between two lick ports, located at opposite ends of a linear maze which alternately dispense a reward (sweetened milk) from the two reward locations or display a punishment (noise). To reach the lick port, mice must pass through a narrowing in which two wings with variable distance from each other are presented to the whiskers. Each visit (= one trial) mice touch the wings and decide whether to lick or not. The paradigm consists of different training

phases in which the relevance and predictability of the whisker stimulus are systematically changed while thalamic ensemble activity is recorded via 64-channel tetrode arrays chronically implanted in the thalamocortical system. Current data shows that successful learning occurs in average after 15-20 sessions (= 8-10 days). The learning speed is strongly dependent on the number of trials per session. Over the complete period, unit activity is measured in four brain areas part of the whisker pathway (POm, ventral posteromedial nucleus (VPM), zona incerta ventralis (ZIv) and barrel field (BF)).

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42 Katharina Ziegler, Jan Burghardt, Ross Folkard, Antonio Gonzalez, Emilio Isaías-Camacho, Jesus Martin-Cortecero, Sailaja Antharvedi-Goda, Sanjeev Kaushalya, Linette Tan, Rohini Kuner, Rebecca Mease, Alexander Groh

Primary somatosensory cortex bidirectionally modulates nociceptive behavior in a layer-specific manner

The role of the primary somatosensory cortex (S1) in pain is debated. While it is widely accepted that S1 encodes sensory components of nociception, it has been difficult to delineate specific modulatory functions of S1 in nociception. In particular, layer-specific contributions of S1 circuits to the modulation of sensory sensitivity to innocuous and noxious input are underexplored. We found that activating corticothalamic neurons in layer 6 (L6) of mouse S1 hindlimb cortex drives spontaneous nocifensive behavior, causes hypersensitivity and aversion. Layer 6 downstream effects recorded with linear multi-channel silicon probes, revealed enhanced thalamic nociceptive responses in the ventro-posterior thalamus, and in parallel, strong suppression of layer 5 (L5) responses in S1, pointing towards an anti-nociceptive function of L5 output. Indeed, activation of L5 neurons reduced sensory sensitivity and normalized inflammatory allodynia and thus modulated nociception in the opposite direction as compared to L6. These results reveal a layer-specific and bidirectional role for S1 in modulating nociception.

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43 Jonas Schimmer, Stephanie Küppers, Julia Lebedeva, Marina Eliava

Oxytocin facilitates sexual behavior in male rats acting at the ventral hippocampus

The hypothalamic neuropeptide oxytocin (OT) modulates a plethora of socio-sexual behaviors. In this study we focused on OT signaling in the ventral hippocampus (vHippo), a central hub of social memory. In our preliminary experiments, implementing cell-type specific adeno-associated viruses (AAVs) we first found substantial innervation of the vHippo from the hypothalamic OT-ergic nuclei. Next, using a combination of OTR-IRES-Cre knock-in rats and viral-based vectors, we identified 3 types of neurons expressing OT receptors (OTR) in the vHippo: 1) Parvalbumin-positive GABA-ergic neurons, 2) Excitatory radiatum giant cells (RGS) and 3) A sub-population of pyramidal cells, residing in CA1 principal cell layer. External application of an OT agonist on acute vHippo slices resulted in generation of EPSCs in both types of OTR-expressing pyramidal neurons and simultaneous IPSPs in OTR-negative pyramidal cells. By chemogenetic activation of vHippo OTR expressing cells we found that male rats show higher interest in urine of their respective mating partner compared to urine of an unknown female in a reference-based olfactory hole-board test. In line, the male rats spent significantly more time sniffing female urine compared to a neutral odor. Our results suggest that OTR neurons of the vHippo facilitate male sexual behavior likely via their specific projections to other brain regions, which process female pheromonal signals.

