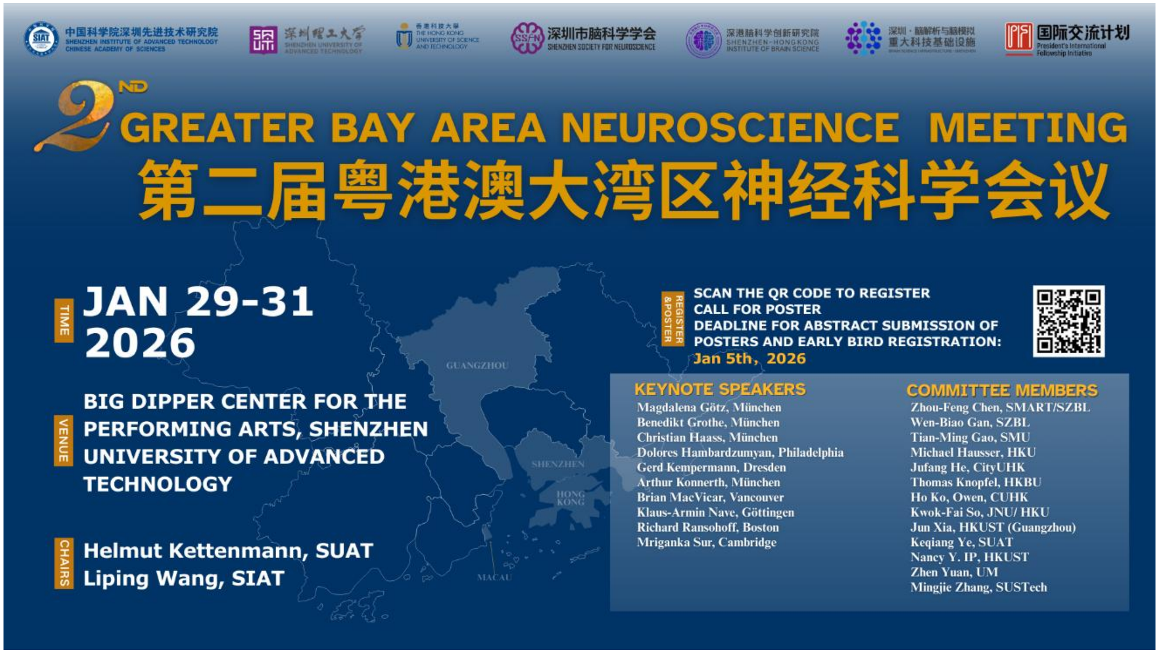

Poster Abstract

海报摘要

1

Intracellular Ca^{2+} dynamics control microglial process motility for surveillance and in response to injury

Sagun Tiwari[1,2], Fan Zeng[1,3], Yan Zhou[1], Alaa Chok[1,2], Alexey Brazhe[4], Alexey Semyanov[5], Kaichuan Zhu[1], Xianyuan Xiang[1,3]*, Helmut Kettenmann[1, 3, 6]*

[1] The Brain Cognition and Brain Disease Institute, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China
[2] University of Chinese Academy of Sciences, Beijing, China.
[3] Bio-X International Institute, Faculty of Life and Health Sciences, Shenzhen University of Advanced Technology, Shenzhen, China
[4] Faculty of Biology, M.V. Lomonosov Moscow State University, Moscow, 119234, Russia
[5] College of Medicine, Jiaxing University, Jiaxing, Zhejiang Province, 314001, China
[6] Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany.
*Equal contribution

Microglia, the resident immune cells of the central nervous system, play pivotal roles in brain surveillance and injury response through highly dynamic process motility. Here, we investigated the role of Ca^{2+} signaling in mediating microglial process motility under both physiological and pathological conditions in vivo. Using dual-laser two-photon microscopy in a transgenic mouse model expressing red fluorescent protein (mCherry) and the Ca^{2+} indicator (GCaMP6m) specifically in microglia, we demonstrated autonomous Ca^{2+} microdomains that operate asynchronously within individual processes, exhibiting significantly more frequent and localized Ca^{2+} transients compared to non-motile structures. Upon focal laser-induced cortical injury, we observed rapid Ca^{2+} waves in microglia, with processes extending toward the lesion site displaying highly correlated Ca^{2+} activity. Attenuation of Ca^{2+} transients by BAPTA, a Ca^{2+} chelator, impaired both homeostatic and laser-induced motility. Importantly, we demonstrated the microglial-specific causal link between Ca^{2+} signaling and motility in a mouse model with microglia-specific genetic overexpression of plasma membrane Ca^{2+} ATPase 2 in vivo. This specific approach demonstrated that impairing intracellular Ca^{2+} signaling in microglia markedly reduces both homeostatic surveillance and the injury response. Together, our findings establish causal evidence that Ca^{2+} signaling is a necessary autonomous driver of microglial process motility in vivo, providing mechanistic insights into neuroimmune dynamics in healthy and injured brains.

2

Functional neurotransmitter and neuropeptide receptor expression in human iPSC-derived microglia

V Poon[1,2], Yan Zhou[3], Helmut Kettenmann[1,4*], Xianyuan Xiang[1*]

[1] Bio-X International Institute, Faculty of Life and Health Sciences, Shenzhen University of Advanced Technology, Shenzhen, China
[2] Medical Neuroscience, Charité University Medicine Berlin
[3] The Brain Cognition and Brain Disease Institute, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China.
[4] Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany.
*Equal contribution

Microglia express a repertoire of receptors, including for neurotransmitters and neuropeptides, which serve for the communication of microglia with their brain environment. While receptor expression and associated microglial functions have been studied extensively in rodents and in microglial cell lines, comparable information for human microglia remains limited. In the present study, we have used human iPSC-derived microglia (iMGL) as a model to analyze whether human microglia exhibit a transmitter and neuropeptide sensing profile that resembles, or differs from, that described in rodents. As a readout of functional expression, we loaded the iMGL with the Ca^{2+} indicator Calbryte520 AM (5 µM) and recorded the Ca^{2+} activity in response to the applied ligand. As a positive control, we applied 1mM ATP, which induced a transient Ca^{2+} increase in 92.75% of the iMGL. Among the ATP-responsive cells, we identified a subset of iMGL that responded with a Ca^{2+} transient to L-glutamic acid (23%), indicating the expression of glutamate receptors. 61% and 24% of cells responded to the agonists of metabotropic glutamate receptors, DCG-IV and xanthurenic acid, respectively. Additionally, 59%, 67%, and 30% of cells responded to the ionotropic glutamate receptor agonists kainic acid, AMPA, and NMDA, respectively. 61% of cells responded to GABA, 58% to the $GABA_B$ receptor agonist baclofen, 68% to the $GABA_A$ receptor agonist muscimol. 45% responded to acetylcholine, 48% and 59% to the muscarinic acetylcholine receptor agonists carbachol and pilocarpine, respectively. Subpopulations of iMGLs responded to serotonin (24%), to norepinephrine (13%), to substance P (60%), to dopamine (26%), and to histamine (37%). We conclude that functional neurotransmitter and neuropeptide receptors are expressed in subpopulations iMGL, similar to what is observed in rodents, implying that human microglia also express the sensors for neuronal activity.

3

DW-pCASL and DTI-ALPS Reveal Blood–Brain Barrier and Glymphatic Dysfunction in Chronic Kidney Disease with Sleep Disturbances

Qiuyi Chen[1], Junfeng Li[1] , Youjia Zeng[2], Fanqi Meng[1], Liantao Hao1， Haodong Qin[3]， Yueyao Chen[1]*

[1] Department of Radiology, Shenzhen Traditional Chinese Medicine Hospital, The Fourth Clinical
[2] Medical College of Guangzhou University of Chinese Medicine, Shenzhen, China
[3] Department of Nephrology, Shenzhen Traditional Chinese Medicine Hospital, The Fourth Clinical Medical College of Guangzhou University of Chinese Medicine, Shenzhen, China
MR Research collaboration,
[4] Siemens Healthineers, Shanghai, China, shanghai, China

Chronic kidney disease (CKD) is frequently accompanied by cognitive impairment and sleep disturbances, suggesting that impaired toxin clearance secondary to renal dysfunction may contribute to central nervous system injury. Sleep disorders are highly prevalent in CKD and are associated with reduced quality of life and disease progression. The blood–brain barrier (BBB) and glymphatic system play critical roles in cerebral fluid homeostasis and metabolic waste clearance, and their dysfunction may underlie CKD-related brain injury.

Despite this, noninvasive imaging evidence of BBB alterations in CKD remains

limited, particularly regarding concurrent BBB and glymphatic changes in patients with sleep disturbances.-- Diffusion-weighted pseudo-continuous arterial spin labeling (DW-pCASL) enables simultaneous quantification of BBB water exchange rate (kw), cerebral blood flow (CBF), and arterial transit time (ATT). Sleep quality is closely linked to glymphatic activity, which can be assessed using diffusion tensor image analysis along the perivascular space (DTI-ALPS).

In this study, we applied DW-pCASL to evaluate BBB function in CKD patients with sleep disturbances. By combining DTI-ALPS and structural MRI, we compared cerebral water exchange, glymphatic function, and choroid plexus volume (CPV) across groups and analyzed their associations with sleep quality.

4

Electroacupuncture Enhances Mesenchymal Stem Cell Therapy via Improved Perfusion and Inflammation Modulation in Peripheral Nerve Injury: An IVIM-MRI Study in Rats

Junfeng Li[1]†, Qiuyi Chen[1]†, Jintong Pan[1,2], Fanqi Meng[1], Wensheng Huang[3], Yingying Liang[4], Xuewen Yu[4], Ruirui Qi[1], Peiyin Luo[1], Haodong Qin[5], Yueyao Chen[1]*, Xiaofeng Lin[6]*

[1] Department of Radiology, The Fourth Clinical Medical College of Guangzhou University of Chinese Medicine (Shenzhen Traditional Chinese Medicine Hospital), Shenzhen, China
[2] Department of Nephrology, Shenzhen Hengsheng Hospital, Shenzhen, China
[3] Department of Radiology, The Seventh Affiliated Hospital, Sun Yat-sen University, Shenzhen, China
[4] Department of Pathology, The Fourth Clinical Medical College of Guangzhou University of Chinese Medicine (Shenzhen Traditional Chinese Medicine Hospital), Shenzhen, China
[5] MR Research Collaboration, Siemens Healthineers, Shanghai, China
[6] Department of Nuclear Medicine, The Seventh Affiliated Hospital, Sun Yat-sen University, Shenzhen, China

Background: Stem cells are widely applied in peripheral nerve repair; however, their therapeutic potential is constrained by immune rejection, inflammatory responses, and a poor regenerative microenvironment. Therefore, reducing the inflammatory response, improving the regenerative environment and dynamically monitoring these processes by imaging techniques are critical. This study examined the effectiveness of electroacupuncture (EA) and bone mesenchymal stem cells (BMSCs) on acute sciatic nerve injury in rats. By employing intravoxel incoherent motion (IVIM) MRI, the study monitored perfusion and explored how EA improves the regenerative environment to optimize stem cell transplantation outcomes.

Methods: Seventy-two rats were randomly assigned to four groups: EA, EA + BMSCs, BMSCs, and PBS. EA was applied at GB30 and ST36. IVIM-MRI (perfusion fraction f), T2WI, histological staining, immunostaining (CD31, IL-1α, IL-10, PPARγ), and SFI were used to evaluate treatment effects.

Results: At 2-4 weeks, the nerve perfusion fraction f in the EA group recovered faster than in the BMSCs group ($P < 0.05$, Figure 3A). By week 4, the EA group showed the greatest myelin regeneration and nerve fiber restoration ($P < 0.05$, Figure 5C and Figure 7). The expression of vascular marker CD31 and anti-inflammatory markers IL-10 and

PPARγ increased ($P < 0.05$, Figure 8A and C-D), while pro-inflammatory marker IL-1α decreased in the EA and EA + BMSCs groups ($P < 0.05$, Figure 8B). Furthermore, f values were strongly correlated with histological and functional outcomes ($P < 0.05$, Supplementary Figure 1).

Conclusion: EA is more effective than BMSCs alone in promoting nerve repair, enhancing blood flow, and reducing inflammation. Moreover, EA enhances the anti-inflammatory effects of BMSCs. The perfusion fraction (f) is a sensitive biomarker for evaluating nerve repair and perfusion restoration.

5

Accelerated brain aging in those with major depression and benzodiazepine use in older but not younger patients receiving repetitive transcranial magnetic stimulation

Sunavsky, Adam[1]; Marzbani, Hengameh[1]; Blumberger, Daniel[2]; Downar, Jonathan[2]; Vila-Rodriguez, Fidel[1]

[1] University of British Columbia
[2] University of Toronto

Machine learning (ML) techniques have utilized neuroimaging to measure 'brain age' in major depressive disorder (MDD). However, most studies offer a cross-sectional approach and do not assess change after treatment such as repetitive transcranial magnetic stimulation (rTMS). Here, we assess the brain-predicted age difference (brain-PAD; the difference between chronological age and estimated brain age), in a large cohort ($N = 655$) of those with MDD pooled from three clinical trials, before and after rTMS treatment, using two different ML algorithms. We tested for age-group differences (<60 vs. ≥60) in brain-PAD and determined whether brain-PAD decreased following rTMS or was associated with treatment response while controlling for clinical and demographic variables. Finally, we tested whether treatment response could predict brain-PAD. Brain-PAD was higher in the older (M = 2.46, 95% CI [0.58, 4.34]) relative to younger (M = −1.78, 95% CI [−2.60, −0.95]; $p < 0.001$) age group while controlling for chronological age; this finding was replicated using $p < 0.10$. Contrary to our hypothesis, younger MDD patients had an average brain-PAD change of +0.41 years after rTMS treatment (within-subjects analysis); this finding was not replicated. Clinically, benzodiazepine use was associated with older appearing brains in the older age group and with lower rTMS response in the younger age group. This study highlights differential aging trajectories in younger relative to older MDD populations and the impact that benzodiazepines may have on accelerated brain aging and lower rTMS response.

6

Neurogenesis Leads Early Development in Zebrafish

Zhengduo Wang[1], Li Tian[2] and Bo Li[1 *]

[1] The Institute for Advanced Studies, Wuhan University, Wuhan 430072, P. R. China.
[2] Department of Physics, Wenzhou University, Wenzhou 325000, P. R. China.

Vertebrate early neurogenesis is a highly conserved process fundamental to brain function and the emergence of intelligence. However, the cellular dynamics bridging gastrulation and organogenesis remain elusive due to observational challenges. We developed a live-cell imaging platform for transgenic zebrafish that provides, for the first time, a continuous reconstruction of early neurogenesis across subcellular to organismal scales. Our analysis reveals that neurogenesis is a precisely orchestrated process. Neuronal cell bodies initially coalesce into discrete, linearly arranged clusters extending from the brain along the spinal cord. From these hubs, axons radiate outward to innervate the central nervous system and peripheral tissues, including the yolk sac surface. A primary pioneer neuron projects from the brain, coursing ventrally in parallel to the body axis. Secondary neurons then interconnect, forming a pervasive network that is subsequently refined through selective axonal apoptosis. The emergence of frequent Ca^{2+} flashes only after structural maturation indicates that functionality is contingent upon an established scaffold. We also observe concurrent material transport and a slow, directional flow of Ca^{2+} along axons, suggesting complementary signaling modalities. Furthermore, neurogenesis exhibits precise spatiotemporal coupling with histogenesis, particularly with the developing lateral line and vasculature. Our work, with refined spatial and time resolution, defines the kinetic pathway of early neurogenesis and underscores the critical interplay of subsystems in embryogenesis, offering fundamental insights for neural health and bio-inspired intelligence.

7

RAB23 regulates lipid homeostasis by affecting Caveolin-1 localization

DU Yunhong, Catherine Hong Huan HOR*

Department of Chemistry, Hong Kong Baptist University

RAB23 is a small GTPase known to regulate vesicle trafficking, and the disruption of RAB23 will lead to Carpenter Syndrome (CS) in humans, with common symptoms including craniosynostosis, polysyndactyly, skeletal defects, obesity, and intellectual disability. Although many researchers study the relationship between these symptoms and the RAB23 intracellular function, the mechanism of the RAB23 defect causing intellectual disability remains unknown. Since aging and neurodegeneration are consistently accompanied by a rise in neutral lipid storage within the nervous system, here we hypothesized that RAB23 can affect the lipid accumulation in the CNS. We culture Rab23 knockout (KO) Mouse Embryo Fibrobalst(MEF) E13.5 from Actin-cre;Rab23f/f mice embryos, and find that the Rab23 KO MEF accumulates more lipid droplets compared with the control group. To investigate the potential underlying mechanism, we perform data mining in RAB23 interactors, and find Caveolin-1 (CAV1), the structural protein of caveolae and a regulator of cellular cholesterol homeostasis, emerges as a candidate regulator at the intersection of the lipid accumulation process. The mislocalization of CAV1 is known to cooperate with LD accumulation; we hence examine the cellular localization of CAV1. The results show that CAV1 mislocalizes in Rab23 KO MEFs, suggesting Rab23 may regulate lipid homeostasis through CAV1 localization. Based on these findings, we can proceed to examine the lipid accumulation in RAB23 KO astrocytes, and test the reactivity of RAB23 KO astrocytes, which can be a hallmark of neurodegeneration. Besides, we will further investigate the role of RAB23 in CAV1 intracellular trafficking in neuronal cells by rescue RAB23, to determine the epistasis of RAB23 in CAV1 localization.