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44 Stephanie Küppers, Jonas Schimmer, Valery Grinevich

Oxytocin improves positive emotional valence to painful stimuli via action in the anterior insular cortex

The hypothalamic neuropeptide oxytocin (OT) exerts not only prominent pro-social and anxiolytic effects, but has also garnered great attention due to its analgesic abilities. The anterior insular cortex (aIC) integrates pain processing and emotional valence and hence is considered as a target structure for OT action in pain conditions. In the present work we aimed to study the effects of endogenous OT release on pain perception and anticipation within the aIC. In order to investigate the underlying circuitry of OT action in the aIC, we employed cell-type specific adeno-associated viruses (AAVs) in combination with a transgenic OTR-IRES-Cre rat line and fluorescence-immuno-histochemistry. Thereby, we described direct axonal projections of hypothalamic OT neurons to the aIC, characterized OT receptor (OTR) expressing neurons of the aIC, and analyzed the intra-insular connectivity of OTR neurons and their long-range projections. To dissect the functional role of OT signalling within the aIC we performed two behavioral experiments while stimulating local axonal OT release utilizing chemogenetics. Firstly, rats with inflammatory pain were subjected to a conditioned place preference (CPP) test, where mechanical pain thresholds were measured simultaneously. Chemogenetic stimulation of OT axons significantly increased times spent in the chamber of the stimulation, while the mechanical pain threshold was not significantly changed. Secondly, animals were presented with the choice to self-administer a sugar solution paired with a light foot-shock in an operant conditioning setup. In line with CPP test results, evoked OT release significantly increased lever pressing behavior despite the simultaneously delivered electric shocks.

45 Francesco Scarlatti, Martin Löffler, Emanuel Schwarz, The IMAGEN Consortium, Herta Flor

A predictive neurosignature of the comorbidity between chronic pain and mood disorders

Mood disorders are frequently observed with chronic pain, with major depressive disorder being the first most common comorbidity and generalized anxiety being the second. The relationship between chronic pain and mood disorders is bidirectional and patients suffering from either condition are more likely to develop the other one than the general population. The comorbidity is associated with worse outcomes and poorer treatment response. Hence, there is a need to understand the mechanisms characterizing this comorbidity and to find biomarkers associated with it. The reward system is altered in chronic pain, depression, and anxiety, and it has been proposed that deficits in the different aspects of reward can underlie the development of both chronic pain and mood disorders. To test this hypothesis, we will analyze task-fMRI data of 1.000 subjects from the IMAGEN dataset, who performed a Monetary Incentive Delay (MID) task. The fMRI data collected at the age of 19 will be used to predict the level of pain, depression, and anxiety at the age of 22, assessed through self-reported questionnaires. Multi-Task Learning will be used to train a model that can contemporarily predict both pain and depression/anxiety. This method will allow us to find the specific neurosignature of the comorbidity, which will be cross-validated on another dataset (CBP-PREDICT) containing chronic pain patients. We will focus on brain regions associated with reward, trying to understand if reward-associated variables can be used to characterize the comorbidity between chronic pain and mood disorders.

46 Maximilian Penzkofer, Susanne Becker, Christian Schmahl, Herta Flor

The relationship of adverse childhood experiences and violent video gaming: effects on pain perception, fear conditioning and pain-related empathy

The video gaming industry counts as one of the fastest growing industries and it grew even faster in the past years due to lockdown measures of the COVID-19 pandemic. 2020 the annual growth was set at above 9% amounting to a 159-billlion-dollar revenue. Violent video games (VVG) are a big factor why this industry got so big over the years. Few research has been done so far to get more insight on violent video gaming and psychological conditions like adverse childhood experiences (ACE). ACEs are a very common burden and stress factor in our nowadays society and can lead to a huge variety of mental and somatic disorders as well as a massive increase in suicide rate. Therefore, this project aims to clarify the connection between VVG and ACEs in terms of pain experience and empathy for pain in others. First results show that violent video gamers endure significantly more pain than nonviolent video gamers or nongamers. Some types of ACEs like neglect and abuse seem to moderate this connection. Further results in terms of brain imaging are still in progress. The first results of the project imply that it would be beneficial for future trauma therapy to look into videogaming behavior of patients when designing exposures or rating empathy capabilities.