8

Causal inference can explain postdictive multisensory illusions

Günaydın G[1-4], Moran JK[1], Rohe T[5,6#], Senkowski D1,4[#*]

[1] Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Psychiatry and Neurosciences, Charité Campus Mitte (CCM), Charitéplatz 1, 10117 Berlin
[2] Bernstein Center for Computational Neuroscience Berlin, Humboldt-Universität zu Berlin, Philippstr. 13, Haus 6, 10115 Berlin, Germany
[3] Humboldt-Universität zu Berlin, Department of Psychology, 10099 Berlin, Germany
[4] Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Einstein Center for Neurosciences Berlin, Charitéplatz 1, 10117 Berlin, Germany.
[5] Fraunhofer Institute for Process Engineering and Packaging (IVV), Sensory Analytics and Technologies, Giggenhauser Str. 35, 85354 Freising, Germany
[6] Institute of Psychology, Friedrich-Alexander-Universität, 91054 Erlangen-Nürnberg, Germany

Information from different sensory modalities is integrated in a temporal window of multisensory processing that can last several hundred milliseconds. Within this window, the processing of a stimulus is influenced not only by preceding and concurrent input, but also by input following a stimulus. A previous study using a beep-flash pair showed that auditory or visual stimuli presented shortly after a stimulus can retroactively influence the perception of the first stimulus, resulting in an illusory or invisible flash (Stiles et al., 2018; PloS One 13:e0204217). A single beep presented between two flash-beep pairs can induce an illusory flash, whereas a single flash presented between two flash-beep pairs can be perceptually suppressed. In this behavioral study (N = 32), we used a Bayesian Causal Inference (BCI) framework to investigate the mechanisms underlying the two multisensory postdictive illusions. We replicated both illusions, found that asynchronous stimuli that fall outside the temporal integration window reduce the illusions, and that the causal inference framework can largely explain cross-modal postdiction better than competing forced-fusion and forced-segregation models. In addition, we present preliminary EEG data that offer insight into the neural mechanisms of the observed multisensory postdictive illusions.

9

Naturally emerging ectopic expression in different neuronal populations mediates various (patho)physiological outcomes

Yu Liu[1], Zixuan Li[1], Pengwei Luan[2,] Zhi Zhang[3], Andrii Domanskyi[4], Yi Zhang[5], Shihao Wu[6], Miao Jing[2], Wenjie Zhang[1], Ilya A. Vinnikov[1]

[1] Department of Prosthodontics, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine; College of Stomatology, Shanghai Jiao Tong University; National Center for Stomatology; National Clinical Research Center for Oral Diseases; Shanghai Key Laboratory of Stomatology; Shanghai Research Institute of Stomatology; School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai, China.
[2] Chinese Institute for Brain Research, Beijing Institute for Brain Research, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China.
[3] School of Life Sciences, Fudan University, Shanghai, China.
[4] Institute of Biotechnology, HiLIFE, University of Helsinki, Helsinki, Finland.
[5] Shanghai Diabetes Institute, Shanghai Key Laboratory of Diabetes Mellitus, Shanghai Clinical Centre for Diabetes, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China.
[6] School of Medicine, Yunnan University, Kunming, Yunnan, China.

Highly controlled pattern of gene expression in various neuronal populations defines their identity and function. Here we describe two exemplary systems when the genes that previously were postulated to be devoid from mature neurons are naturally abundant in specific neuronal populations. Indeed, we demonstrate the cytoprotective function of the stem-cell specific mir-290-295 cluster in mature dopamine neurons, where it is naturally and exclusively expressed as the most abundant of all microRNAs. A counterexample constitutes paraventricular nucleus of the hypothalamus in high fat diet-fed mice or diabetic human patients, where oxytocin neurons start expressing P2y12 purinergic receptor, which is characteristic exclusively for hematopoietic lineage cells. Activation of this receptor receiving ATP signals from astrocytes leads to exacerbation of metabolic phenotypes such as hyperglycemia and obesity. This purinergic signaling constitutes an excellent druggable target, which we successfully validated both in rodents and primates. As such, we postulate that these and other not yet discovered examples of "ectopic", but natural expression specific for distinct neuronal populations might have a tremendous functional relevance both in health and disease.

10

The behavioral and neural differences between direct and vicarious mentalizing

Jingmin Qin[1,2], Xinze Wang[1], and Haiyan Wu[1,2*]

[1] Centre for Cognitive and Brain Sciences and
[2] Department of Psychology, University of Macau, Macau, China

Abstract: Mentalizing, the ability to understand others' mental states, is fundamental to human social cognition; while traditional research focuses on direct inference, real-world reasoning often involves various ways. Understanding how individuals use self-related and vicarious mentalizing during social interactions remains a challenge. Our study investigates the mechanisms underlying direct mentalization and vicarious mentalization of others' mental states, combining with mouse tracking and electroencephalography (EEG). Here, we designed a novel triadic mentalizing task where participants integrated self-acquired evidence with an Agent's inferences. In this task, the core variable, confidence of the agent, can be derived from the motor trajectories. With computational model based on the weights of self and other's evidence and confidence, our results reveal adaptive strategies in weighing self-generated and agent-provided inference, adjusting reliance based on the strength of self-evidence and agent reliability. Critically, EEG analyses identified distinct neural signatures: beta oscillation reflected the strength of self-related and agent-related information during observing phases, while representational similarity analysis (RSA) provided neurophysiological evidence for the dynamic neural encoding of agent-dependence across observing and decision phases. This work shows how people flexibly rely on the two ways in predicting the state of others, offering new insights into the enhancement of interpersonal adaptability.

11

DNMT3a knockdown in the medial prefrontal cortex blocks psilocybin's lasting sociability rescuing effect in a mouse model of autism

Jonathan Qing Feng Tan[1], Jocelyn Sze Lyn Toh[1], Thomas Ernst Knöpfel[2], Chenchen Song[1]

[1] Nanyang Technological University, Singapore,
[2] Hong Kong Baptist University, Hong Kong

The serotonergic psychedelic psilocybin can induce lasting therapeutic behavioural alterations following a single systemic dose, exerting effects beyond its bioavailability. The mechanisms underlying this sustained behavioural rescuing remain unclear. We hypothesise that the lasting therapeutic effect of psilocybin is mediated through epigenetic mechanisms. To test this, we focus on examining whether interfering with DNA methylation dynamics – specifically, DNA methyltransferase 3a (DNMT3a) function – can block psilocybin's (lasting) therapeutic effect.
To this end, we designed targeted short hairpin ribonucleic acid (shRNA) to knockdown DNMT3a. We first functionally validated shRNA knockdown efficiency in vitro in cultured mouse cell line, and identified the most effective DMNT3a-targeting shRNA with minimal effect against other DNMT isoforms. We then packaged this shRNA construct into adeno-associated viruses (AAVs), to examine whether knocking down DNMT3a in vivo can block psilocybin's reported lasting effect at rescuing sociability deficits in the Cntnap2-KO mouse model of autism spectrum disorder.
We stereotaxically delivered AAVs expressing either DMNT3a-targeting shRNA or scrambled shRNA control bilaterally into the medial prefrontal cortex (mPFC) of six-week-old mice from the Cntnap2-KO colony. Following two weeks expression time, we systemically administered a single dose of psilocybin or saline vehicle control to these mice, and evaluated their social behaviour using the three-chamber test at one day, one week, and two weeks post-treatment. We observed that the Cntnap2-KODNMT3a-KD mice displayed low sociability following psilocybin treatment, whereas Cntnap2-KOScrambled mice that received psilocybin displayed higher sociability across all post-treatment time points. This observation indicated that DNMT3a knockdown prior to psilocybin treatment blocks the sustained behavioural rescuing effects of psilocybin. Further, we did not observe significant difference in sociability in vehicle-treated isogenic control mice (i.e. Cntnap2-WT mice from the same colony) expressing either DNMT3a shRNA or scrambled shRNA, indicating that the observed effects were not due to DNMT3a shRNA expression itself.

Together, our observations provide preliminary evidence that DNA methylation mechanisms in the mPFC are required for the lasting sociability rescuing effects of psilocybin in a mouse model of autism. This highlights epigenetic regulation as a potential contributor to long-lasting behavioural plasticity through which psilocybin may exert its lasting therapeutic effects.

12

Cell type-resolved methylomes related to sociability

Lin Wang[1], Chang Ming[1], Jocelyn S.L.Toh[2], Jonathan Q.F. Tan[2], Chenchen Song[2], Thomas Ernst Knöpfel[1]

[1] Hong Kong Baptist University, Hong Kong
[2] Nanyang Technological University, Singapore

Individual differences in social behavior are not fully explained by genetic variation. Environmental influences across neurodevelopment shape individual personalities, and these effects are reflected at the level of epigenetic status. DNA methylation, as a core epigenetic mechanism, finely regulates gene expression, and influences neuronal circuits in brain regions such as the frontal cortex and governs social behavior. Clinically, social behavior is a key behavioral phenotype altered in autism spectrum disorder (ASD), but human association studies lack brain cell-type-specific methylation data, making it difficult to determine which epigenetic signatures truly reflect sociability. Therefore, many important questions remain: How does genetic risk versus environmental exposure contribute to ASD-related DNA methylation? Are methylation changes associated with low sociability reversible when sociability is behaviorally rescued using therapeutic interventions?
Towards addressing these questions, we isolate inhibitory and excitatory neurons from the frontal cortex of mouse models of varying sociability and their isogenic controls, then quantify DNA methylation levels of candidate genes. We compare these epigenetic profiles across genetic, teratogenic, and environmentally induced low sociability models. We further compare the DNA methylation profiles with mice that have had their sociability pharmacologically rescued, to identify DNA methylation profiles that reflect sociability reversibility.
By comparing across different etiologies, neuronal cell types, and intervention states, we aim to identify DNA methylation signatures that correlate with sociability deficits and that are common or distinct to different models. Our findings provide cell-type-specific epigenetic candidate biomarkers for sociability and establish an experimental framework for testing causal relationships between DNA methylation changes and social behavior, bridging basic mechanisms with translational applications.

13

Structural Signatures of Cocaine Addiction-Like Behavior in Rats

Marvin Urban[1,2], Felix Hörner[3], Wolfgang Weber-Fahr[3], Marcus Meinhardt[1], Rainer Spanagel[1]

[1]Institute of Psychopharmacology, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany
[2] Interdisciplinary Center for Neurosciences, University of Heidelberg, Germany
[3] Department of Neuroimaging, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

Cocaine use disorder (CUD) is a mental health condition that affects around 20% of regular consumers and is associated with significant physiological and behavioral dysfunction. Assessing biological drivers of vulnerability and resilience to CUD is challenging due to inter-individual variability in psychological traits and early-life conditions. The 0/3Crit model is a highly translational rodent model that mimics clinical diagnostic criteria by capturing different behavioral dimensions, such as motivation for cocaine, persistence of drug seeking, and compulsivity. Based on their scores in these domains, animals can be classified as addicted (3Crit) and non-addicted (0Crit), i.e., vulnerable and resilient. Structural abnormalities in CUD patients are well established. Thus, using the 0/3Crit model in combination with longitudinal structural MRI, we explored the neurobiological underpinnings of resilience and vulnerability to addiction-like behavior in 48 male Sprague-Dawley rats.
We employed voxel-based morphometry on T2-weighted anatomical scans and tract-based statistics on diffusion-weighted images (b = 1000s/mm2) to track differences between resilient and vulnerable rats before cocaine exposure, after 15 days of self-administration, and after 50 days of self-administration. No significant differences in gray matter volume (GMV) were observed at baseline. However, addiction-prone rats exhibited earlier onset and more widespread GMV increases within the frontostriatal network, particularly the caudate putamen, the insula, and prefrontal areas. In several of the affected regions, GMV increases correlated with addiction-like behavior, suggesting a functional relevance of gray matter abnormalities in this model. While the observed effects showed substantial anatomical overlap in both phenotypes, the onset and magnitude of cocaine-induced GMV growth differed. White matter tracts of the external and internal capsule, on the other hand, displayed a baseline trend ($p < 0.1$) of elevated mean diffusivity (MD) in the resilient animals. After 50 days of CSA, however, the resilient rats show decreases in MD in these tracts, while the vulnerable rats do not differ

from controls. MD is sensitive to local membrane density and extracellular matrix organization. Thus, this finding could imply that resilient animals react to cocaine exposure with some sort of protective glial or extracellular matrix remodeling.

Taken together, this data suggests divergent trajectories in response to repeated cocaine exposure, comprising excessive frontostriatal neuroplasticity in vulnerable animals and protective microstructural adaptations in resilient rats. Some of the effects seen in this experiment have been observed in other preclinical and, in part, clinical studies. Molecular and histological experiments informed by these findings could help pinpoint the mechanisms underlying differences in cocaine addiction vulnerability and identify potential targets for intervention.

14

Age-Related Differences in Interoceptive Accuracy: A Functional Near-Infrared Spectroscopy (fNIRS) Study

Feiping Yu [a], Xinyi Liu [a], Xiaolei Li [a], Yuli Li [a], Xuan Zhang [a], Ping Li [a], Meiling Qi [a,*]

[a] School of Nursing and Rehabilitation, Shandong University, Jinan 250012, Shandong, China.

Interoceptive accuracy (IAcc) declines with aging, yet its underlying neural mechanisms remain unclear. This study utilized functional near-infrared spectroscopy (fNIRS) to investigate age-related differences in cortical activation and functional connectivity of cortical regions during interoceptive tasks to uncover neural mechanisms that may explain age-related differences in IAcc. This study recruited 29 young adults (Mage=23.76) and 25 older adults (Mage=64.32) to undertake a breath-focused interoceptive task and a resting-state scan. IAcc was assessed by the heartbeat perception task. This study showed that young adults exhibited significantly greater activation in the SMC, PFC, IFG, S1-L, and M1-R during the interoceptive task compared to older adults. Critically, higher IAcc was positively correlated with the IFG-L activation. Older adults demonstrated significantly stronger functional connectivity between S1-L and IFG-R, PFC-R, as well as PFC-L during the interoceptive task relative to the resting state. Importantly, between-group comparisons revealed a trend enhanced functional connectivity in older adults for M1-L/M1-R, S1-L/IFG-R and PFC networkers before the FDR correction for multiple comparisons. These findings suggest that age-related cortical changes may compromise the neural efficiency of interoceptive networks. This research provides novel insights into the age-specific neural patterns underlying IAcc decline.

15

Deletion of Rab23 Impairs Hypothalamic Ciliogenesis and Leptin Signaling in Appetite Regulation

Wai Lam Tung[1], Feixu Jiang[1], Bor Luen Tang[2], Catherine Hong Huan Hor[1]

[1] Department of Chemistry, Hong Kong Baptist University, Hong Kong, China
[2] Department of Biochemistry, National University of Singapore, Yong Loo Lin School of Medicine, Singapore

Carpenter Syndrome (CS) is a rare genetic disorder characterized by various clinical manifestations, including obesity, craniofacial malformations, and polydactyly. Mutations in the RAB23 gene, which encodes a small GTPase, are the causative factor for CS, yet the mechanisms linking RAB23 dysfunction to disordered energy balance remain poorly explored. Here, we investigated the role of Rab23 in central control of feeding by selectively deleting Rab23 in neural progenitor cells using Nestin-Cre. Mice with CNS-specific Rab23 deficiency developed hyperphagia-driven obesity, excessive intake rather than reduced energy expenditure. Mutant mice exhibited leptin resistance, with blunted leptin response and diminished Stat3 phosphorylation in the hypothalamus, alongside reduced Mc4r expression. Consistent with melanocortin pathway disruption, Nes-CKO mice showed impaired feeding suppression in response to MC4R agonists (melanotan II and setmelanotide). Rescue experiments demonstrated that reintroduction of Rab23 in hypothalamic neurons restored Mc4r expression and neuronal activation, with a GTP-locked Rab23 variant providing significant rescue. Moreover, targeted AAV-mediated Mc4r overexpression in the hypothalamus reinstated normal agonist responses and reversed metabolic deficits in vivo. In cell-based assays, RAB23 depletion increased MC4R surface abundance and impaired agonist-induced internalization, indicating that Rab23 governs receptor trafficking dynamics in addition to modulating hypothalamic signaling. Together, these findings identify Rab23 as a critical regulator of the leptin–melanocortin signaling pathway, acting through both neuronal leptin responses and MC4R trafficking, and provide a potential mechanism for CS-associated obesity.

16

TMEM63B functions as a mammalian hyperosmolar sensor for thirst

Wenjie Zou[1,8], Xingyu Chen[1,2,8], Jiamin Ruan[1,3,8,] Siqi Deng[1,4,8,] Huize Wang[1], Wuqiang Zhan[1], Jingxin Wang[5,6], Zhiyong Liu[5,7], Zhiqiang Yan[1*]

[1] Institute of Molecular Physiology, Shenzhen Bay Laboratory, Shenzhen 518132, China
[2] Department of Chemistry, Southern University of Science and Technology, Shenzhen 518055, China
[3] School of Basic Medical Sciences, Capital Medical University, Beijing 100069, China
[4] Division of Life Science, The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong, China.
[5] Institute of Neuroscience, State Key Laboratory of Neuroscience, CAS Center for Excellence in Brain Science and Intelligence Technology, Chinese Academy of Sciences, Shanghai 200031, China
[6] University of Chinese Academy of Sciences, Beijing 100049, China
[7] Shanghai Center for Brain Science and Brain-Inspired Intelligence Technology, Shanghai 201210, China
[8] These authors contributed equally to this work.