47 Viktoria Greeck, S Williams, Ricarda Diem, Hilmar Bading, Richard Fairless

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

Multiple Sclerosis (MS) is a chronic autoimmune disease of the central nervous system characterized by acute inflammatory attacks as well as slow primary and secondary neurodegeneration in the grey and white matter. Although a range of immunomodulatory treatments exist, neuroprotective therapies are currently lacking. Glutamatergic excitotoxicity has been proposed as a contributing factor to pathophysiological processes in MS and its animal model, experimental autoimmune encephalomyelitis (EAE). However, the precise role of glutamate receptors on neurons and non-neuronal cells of the immune system and blood-brain barrier in MS/EAE remains unclear. In this project, we aim to assess the contribution of the NMDA receptor to EAE disease progression in the C57BL6 mouse model. Specifically, the neuroprotective potential of the novel therapeutic agent FP802 will be assessed. FP802 has been shown to disrupt NMDAR-mediated excitotoxicity in neurons, while leaving physiological glutamate signaling intact, through disruption of a newly characterized NMDAR-TRPM4 death complex. So far preliminary results indicate that while therapeutic FP802 treatment (from disease onset) did not significantly affect disease course, prophylactic FP802 treatment (prior to disease induction) significantly delayed disease onset and reduced disease severity. Retinal ganglion cell degeneration, a commonly observed early event in EAE, was not significantly reduced in FP802-treated animals. Immunohistochemical assessments of demyelination, axonal injury and immune cell infiltration of the optic nerves and spinal cord are currently ongoing. Collectively, FP802 treatment can have a positive impact on EAE disease progression, but the identity of the cellular targets are subject to on-going investigation.

48 Nadja Lehmann, Stefan Markovic, Christian Thome, Maren Engelhardt

Development of AcD neurons in the murine hippocampus and primary somatosensory cortex

Cortical principal neurons receive synaptic input at their dendrites and convey them to the soma. After integration, the axon initial segment (AIS) initiates action potentials. Classically, the axon emerges from the soma. However, in axon-carrying dendrite (AcD) neurons, the axon originates from a basal dendrite resulting in potentially distinct functional

implications. Although AcD neurons have been described in numerous cortical regions and across species, their developmental profile remains largely unknown. Therefore, this project focuses on the early maturation profile of AcD neurons in comparison to nonAcD neurons in the murine ventral hippocampus (vHC) and primary somatosensory (S1) cortex. Parameters include the occurrence of both subtypes across numerous ages, as well as the maturation of AIS-specific features (e.g., expression of voltage-gated sodium channels and the emergence of GABAergic axo-axonic synapses (AAS)). Data show that in vHC, the number of AcD neurons steadily increases until P28, while in S1, AcD neurons peak at P16. AcD neurons show shorter Nav1.6 channel distribution than nonAcD neurons in both brain regions. Live-cell imaging in cortical slice cultures reveals a striking morphological dynamic in pyramidal neurons, which transform from one type to the other during development. Preliminary data also indicate that the development of AcD neurons has an activity-dependent component. In summary, our data imply that entire cells exhibit morphological plasticity, the regulating mechanisms of which remain to be studied *in vivo*.

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49 Isabella Boccuni, Andreas Draguhn, Claus Bruehl, Richard Fairless

Contribution of ambient glutamate and glutamate transporters to retinal ganglion cell vulnerability in experimental multiple sclerosis

Glutamate transporter (GluT)-mediated neurotransmitter uptake allows for low, but relevant, ambient glutamate levels in neuronal tissue which can activate extrasynaptic NMDARs (esNMDARs). In pathological situations, esNMDARs have been shown to be major initiators of neurodegeneration. Here we analyzed the regulation of ambient glutamate around retinal ganglion cells (RGCs). These cells express consistent pools of esNMDARs, and are vulnerable during early stages of multiple sclerosis, possibly triggered by tonic excitation of esNMDARs. Patch clamp electrophysiology was performed on rat retinal whole-mount preparations to study αON- and αOFF-RGC subtypes, since we previously demonstrated αOFF-RGCs to be more vulnerable than αON-RGCs to degeneration during experimental autoimmune encephalomyelitis (EAE), a disease model of multiple sclerosis. αRGCs were classified according to their ON/OFF light responses and ambient glutamate-induced tonic currents were measured in both healthy and EAE conditions. Under these conditions, tonic currents were observed in both αON and αOFF RGC subtypes, which were partially blocked by application of the NMDAR antagonist MK-801. Perfusion of the retina with TBOA, a pan-GluT inhibitor, induced an increase in the tonic current of between 50-150-fold. However, both the magnitude of the TBOA-induced currents and the relative esNMDAR-mediated components were different between αON/OFF-RGC subtypes in healthy and EAE conditions. These data suggest the physiological presence of ambient glutamate around αRGCs. Impaired GluT function under neuroinflammatory conditions may lead to an increase of ambient glutamate causing over-activation of esNMDARs. A differential proportion of activated NMDARs between the αON/OFF-RGC subtypes could underlie their type-specific vulnerability in multiple sclerosis.