Thirst drives animals to reinstate water homeostasis by fluid intake. An increase of blood osmolality is thought to induce thirst by activating a thirst receptor expressed in the subfornical organ (SFO), but the molecular identity of this receptor remains elusive. Here, we provide behavioral and functional evidence to show that TMEM63B functions as a mammalian thirst receptor in the SFO and mediates osmotic and dehydrated thirst. First, we showed that TMEM63B is expressed in SFO excitatory neurons and required for the neuronal responses to hypertonic stimulation. Heterologously expressed TMEM63B is activated by hypertonic stimuli and point mutations can alter the reversal potential of the channel. More importantly, purified TMEM63B in liposomes establishes osmolarity-gated currents. Finally, Tmem63b knockout mice have profound deficits in thirst, and deleting TMEM63B within the SFO neurons recapitulated this phenotype. Taken together, these results provide a molecular basis for thirst and demonstrate TMEM63B is the long-sought mammalian thirst receptor.

17

EFFECTS OF TRANSCUTANEOUS ELECTRICAL ACUPOINT STIMULATION ON AUTISM: BEHAVIORAL THERAPEUTICS EFFICACY AND OXYTOCIN MECHANISMS IN THE CENTRAL NERVOUS SYSTEM

Xiaoxi Wang[1], Rong Zhang[2*]

[1] Institute of Acupuncture and Moxibustion, China Academy of Chinese Medical Sciences, Beijing 100000, China
[2] Neuroscience Research Institute, Peking University, Beijing, 100871, P. R. China.

Autism spectrum disorder (ASD) is a mental development disorder in children characterized by core symptoms such as impaired social interaction, stereotyped behaviors, and abnormal sensations. Its mechanism is believed to be related to oxytocin (OXT). Transcutaneous electrical acupoint stimulation (TEAS) can produce various neurotransmitters including OXT by stimulating the skin, and OXT is also regarded as a potential treatment for ASD. This study explored the effect and mechanism of TEAS in the treatment of ASD through animal experiments and clinical trials. Through a valproic acid (VPA)-mediated rat model of autism, TEAS intervention was administered in the early stage of life to observe the behavioral and OXT system changes of adolescent offspring. Then, behavioral changes were observed under the condition of blocking oxytocin or oxytocin receptors. Additionally， clinical research observed the effect of TEAS on the gut microbiota of ASD children through 16s RNA technology. Therefore, we hypothesize that TEAS may work through the oxytocin system to improve symptoms of ASD. In this article, we will use the VPA induced rat model to investigate the therapeutic mechanism of TEAS in ASD.

18

Neuroinflammatory Activation of Microglia Induces Cytokine Release that Drives Piezo1 Expression in Astrocytes

Yanyang Bai[1,2], Hyun B. Choi[1], Stefan Wendt[1], Morgan Towriss[3], Ada J. Lin[4], Christopher J. Groten[1], Zhonghua Lu[2], Haakon B. Nygaard[4] and Brian A. MacVicar[1]

[1] Department of Psychiatry, Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, V6 T 1Z3, Canada
[2] Research Center for Primate Neuromodulation and Neuroimaging, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, 518000, China
[3] Department of Biochemistry and Molecular Biology, Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, V6 T 1Z3, Canada
[4] Division of Neurology, Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, V6 T 1Z3, Canada

Numerous brain disorders are associated with structural alterations in the tissue microenvironment of the brain that can be sufficient to initiate mechanosensory signaling pathways and impact neuropathology. However, the precise mechanisms regulating mechanosensory signaling in brain pathology remain uncertain. In our study, we examined how the expression of astrocyte Piezo1, a mechanosensitive ion channel, is modulated by inflammatory triggers both in vivo and in vitro. To do this, we used rodent primary astrocyte and microglia cultures as our cell models. Using qPCR and Western blotting we found that the addition of either LPS or oligomer Aβ (oAβ) to astrocyte cell cultures had either no effect or only marginal effects on Piezo1 expression. However, when LPS or oAβ were added to microglia cultures, not only did Piezo1 expression increase in microglia, but the conditioned media from these microglia cultures also significantly increased Piezo1 expression in astrocytes. We found that the proinflammatory cytokines interleukin 1α, interleukin 1β, and TNFα were released from microglia after LPS or oAβ treatments. The addition of these cytokines to astrocyte cell cultures was sufficient to significantly increase Piezo1 expression and Piezo1-mediated Ca2+ responses in astrocytes. Futhermore, depleting microglia significantly decreased astrocyte Piezo1 expression in the brains of 5xFAD mice. We therefore conclude that the upregulation of Piezo1 in astrocytes is triggered by microglial activation and the subsequent cytokine release from microglia. We propose that microglia-astrocyte cross talk plays a critical role in facilitating astrocyte mechanosensation by increasing Piezo1

expression in astrocytes thereby modulating homeostatic functions of astrocytes in neuroinflammation.

19

Consciousness Theory Based on Holism: Reconciling the Hard Problem of Consciousness and Explanatory Gap

Jicheng Chen[1,2]

[1] Department of Cardiovascular Medicine, Longhua District Central Hospital, Shenzhen, Guangdong, China
[2] School of Life Sciences, Shanghai Jiao Tong University, Shanghai, China

Natural science is founded on physicalism. We regard physical reality as the only objective reality, while inner experience is treated as an epiphenomenon, with the two viewed as independent. This cognitive paradigm has successfully explained many natural phenomena. However, discoveries in fields such as quantum mechanics and psychology challenge this basic notion—often taken as an axiom. Yet, the inner and the outer appear to be distinct categories of existence, and there remains a lack of scientific methods to demonstrate a transformative relationship between them. This has led to a series of consciousness-related dilemmas marked by the hard problem of consciousness and the explanatory gap. We propose that holism offers a potential solution. According to holism, objective reality and causality are not absolute but are inseparable from the (cognitive perspective of the) subject. The physicalist stance that treats the inner and the outer as independent is an underlying assumption based on the third-person perspective (3PP), not an axiom. From the 3PP, the physical properties of things are described, and interactions between objects are confined to spacetime. The 3PP excels at describing the laws of motion governing simple systems, but it simultaneously introduces limitations such as the explanatory gap. In contrast, from the first-person perspective (1PP), the holistic attributes of things are experienced by the subject and can generate objective causal efficacy. Interactions under the 1PP exhibit non-local characteristics unrestricted by spacetime. The 1PP excels at revealing the laws governing complex phenomena and can resolve issues like the explanatory gap. Although superficially opposed, these two cognitive perspectives are functionally complementary. Therefore, a generalized complementarity principle grounded in holism provides a potential pathway for reconciling the contradiction between quantum theory and relativity, offers a falsifiable scientific methodology for the "subjective" interpretation of quantum theory, and helps, to some extent, harmonize core disagreements among different theories of consciousness. To test these hypotheses, we propose an experimental model to demonstrate that, holistic

interaction without a physical medium from the 1PP is an objective way of interaction between things, the inner experience, as a holistic attribute of things, can also produce objective causal efficacy.

20

Ultrasound combined with urokinase under key-shaped bone window enhances blood clot lysis in an in vitro model of spontaneous intracerebral hemorrhage

Min Cui[1]

[1] Department of Neurosurgery, Chongqing Emergency Medical Center, Chongqing University Central Hospital, Chongqing, 400010, China

Objective

Minimally invasive surgery for spontaneous intracerebral hemorrhage is impeded by inadequate lysis of the target blood clot. Ultrasound is thought to expedite intravascular thrombolysis, thereby facilitating vascular recanalization. However, the impact of ultrasound on intracerebral blood clot lysis remains uncertain. This study aimed to explore the feasibility of combining ultrasound with urokinase to enhance blood clot lysis in an in vitro model of spontaneous intracerebral hemorrhage.

Methods

The blood clots were divided into four groups: control group, ultrasound group, urokinase group, and ultrasound + urokinase group. Using our experimental setup, which included a key-shaped bone window, we simulated a minimally invasive puncture and drainage procedure for spontaneous intracerebral hemorrhage. The blood clot was then irradiated using ultrasound. Blood clot lysis was assessed by weighing the blood clot before and after the experiment. Potential adverse effects were evaluated by measuring the temperature variation around the blood clot in the ultrasound + urokinase group.

Results

A total of 40 blood clots were observed, with 10 in each experimental group. The blood clot lysis rate in the ultrasound group, urokinase group, and ultrasound + urokinase group (24.83 ± 4.67%, 47.85 ± 7.09%, 61.13 ± 4.06%) was significantly higher than that in the control group (16.11 ± 3.42%) ($p = 0.02$, $p < 0.001$, $p < 0.001$). The blood clot lysis rate in the ultrasound + urokinase group (61.13 ± 4.06%) was significantly higher than that in the ultrasound group (24.83 ± 4.67%) ($p < 0.001$) or urokinase group (47.85 ± 7.09%) ($p < 0.001$). In the ultrasound + urokinase group, the mean increase in temperature around the blood clot was 0.26 ± 0.15˚C, with a maximum increase of 0.38 ± 0.09˚C. There was

no significant difference in the increase in temperature regarding the main effect of time interval ($F = 0.705$, $p = 0.620$), the main effect of distance ($F = 0.788$, $p = 0.563$), or the multiplication interaction between time interval and distance ($F = 1.100$, $p = 0.342$).