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50 Märt Rannap, Shinya Ohara, Menno P. Witter, Andreas Draguhn, Alexei V. Egorov

Structural and functional organization of the hippocampal-medial entorhinal output circuit

The medial entorhinal cortex (MEC) constitutes a major interface between the hippocampus and neocortex. Multimodal sensory information enters the hippocampus via superficial MEC layers (LII and LIII) while deep layers (LVa, LVb and LVI) receive the processed hippocampal output. The MEC deep layers are functionally distinct, with LVa containing telencephalically projecting, LVb locally projecting and LVI hippocampally back-projecting neurons. How hippocampal afferents innervate the individual deep layers along the longitudinal axis remains largely unknown. Previous studies have primarily investigated dorsal hippocampal-MEC LV projections, revealing a strong preference for LVb. As intrinsic LVb-to-LVa connections in MEC are sparse, this questions the efficiency of hippocampal information transfer to LVa and thus the neocortex and thereby challenges current understanding on memory consolidation. Using anterograde tracing and *in vitro* electrophysiology in mice, we systematically examined projections from both the dorsal and ventral hippocampus to MEC throughout its dorsoventral axis. Our data reveal profound differences in the respective connectivity patterns. First, dorsal hippocampal projections are confined to dorsal MEC where they preferentially target LVb over LVa as previously reported. In contrast, ventral hippocampal projections extend throughout the dorsoventral axis of MEC, innervating all three layers with similar strength in ventral but mainly LVa and LVI in dorsal MEC. These data indicate different and largely complementary interactions of dorsal and ventral hippocampal outputs with MEC circuits along the dorsoventral axis. They further implicate the ventral hippocampus in regulating LVa-mediated entorhinal-neocortical outputs from both dorsal and ventral MEC which likely plays an important role in memory consolidation.

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51 Nikolas Stevens, Andreas Draguhn, Martin Both, Christian Thome

Mouse CA1 pyramidal cells with dendritic axon origins receive specialized interhemispheric input at their basal dendrites

A large fraction of hippocampal pyramidal cells have axons emerging from a basal dendrite rather than from the soma. We have previously shown that axon-carrying dendrites (AcDs) constitute a privileged pathway for action-potential

generation. Here, we studied dendritic features and the input connectivity of CA1 pyramidal cells in the adult mouse hippocampus. We found considerable variability between hippocampal sub-regions ranging from 70% AcD-morphology in the central CA1 of medial portions of the hippocampus down to 5% in dorsal CA1. The total size of basal or apical dendritic trees was not different between the two cell types. However, AcDs are larger than nonAcDs. To assess the innervation of AcD and nonAcD cells by CA3 neurons, we used optogenetic stimulation of virus-targeted presynaptic fibres combined with patch-clamp recordings. Inputs from contralateral CA3 onto the basal dendritic tree elicited stronger excitatory postsynaptic currents and potentials in AcD cells compared to nonAcD neurons. We verified that this region shows selectivity for *stratum oriens* in which basal dendrites reside. Stimulation targeting apical dendrites in *stratum radiatum* showed no differences between the two cell types. Similarly, inputs from ipsilateral CA3 and CA2 were not different. We also found that CA3 axons build more putative synapses with AcD cells and have an additional preference for AcD branches. In summary, our data show that synaptic connectivity is distributed asymmetrically between cells with and without a privileged dendritic input branch. This further demonstrates the specialized role of cells with dendritic axon origins within the hippocampal network.

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52 Christopher Koch, Katja Bauer, Francesco Ciccolini

Investigating HES1 as a neural stem cell activator

Apical and basal neural stem cells (NSCs) in the ventricular-subventricular zone (VZ-SVZ) are the origin of adult-born olfactory bulb interneurons, which in rodents play critical roles in olfaction and the olfaction-dependent behavior. Recently, the relationship between apical and basal NSCs were more closely investigated, revealing functional differences between the two stem cell niches. Specifically, that apical NSCs act as proliferation gatekeepers by regulating Notch signaling in the postnatal VZ-SVZ. Here I sought to further elucidate the signaling involved in apical and basal NSC activation. Through the use of *ex vivo* VZ-SVZ primary cell tracking, pharmacological inhibition of Notch signaling via Dapt, and inhibition of Notch target gene Hes1 via infection with adeno-associated virus (AAV) encoding for Hes1 shRNA, I found Hes1 to be a significant player in promoting cell division in basal NSCs.

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53 Katja Baur, Carmen Carrillo García, Şeydanur Şan, Gabriele Hölzl-Wenig, Claudia Mandl, Francesca Ciccolini

Growth/differentiation factor 15 controls apical niche homeostasis in the developing SVZ

At late embryonic ages, the expression of growth/differentiation factor (GDF) 15 increases in the germinal epithelium of the murine ganglionic eminence (GE). However, the function of GDF15 in this region is unknown. We here show that absence of GDF15 leads to an increase in proliferation of apically and subapically dividing progenitors in the GE. This is associated with faster cell cycle progression in both progenitor groups, and an increase in the total number of cycling progenitors. Enhanced proliferation of apically dividing precursors leads to a permanent significant increase in the number of ependymal and neural stem cells (NSC). In contrast, the extra proliferation of subapically dividing progenitors leads to a transient increase in the number of neuronal progenitors, which seem to be compensated by apoptosis. Application of exogenous GDF15 rescued the effect of the genotype on the expression of EGFR and decreased proliferation in the mutant GE. Taken together, our results indicate that GDF15 affects proliferation and growth factor responsiveness of progenitors in the developing GE which leads to a permanent change in the number of ependymal and NSCs.

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54 Nadja Sharkov, Matthias Klumpp, Nikolas Stevens, Christian Thome, Janina Kupke, Andreas Draguhn, Ana Oliveira, Martin Both

Different involvement of axon-carrying dendrite versus canonical neurons during learning processes

The hippocampus is important for the formation of declarative memories. It generates distinct network oscillations, during which functional ensembles are specifically activated. The formation of coherently active ensembles requires integration of multiple synaptic inputs within single neurons. According to current understanding, dendritic excitatory synaptic potentials are integrated at the soma which is directly connected with the axon. Signal flow to the axon can be blocked by perisomatic inhibition which is particularly active during network oscillations. Recently, we have shown that in about 50% of hippocampal CA1 pyramidal neurons the axon emerges from a basal dendrite (AcD, ‘axon-carrying dendrite’). This particular dendrite is largely independent from somatic signal integration and can efficiently convert excitatory inputs into APs, even under conditions of strong perisomatic inhibition. We therefore hypothesize that AcD cells are more active during states of strong perisomatic inhibition. Based on this mechanism, AcD and canonical cells might be differently involved in the formation and consolidation of episodic memory. To test this hypothesis, we trained mice on a spatial memory task (m-maze). Active neurons are expected to express immediate early genes (e.g., cFos), and can be identified by *ex vivo* staining. Additional, staining of the axon initial segment enabled us to sort task-activated neurons into AcD and canonical cells. Interestingly, nonAcD cells have a two times stronger expression of cFos than AcD after 7 days of training. While the underlying mechanisms are presently unclear it does, however indicate distinct roles of AcD and non-AcD cells during formation and consolidation of memory.

55 Dorothea Schall, Claudio Acuna, Gudrun A. Rappold, Simone Berkel

Investigation of KCNQ1 function in human neurons with a focus on insulin signaling