Conclusions

Our study provides evidence supporting the enhancement of blood clot lysis in an in vitro model of spontaneous intracerebral hemorrhage through the combined use of ultrasound and urokinase. Further animal experiments are necessary to validate the experimental methods and results.

21

Hindbrain Astrocytes Rapidly Initiate Goal-Directed Hunting Behavior in Larval Zebrafish

Guangnan Tian*[1], Kai Fu*[1], Kenny K.Y. Chen*[2], Gewei Yan[3], Thomas Ka Chung Lam[4], Jianan Qu[3], Julie L. Semmelhack[1]

[1] The Hong Kong University of Science and Technology, Division of Life Science, Hong Kong S.A.R., China
[2] The Hong Kong University of Science and Technology, Department of Computer Science and Engineering, Hong Kong S.A.R., China
[3] The Hong Kong University of Science and Technology, Department of Electronic and Computer Engineering and State Key Laboratory of Molecular Neuroscience, Hong Kong S.A.R., China
[4] EPFL, Brain Mind Institute & Interfaculty Institute of Bioengineering, Lausanne, Switzerland

Astrocytes are increasingly recognized as key modulators of neural circuit dynamics and behavior. In larval zebrafish, radial astrocytes have been shown to mediate both physiological and pathological behavioral state transitions—such as futility-induced passivity and epilepsy—typically over timescales of seconds to minutes. However, whether astrocytes can initiate fast, goal-directed behaviors remains unknown. Here, we identify a population of hindbrain radial astroglia that exhibits rapid activity during visually evoked hunting behavior, but not during futility-induced passivity. Optogenetic stimulation of these astrocytes reliably triggers hunting behavior with a latency of approximately 50 milliseconds. To investigate the underlying circuit mechanisms, we recorded neuronal calcium responses following astrocyte stimulation. We found that hindbrain neurons activated during visual prey capture also respond during astrocyte-induced hunting events, suggesting that astrocytes may initiate hunting by activating hindbrain premotor neurons. These findings reveal a previously unrecognized role for astrocytes in rapidly initiating innate behavior and suggest that astrocyte-neuron interactions in the hindbrain contribute to fast sensorimotor transformations.

22

Steric Confinement-Induced Emission Probe for Monitoring Protein Conformations in Live Cells

Huixia Jia[1,2,3#], Lin Yang[4#], Yu Yang[1,2,3], He Liu[1,2,3], Jia Liu[5], Chunfeng Shang[6], Zengru Di[1,2,3], Benzhao He[4*], Ke Zhang[1,2,3*]

[1] Department of Systems Science, Faculty of Arts and Sciences, Beijing Normal University, Zhuhai, 519087, China.
[2] International Academic Center of Complex Systems, Beijing Normal University, Zhuhai, 519087, China.
[3] School of Systems Science, Beijing Normal University, Beijing, 100875, China.
[4] Department of Chemistry, Faculty of Arts and Sciences, Center for Advanced Materials Research, Beijing Normal University, Zhuhai, 519087, China.
[5] Shanghai Institute for Advanced Immunochemical Studies and School of Life Science and Technology, ShanghaiTech University, Shanghai 201210, China.
[6] Guangdong-Hongkong-Macau Institute of CNS Regeneration, Ministry of Education CNS Regeneration Collaborative Joint Laboratory, Jinan University, Shenzhen, 518053, China.

Protein conformational changes drive signal transduction to regulate cellular activities, yet monitoring of these changes in living cells remains challenging. Here, we introduce BIOSCE (BIOprobe based on Steric Confinement-induced Emission), a technique that enables tracking of individual protein conformations in living cells across millisecond-to-minute timescales. BIOSCE reports protein conformational changes via steric confinement–induced luminescence switching from non-luminescent to luminescent states. We demonstrate that BIOSCE rapidly senses calmodulin conformational changes triggered by intracellular calcium fluctuations. The BIOSCE platform achieved millisecond-resolution monitoring of single-protein conformations within cellular signaling pathways, as evidenced by its sensitive detection of rapamycin-dependent FKBP (FK506-binding protein)-FRB (FKBP-rapamycin binding) interactions regardless of the labeled partner. Furthermore, we applied BIOSCE to track the spatial distribution of SNAP25 (25 kDa synaptosomal nerve-associated protein) during botulinum neurotoxin A (BoNT/A) intoxication, revealing differential catalytic processing of its cleavage fragments. This generalizable approach provides a robust platform for investigating single-molecule conformational changes with high spatiotemporal resolution and enables direct evaluation of transient cellular events.

23

Pathological Phagocytosis Hijacks Oligodendrocyte Precursor Cells to Drive Remyelination Failure

Jia-Yi He[1]†, Qing-Qing Sun[1]†, Xiao-Wen Li[1]†, Wei-Feng Zhang[1]†, Hirohide Takebayashi[2], Rui-Min Yan[1], Fang Zhou[1], Rui Gao[1], Xiao-Hui Li[1], Wen-Yue Li[1], Rui Zhang[1], Hao-Ran Xue[1], Li-Bin Wang3, Xing Li[1*], Yuan Zhang[1*]

[1] National Engineering Laboratory for Resource Development of Endangered Crude Drugs in Northwest China, The Key Laboratory of Medicinal Resources and Natural Pharmaceutical Chemistry, The Ministry of Education, College of Life Sciences, Shaanxi Normal University, Xi'an, Shaanxi, China;
[2] Center for Anatomical Studies, Graduate School of Medicine, Kyoto University, Kyoto, Japan;
[3] Department of Medical Experimental Center, Shenzhen Nanshan People's Hospital, Shenzhen, Guangdong, China.

Neuroinflammatory demyelination, a hallmark of brain aging and neurological disorders, features persistent myelin debris accumulation at lesion sites. While oligodendrocyte precursor cells (OPCs) are essential for remyelination, their differentiation is arrested in pathological contexts. Yet the cellular and molecular processes regulating the impaired differentiation of these cells throughout the course of the disease are poorly understood. Here, we identify a disease-specific subset of phagocytic OPCs that engulf myelin debris (termed as myelin-debris-associated OPCs; MAO) that fail to mature into myelinating oligodendrocytes. Single-cell transcriptomics across human, marmoset, and murine demyelinating lesions revealed that these phagocytic OPCs exhibit marked upregulation of the low-density lipoprotein receptor (LDLR). Genetic ablation of LDLR specifically in OPCs abolished myelin debris engulfment, rescued differentiation arrest, and enhanced functional recovery in demyelination models. Mechanistically, LDLR activation triggered lanosterol synthase (LSS)-mediated cholesterol biosynthesis, leading to lipid droplet accumulation and inflammasome activation. Conditional knockout of LSS or its pharmacological inhibition restored cholesterol efflux via the 24,25-epoxycholesterol/LXRβ/ABCA1 axis, suppressed NLRP3 inflammasome activity, and accelerated remyelination. Our findings unveil an LDLR-LSS signaling nexus that locks OPCs in a phagocytic state, providing a therapeutic strategy to reverse remyelination failure.

24

Selective abundance of the stemness-promoting cluster miR-290-295 within the adult substantia nigra dopamine neurons is neuroprotective via preservation of protein synthesis

Zixuan Li[1], Yang Xu[1], Nicola Murgia[1], Nikolay Kovzel[1], Dicksan Ng[1], Yu Liu[1], Xuejia Kang[1], Andrii Domanskyi[2], Wenjie Zhang[1], Ilya A. Vinnikov[1*]

[1] Department of Prosthodontics, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine; College of Stomatology, Shanghai Jiao Tong University; National Center for Stomatology; National Clinical Research Center for Oral Diseases; Shanghai Key Laboratory of Stomatology; Shanghai Research Institute of Stomatology; School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai, China.
[2] Institute of Biotechnology, HiLIFE, University of Helsinki, Helsinki, Finland.

The ventral midbrain regulates locomotor activity, reward-related locomotion, and other vital functions, with dopamine (DA) neurons playing a central regulatory role. From early development to maturity, the functional integrity of these DA neurons relies critically on the coordinated expression of both coding and non-coding genes. For example, in rodent stem cells, the miR-290-295 cluster is the predominant expressed microRNA and is essential for maintaining stemness. During differentiation into terminally differentiated lineages such as neurons, cells typically downregulate the transcription of these stem cell-specific microRNAs. In this study, we report that within the adult *substantia nigra pars compacta* (SN), the miR-290-295 cluster is specifically expressed in DA neurons (SN^{DA}). This cluster prevents locomotor deficits and sustains adequate expression of enzymes involved in DA biogenesis, including tyrosine hydroxylase (TH), dopa decarboxylase (DDC), and DA transporter (DAT). Notably, global knockout of the miR-290-295 cluster results in a reduced number of SN^{DA} neurons in adult mice. Using *in vitro* and *in vivo* DA cell-specific loss-of-function models, we demonstrated that miR-292a-3p, the most abundant microRNA in this cluster, directly targets *Pten*, a phosphatase that antagonizes the neuroprotective phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)-Akt-mechanistic target of rapamycin kinase (mTOR) pathways, which regulate translation initiation. Mechanistically, when labeled with the click chemistry-compatible methionine analogue L-azidohomoalanine, SN^{DA} neurons deficient in the miR-290-295 cluster exhibited a severe impairment in protein synthesis which is critical for DA biogenesis. Our finding provides the first evidence that stem cell-specific, stemness-promoting

microRNAs are selectively expressed in a distinct population of mature neurons to maintain their physiological functions in adulthood. This suggests that similar epigenetic disinhibition mechanisms may also be crucial for other terminally differentiated cells across species.