KCNQ1, a component of a voltage-dependent potassium channel, has been linked to disorders with impaired insulin signaling (so-called insulinopathies) regarding the soma and the brain. However, the function of KCNQ1 in the human brain is unknown. In this project, we aim to elucidate the function of KCNQ1 in human neurons, with a focus on insulin signaling. It is already known that KCNQ1 inhibits insulin secretion in the pancreas. Furthermore, the KCNQ1 gene has been linked to insulinopathies like type 2 diabetes, obsessive compulsive disorder, and Alzheimer's disease. For this reason, we will use human induced pluripotent stem cells (hiPSCs) as a model system, and differentiate them into neuronal stem cells (NSCs) and cortical neurons. We have already established a neuronal differentiation protocol and analyzed KCNQ1 expression in hiPSCs, NSCs, and neurons. All analyzed cell types show KCNQ1 expression. To elucidate KCNQ1 function, we generated KCNQ1 knockout hiPSC lines using CRISPR/Cas9 genome editing. In the next step these hiPSCs will be differentiated into NSCs and further into cortical neurons. The KCNQ1 knockout cells will be compared to isogenic control cells by analyzing different cell properties, the transcriptome, protein expression and phosphorylation of members of the insulin signaling pathway, and the neuronal function. Elucidation of the function of KCNQ1 in the brain will advance our knowledge of insulin signaling, and potentially also the molecular details of certain neuropathologies.

56 Calvin Thommek, C. Peter Bengtson, Hilmar Bading

The role of excitatory amino acid transporters in excitotoxicity

The tight regulation of glutamatergic signaling including all its components is essential to maintain physiological balance between excitation and inhibition in the central nervous system playing an important role in the prevention of excitotoxicity. Particularly, the clearance of glutamate by excitatory amino acid transporters (EAATs) from the synaptic cleft after action potential mediated glutamate release is crucial to prevent the over-activation of postsynaptic glutamate receptors, such as NMDA receptors. Prolonged activation of NMDA receptors due to increased levels of extracellular glutamate was shown to induce death-signaling pathways eventually leading to neuronal loss and neurodegeneration. Elevated levels of extracellular glutamate and dysfunction of the glutamatergic system are associated with several neurological pathologies such as Alzheimer’s disease or epilepsy, but also with psychopathologies such as schizophrenia and bipolar disorders. In particular, the two glial glutamate transporters, EAAT1 and EAAT2, seem to play a key role in the the removal of glutamate form the synaptic cleft, while the role of the neuronal EAAT3 is still poorly understood and remains to be discovered. In this study, we aim to use whole-cell patch clamp recordings of hippocampal CA1 neurons in acute brain slices to investigate the role of different transporters in the context of NMDA receptor mediated excitotoxicity and how different subtypes are contribute to glutamate clearance. In particular, we try to examine to what extent the neuronal glutamate transporter EAAT3 is involved, which has so far been overshadowed by glial glutamate transporters EAAT1 and EAAT2.

57 Nikolaus Goessl, Anna M. H. Hertle, Kristina Battis, Wojciech Ambroziak, Sebastian Marty, Jan Siemens, Hilmar Bading

NPAS4 in Chronic Pain

The nervous system detects and processes a wide range of mechanical and thermal stimuli that can generate acute pain. In the setting of persistent nerve injury or inflammation, the nervous system can undergo lasting plastic changes that result in increased pain signals and hypersensitivity, generating a chronic pain state. Changes in the excitatory-inhibitory balance of networks of the dorsal spinal cord have been shown to play an important role in the chronification of pain. However, our understanding of this dysregulation at the network level remains incomplete. The activity-dependent transcription factor NPAS4 has been shown to be crucial for maintaining the excitatory-inhibitory balance in neuronal networks of the central nervous system. In the spared nerve injury (SNI) model of neuropathic pain, we found, contrary to other immediate early genes, NPAS4 expression in the dorsal spinal cord is decreased. To further investigate the role of NPAS4 in chronic pain, we employed recombinant adeno-associated viral vectors to overexpress NPAS4 in the L4-L5 segments of the mouse dorsal spinal cord. Both in the neuropathic SNI model and a CFA-induced inflammation model, NPAS4 overexpression resulted in a clear decrease in mechanical hypersensitivity. To determine whether NPAS4 expression similarly rescued changes in inhibitory synaptic transmission, we performed electrophysiological recordings to measure the frequency and amplitude of miniature inhibitory synaptic currents in the spinal cord dorsal horn. Altogether, our data suggest that decreased NPAS4 levels in models of chronic pain lead to impaired inhibitory neuronal signaling, therefore increasing mechanical hypersensitivity, and that gene therapeutic approaches targeting NPAS4 signaling pathways may be effective for ameliorating or preventing chronic pain phenotypes.

58 Silvia Gleitze, Pedro Lobos, Andrea Paula-Lima, Cecilia Hidalgo

Iron chelation and ryanodine receptor inhibition offer protection against ferroptosis in primary hippocampal neurons

Introduction: Ferroptosis, a newly described cell death pathway, is characterized by iron-mediated lipid peroxidation, decreased levels of the intracellular antioxidant glutathione and altered mitochondrial morphology. Inhibition of glutathione peroxidase 4 (Gpx4), a key antioxidant enzyme that reverses lipid peroxidation, promotes ferroptosis in different cell types, albeit no information is available regarding ferroptosis in primary hippocampal neurons. Moreover, the role of Ca^{2+} signaling in ferroptosis, and in particular the contribution of Ca^{2+} release from the endoplasmic reticulum (ER), have not been reported. Aims: We propose that Gpx4 inhibition induces ferroptosis in primary hippocampal neurons, and that the dysregulation of the cellular redox state observed in ferroptosis perturbs Ca^{2+} signaling through excessive activation of ryanodine receptor (RyR) channels. Material and Methods: Primary hippocampal neurons were treated with a selective Gpx4 inhibitor (by RSL3) and ferroptosis was characterized by analyzing cell viability, cell morphology and lipid peroxidation. The effects of iron chelation by deferoxamine (DFO), and RyR inhibition by ryanodine were evaluated. Results: Incubation with RSL3 (15 μM) for 24 hours induced 50% cell death, dendritic damage and lipid peroxidation which was reduced by previous incubation with DFO. Moreover, suppression of RyR activity offered partial protection in RSL3-treated hippocampal neurons. Conclusion: Gpx4 inhibition induced ferroptosis in primary hippocampal cultures, as evidenced by the protection offered by the iron chelator DFO. Suppression of RyR activity mitigated the effects of Gpx4 inhibition, indicating that Ca^{2+} contributes to ferroptosis in some neurodegenerative diseases.

59 Zihong Zhang, Jing Yan, Celia Garcia Vilela, Anna M. H. Hertle and Hilmar Bading

The role of calpain in the degradation of the NMDA receptor and neurotoxicity

N-methyl-D-aspartate receptors (NMDARs) are glutamate-gated calcium-permeable channels that are composed of two GluN1 subunits and two GluN2 subunits. The NMDAR-dependent excitotoxicity depends on the location of NMDAR: stimulation of synaptic NMDARs results in the formation of a neuroprotective ‘shield’, whereas stimulation of extrasynaptic NMDARs promotes cell death. Recently, our group has proved that Transient receptor potential (TRP) melastatin 4 (TRPM4) is a cation channel that is only expressed at the extrasynaptic site and can bind to the NMDA receptor which we called NDMAR/TRPM4 complex leading to cell death. We found that activation extrasynaptic NMDARs participate in the degradation of GluN2A and GluN2B subunits, but not GluN1 subunits and TRPM4. Additionally, only activated synaptic NMDARs, the protein expression of GluN1, GluN2A, GluN2B, and TRPM4 has no changes. Here, we propose the existence of a third protein that can only lead to GluN2A and GluN2B degradation when activated extrasynaptic NMDARs and focus on m-calpain as a candidate. Calpains are a family of non-lysosomal cysteine protease. In the brain, m-calpain is activated downstream from extrasynaptic NMDARs, leading to neurotoxicity. We found that m-calpain inhibitor can inhibit the degradation of GluN2A and GluN2B. Additionally, we found disrupting the NMDAR/TRPM4 complex can lead to a decrease in the calpain-mediated generation of breakdown products (BDPs) targets of proteins such as fodrin. These findings suggested that the regulation between calpain and the NMDAR/TRPM4 complex plays an important role in NMDA-mediated neurotoxicity.