25

Detecting Rare Variants in PCDHGB1 in Dystonia

Junyu Lin MD#, Chunyu Li MD#, Dejiang Pang MD#, Ruwei Ou MD, Qianqian Wei MD, Yi Xiao MD, Tianmi Yang MD, Yuan Gao MD, Qirui Jiang MD, Shichan Wang MD, Jiyong Liu MD, Yushan Tan MD, Jingxuan Huang MD, Yuanzheng Ma MD, Wei Song MD, Xueping Chen MD, Jing Yang MD, Bi Zhao MD, Yiyuan Cui, Ying Wu MD, Huifang Shang MD*

Department of Neurology, Rare Disease Center, Laboratory of Neurodegenerative Disorders, West China Hospital, Sichuan University, Chengdu, Sichuan Province, China

Background: Recently, PCDHGB1 has been identified to be a novel causative gene of dystonia predominantly affecting the cervical muscles. However, no large cohort study has been conducted to confirm the association.

Objectives: We aimed to systematically evaluate the genetic associations of PCDHGB1 with dystonia in a large Chinese dystonia cohort.

Methods: We analyzed rare variants of PCDHGB1 in 878 dystonia patients with whole exome sequencing. The over-representation of rare variants in patients was examined with Fisher's exact test at allele and gene levels.

Results: A total of 28 rare variants in PCDHGB1 were identified in 59 individuals, including three frameshift substitution variants and 25 missense variants. Among the 28 rare variants, 19 variants were ultra-rare (MAF < 0.001) in the East Asian population from gnomAD and ChinaMAP, and 16 variants were predicted to be damaging by at least 5 in-silico prediction tools. Among the 59 variants carriers, 45 patients presented with isolated dystonia, and 14 presented with combined dystonia. Seven patients carried the frameshift substitution variant p.S178Cfs*21, 10 patients carried the variant p.M111V, including 3 patients from one family. At variant level, p.S178Cfs*21 was significantly associated with a higher risk of dystonia, while p.D591G and p.P773S were nominally associated with a higher risk of dystonia. Gene-based burden analysis detected enrichment of ultra-rare variants and ultra-rare damaging variants of PCDHGB1 in dystonia.

Conclusions: Our study supplemented the evidence on the role of PCDHGB1 in dystonia, and expanded the genotypic and phenotypic spectrum of PCDHGB1.

26

PLAUR Exacerbates Neuroinflammation in Diabetic Ischemic Stroke by Driving Neutrophil-Mediated Blood-Brain Barrier Disruption and Reprogramming Microglial Metabolism

Si-qi Ma[a], Yan Lu[b], long Wang[c], Shi-min Yin[d]

[a] The Postgraduate Training Base of Jinzhou Medical University ,The PLA Rocket Force Characteristic Medical Center, Beijing, China.
[bc]Department of Neurology, The PLA Rocket Force Characteristic Medical Center, Beijing, China.
[d] Department of pain treatment, the First Medical Center, Chinese PLA General Hospital, Beijing, China.

Background

Diabetic ischemic stroke can lead to more severe brain damage. Although the urokinase-type plasminogen activator receptor (PLAUR) is associated with inflammatory responses and cell migration, its specific role in diabetic stroke remains unclear.

Methods

We established a streptozotocin-induced diabetic tMCAO mouse model. PLAUR expression was analyzed using microarray, Western blot, and immunofluorescence. We assessed cerebral infarct volume, brain water content, neurological deficits, and BBB integrity. Neutrophil infiltration (flow cytometry), inflammatory mediators, microglial polarization, and metabolic reprogramming (glycolytic proteins, ECAR/OCR) were investigated in vivo and in vitro.

Results

PLAUR was significantly upregulated in the brain tissues of mice with diabetic stroke, with a predominant expression in microglia. Knockdown of PLAUR resulted in reduced infarct volume, promoted functional recovery, and preserved the integrity of the blood-brain barrier (BBB), which was attributed to the restoration of tight junction protein

expression and function.PLAUR enhances neutrophil infiltration and pro-inflammatory mediator release, with a consequent exacerbation of neutrophil extracellular trap (NET) formation.

Conclusion

PLAUR plays a crucial role in the neuroinflammatory process of diabetic ischemic stroke, exacerbating brain injury through mechanisms such as blood-brain barrier disruption, neutrophil recruitment, microglial polarization, and NETosis (neutrophil extracellular trap formation).

Key words

Diabetic Ischemic Stroke; PLAUR; Neutrophil; Blood-Brain Barrier; Neuroinflammation

27

Study on the Regulation of Amnesia and Its Mechanism by Circadian Rhythm Disorders in AD Mice Through the Hippocampal Bmal1-Rac1/Cdc42 Pathway

Jie Pan1,Tianyi Zhao[1],Qi Kong[1],Luyao Xu[1],Hanwen Deng[1],Rulin Zhao[1] Rongrong Mao[1*]

[1] School of Basic Medical Sciences,Kunming Medical University, Kunming, China

Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder characterized by memory loss as a core feature, with limited clinical treatments available. Circadian rhythm disturbances are common symptoms in AD patients, but it remains unclear whether they precede memory loss and their relationship to memory impairment. Bmal1 is a clock gene closely associated with circadian rhythms; its deletion leads to circadian rhythm disturbances and memory damage, though the underlying mechanisms are unknown. Ras-related C3 botulinum toxin substrate 1 (Rac1) and cell division cycle protein 42 (Cdc42) are important members of the small G protein family. Studies have found that in both AD patients and mice, hippocampal Bmal1 expression levels are significantly decreased, while Rac1 and Cdc42 activity is markedly increased, and increased Rac1/Cdc42 activity acceler.

28

Exercise Mitigates Memory LOSS in a Mouse of Alzheimer Disease Probably via Suppression of IRF8

Weiyuan Fang[1], Jiehong Huang[1], Lingjing Jin[1,#], Yunping Song [1,#]

[1].Department of Neurology and Neurological Rehabilitation, Yangzhi Rehabilitation Hospital (Shanghai Sunshine Rehabilitation Center), School of Medicine, Tongji University, Shanghai, China.

Objective: The typical pathology of AD patients involves abnormal deposition of β-amyloid protein (Aβ) in the brain. Its overactivation of glial cells and brain immune responses leads to irreversible neuronal damage and apoptosis, which is considered one of the mechanisms for the onset and progression of AD. In recent years, multiple studies have confirmed that running exercise training can, to a certain extent, slow down the progression of AD, together with improvements observed in inflammatory microenvironments. However, the specific molecular pathways remain to be investigated.

Methods: Nine-month-old APP/PS1 mice and C57 mice were divided according to body weight and cognitive memory ability into an AD non-exercise group (n=6), an AD exercise group (n=6), and a control group (n=6). The exercise regimen for the AD exercise group mice was as follows: initial speed 5 m/min for 1 minute, level one speed 10 m/min for 40 minutes, once a day, 5 days per week, for a duration of 3 months. During training, the other two groups of mice were placed in the same environment but did not undergo exercise intervention. After 3 months, Y-maze and novel object recognition tests were conducted on the mice, and brain samples were collected. Western blot was used to quantify Aβ expression in the brain, and immunofluorescence staining was used to analyze the number of microglia, chemokine receptor expression, and other factors in each group of mice.

Results: Analysis of the spontaneous alternation index in the Y-maze showed that, compared with wild-type mice, the AD non-exercise mice had a significantly lower index ($p = 0.002$), but it significantly recovered after 3 months of training ($p = 0.001$). The WB experiment results showed that the expression level of hippocampal β-amyloid protein in AD non-exercise mice was significantly higher than that in C57 mice ($p = 0.001$) and AD exercise mice ($p = 0.032$). Further analysis of hippocampal slices revealed no significant differences in the number of microglia among the three groups. However, the ratio of Iba-1/IRF8 double-positive cells to Iba-1 positive cells showed significant differences, with

this parameter being significantly higher in AD non-exercise mice compared to the C57 group ($p = 0.018$) and the AD exercise group ($p = 0.038$), respectively.

Conclusion: Three months of running exercise training improved memory in APP/PS1 mice and reduced β-amyloid deposition in the hippocampus. These effects may be related to the suppression of IRF8-mediated excessive immune responses. This study also proposes the possibility in regulating IRF8 to mitigate AD progression.