60 Rowena Groeneveld, Beate Throm, Kevin Allen

Object-vector cells in two mouse models of Alzheimer’s disease

A population of neurons in the medial entorhinal cortex encodes the distance and direction of objects relative to an animal's position. These cells, referred to as object-vector cells, are found in the same brain area as grid cells and speed cells. Whereas spatial coding by grid cells and speed cells is impaired in mouse models of Alzheimer’s disease (AD), object-vector coding has yet to be investigated in these models. The aim of this project is to test whether object-vector coding is impaired in two mouse models of AD, namely the Tau P301S and 5xFAD models. We recorded the activity of neurons from the medial entorhinal cortex of Tau P301S, 5xFAD, and control mice using silicon probes. Recordings were performed while mice explored an open-field environment containing an object that was moved between two locations. We established a procedure to quantify object-vector coding which involves calculating object-centered firing rate maps. In object-vector cells, the position of the cell firing field relative to the object remains stable when the object is moved in space. We will now apply this procedure to our dataset to quantify object-vector coding in Tau P301S, 5xFAD, and control mice.

61 Ilknur Coban, Rangel Leal Silva, Annika Wenzel, Manuela Simonetti, Amit Agarwal

Alpha2a adrenergic receptors on astrocytes regulate neuropathic pain

In response to a peripheral nerve injury, astrocytes exhibit aberrant Ca^{2+} signaling, which leads to excessive release of synaptogenic factors and induces rewiring of neural circuits of pain. In astrocytes, activation of adrenergic receptors

induces Ca^{2+} signals, and the drugs (e.g., dexmedetomidine, Dex) that activate alpha 2 adrenergic (Adra2) receptors have an analgesic effect. We want to investigate whether Adra2 receptors are expressed by astrocytes, and do these receptors modulate chronic pain? First, histological analysis on mice expressing LacZ reporter under the control of Adra2a promoter showed that astrocytes express Adra2a receptors. Second, we imaged Ca^{2+} signals and observed that bath application of 1µM Guanfacine, a sub-type specific Adra2a receptors agonist, led to an increase in Ca^{2+} signals in astrocytes. Third, we generated a novel Adra2a floxed mouse line, and crossbred them to mGFAP-Cre mice to generate mGFAP-Cre, $Adra2a^{-/-}$ mutants. We performed spared nerve injury (SNI) on 8-10 weeks old control and mutant mice. The behavioral analysis on control and Adra2a mutant mice didn't show any difference in the mechanical sensitivity. However, we observed that Adra2a mutant mice did not develop cold allodynia. To test the contribution of astrocytic Adra2a on the analgesic action of Adra2 agonists, we injected mice with Dex (10µg/kg) 21 days post-SNI. The behavioral analysis indicates that Dex did not show an analgesic effect on Adra2a mutant mice as it does in control mice, thereby, indicating a potential link between astrocytic Adra2a receptors and chronic pain. Our ongoing studies will further shed light on the mechanisms of the role of astrocytic Adra2a in pain modulation.

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62 Laura Kärtner, Moritz Spangemacher, Lea Mertens, David Erritzoe, Gerhard Gründer

Psychedelic microdosing: more than "just" placebo?

Background: Psychedelic microdosing describes the ingestion of subperceptual or low-threshold doses of classical psychedelic substances. This phenomenon has gained considerable media attention in recent years due to its alleged positive effects on mental health and creativity. It is precisely this media attention that has likely contributed to the formation of strong opinions and expectations about the positive effects of microdosing in popular culture and created a large potential for placebo effects. Conflicting findings in the current research base seem to support this assumption.
Methods: In this discussion, we compare different levels of evidence from modern scientific findings on the effects of psychedelic microdosing (observational studies vs. randomized controlled trials). We discuss observed acute, post-acute, and long-term effects of microdosing, as well as design limitations (e.g., expectancy effects or possible unblinding effects).
Results: While results from uncontrolled observational studies support the anecdotally reported positive effects of microdosing-but cannot provide evidence beyond the placebo effect-modern randomized controlled trials (RCTs) failed to find strong evidence of positive effects on mood and cognition. Because the latter primarily studied healthy subjects and primarily administered single doses, the generalizability of the results is very likely limited.
Conclusions: Although psychedelics are an important tool for investigating placebo effects, placebo effects in psychedelic studies (both high and low dose) currently remain largely unexplored. Further research on placebo effects in psychedelics studies should be conducted in the future. In this context, we discuss open questions regarding the applicability (yet to be tested) of microdosing in a therapeutic context and how potential "placebo-enhancing" properties and a synergistic context design could be used to investigate a possible therapeutic efficacy of microdosing (especially in clinical populations).