29

Encoding of Object Identity (‘what’) and Spatial Location (‘where’) During Action Selection in Larval Zebrafish

Yuxin Tong[1], Peixiong Zhao[1], Ling Fung Chan[1], Julie L. Semmelhack[1]

[1] Division of Life Science, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong S.A.R., China

Distinguishing ecologically relevant objects, such as prey and predators is critical for selection of appropriate hunting or defensive behaviors, and therefore for survival. While discrimination relies on specific visual features, how the brain integrates these features into object identity (‘what’) and coordinates them with spatial information (‘where’) to guide behavior remains unclear. Furthermore, it is unknown if the spatial maps within the optic tectum operate independently from object identity. To investigate these questions, we presented a battery of prey- and predator-like visual stimuli to systematically map feature tuning across sensory populations using volumetric calcium imaging. We identified two functionally and anatomically distinct neural classes encoding identity: prey identity neurons, distributed primarily in the pretectum and selectively tuned to ethological features such UV wavelengths and ON stimuli, and predator identity neurons, located mostly in the dorsal thalamus and nucleus isthmi, which are tuned to predator features and respond to large, dark silhouettes anywhere in the visual field. In contrast, location-encoding neurons were concentrated in the optic tectum. These neurons responded non-selectively to all stimuli within their visual field, and exhibited much smaller spatial receptive fields compared to identity neurons.

We next investigated how these circuits responded during a behavioral conflict paradigm where prey and predator stimuli were presented simultaneously. This condition resulted in the suppression of hunting behavior in favor of freezing. The behavioral shift was mirrored by a selective suppression of the pretectal prey identity neurons, whereas activity in thalamic predator neurons and tectal location-encoding neurons was preserved. We conclude that prey identity neurons are subject to targeted modulation in the presence of a predator, and may thus play a key role in the discrimination of prey objects. The functional segregation between location and prey and predator identity neurons allows the animal to maintain a precise spatial map of all relevant objects via the tectum, while selectively gating command signals through identity centers to determine the final behavioral output.

30

Explainable ML and Network Toxicology Reveal BaP-Driven Signatures in HICH

Sha Yang[1], Xiangqian Ren[3], Liangzhan Chu[3], Guoqiang Han[2], Jiqin Zhang[*4], Ying Tan[*2]

[1] Guizhou University Medical College, Guiyang 550025, Guizhou Province, China.
[2] Department of Neurosurgery, Guizhou Provincial People's Hospital, Guiyang, China
[3] Department of Neurosurgery, Affiliated Hospital of Guizhou Medical University, Guiyang, China
[4] Department of Anesthesiology, Guizhou Provincial People's Hospital, Guiyang, China

Objective: Benzo[a]pyrene (BaP), a ubiquitous polycyclic aromatic hydrocarbon, is pro-inflammatory and neurotoxic, but its mechanistic contribution to hypertensive intracerebral hemorrhage (HICH) remains unclear. We aimed to delineate BaP-associated pathogenic mechanisms in HICH and identify key molecular targets and immune pathways.

Methods: BaP-related targets were predicted using five databases (ChEMBL, PharmMapper, STITCH, SEA, TargetNet) and unified to official human gene symbols. Peripheral-blood transcriptomes from HICH patients and hypertensive controls were analyzed to define differentially expressed genes (DESeq2; FDR < 0.05, $|\log2FC| \geq 0.585$). Overlapping genes between BaP targets and HICH DEGs were functionally annotated (GO/KEGG) and prioritized using an explainable machine-learning framework (11 algorithms; 130 model configurations with nested cross-validation, strict quality control, and imbalance handling). The best model was interpreted by SHAP. Molecular docking evaluated BaP–protein binding. Immune infiltration was estimated by CIBERSORT, followed by gene–immune correlation analyses. GSVA/GSEA assessed pathway activities. An adverse outcome pathway (AOP) linking BaP exposure to HICH was constructed. In vitro, BV2 microglia were treated with hemin (ICH-like injury) with/without BaP (1–3 μM); cell viability (CCK-8) and qRT-PCR (cytokines and key genes) were assessed.

Results: We identified 247 predicted BaP targets and 4,564 HICH-associated DEGs; their intersection yielded 34 candidate mediators. The optimal diagnostic model (stepwise GLM + plsRglm) achieved AUCs of 0.986 (training) and 0.919 (validation) and selected eight core genes: PYGL, SRP54, CRAT, CD22, RET, CDK1, GPR35, and PIM1, with PYGL showing the highest SHAP contribution. Docking supported stable BaP binding to all eight proteins (binding energies −8.7 to −13.0 kcal/mol), strongest for CDK1, RET,

and PIM1. Immune deconvolution indicated increased neutrophils, activated dendritic cells, and γδ T cells, with reduced naïve B cells and Tregs in HICH; key genes (e.g., PYGL) correlated positively with pro-inflammatory innate immune subsets and negatively with adaptive/immunoregulatory cells. Enrichment analyses highlighted immune-regulatory and metabolic programs (e.g., antigen processing/presentation, PPAR signaling, detoxification-related pathways). In BV2 cells, BaP aggravated hemin-induced cytotoxicity and inflammatory cytokine transcription and amplified dysregulation of the eight targets.

31

Multiscale dynamic characteristics and neural circuit regulation of depressive disorder

Yu Yang [1,2,3#], Huixia Jia[1,2,3#], He Liu[1,2,3*]

[1] Department of Systems Science, Faculty of Arts and Sciences, Beijing Normal University, Zhuhai, 519087, China.
[2] International Academic Center of Complex Systems, Beijing Normal University, Zhuhai, 519087, China.
[3] School of Systems Science, Beijing Normal University, Beijing, 100875, China.

The role of the cerebrospinal fluid (CSF) in the pathogenesis of major depressive disorder remains poorly understood. Here, we report a pathogenic "Cortex-to-CSF" volume transmission axis triggered by chronic stress. We observed that stress induces an aberrant upregulation of epithelial marker genes in the choroid plexus. Crucially, during the pre-depressive prodromal phase, this is coupled with a massive reduction of cortical $GAD1^+$ inhibitory neurons and a depletion of their intracellular calcium buffer, Calbindin-D28k (CB). Rather than undergoing intracellular degradation, cytosolic CB leaks into the CSF, transforming from a neuroprotectant into an extracellular "depression-inducing" agent. Indeed, intracisternal injection of CB into naïve mice is sufficient to recapitulate depressive phenotypes, whereas removing most CSF from the ventricles prevents the transition to depression. These findings identify ectopic CSF-CB as a critical liquid-phase mediator, suggesting that depression involves a systemic self-intoxication mechanism initiated by cortical disinhibition.

32

Social Isolation Promotes the Burden of α-Synuclein Pathology via Suppressing Oxytocin Signaling and Glymphatic Influx

Jin-Bao Zhang[1,#], Ruo-Yu Li[1,#], Xi-Ning Ren[1,#], Yun-Jiao Zhou[1], Bing-Yu Li[1], Yun-Ping Song[1,*], and Ling-Jing Jin[1,*]

[1] Department of Neurology and Neurological Rehabilitation, Yangzhi Rehabilitation Hospital (Shanghai Sunshine Rehabilitation Center), School of Medicine, Tongji University, Shanghai, China.

Objective: Social isolation (SI) is associated with an increased risk of Parkinson's disease (PD), yet the underlying mechanisms remain elusive. Oxytocin (OXT), a neurohormone critical for social bonding and stress resilience, is suppressed under conditions of SI. Recent evidence suggests OXT possesses neuroprotective properties and plays a role in regulating waste clearance pathways. This study aimed to investigate whether SI exacerbates αSyn aggregation and dopaminergic neuron loss by impairing OXT-dependent glymphatic function, and to evaluate the therapeutic potential of OXT administration and exercise in counteracting this impairment.

Methods: A PD mouse model was generated through unilateral striatal injection of αSyn preformed fibrils (PFFs). Mice were subjected to either chronic social isolation or group housing. Motor behaviours, αSyn pathology, dopaminergic neuron integrity, and OXT levels were assessed 5 months post-injection. Glymphatic function was evaluated by analysing AQP4 polarity, fluorescent tracer clearance, and in vivo DCE-MRI. Rescue strategies involved intranasal OXT administration and voluntary treadmill exercise.

Results: Compared to PFFs group, the PFFs+SI group exhibited significantly more severe motor deficits, robust αSyn pathology, and approximately 30% greater dopaminergic neuron loss. Furthermore, SI disrupted the normal perivascular localization of AQP4, indicating a loss of AQP4 polarity. This structural impairment was associated with significantly reduced clearance of both fluorescent tracers and fluorescent-labeled αSyn PFFs from the brain parenchyma. ELISA revealed that SI markedly reduced OXT levels in brain regions including the hypothalamus and striatum. Intranasal OXT administration restored both AQP4 polarity and glymphatic tracer clearance, indicating that OXT deficiency mediates SI-induced impairment of glymphatic function. Additionally, voluntary treadmill exercise elevated endogenous OXT levels, reversed AQP4 polarity loss, and improved glymphatic influx in PD mice, as demonstrated by DCE-MRI.

Conclusion: We demonstrated that SI exacerbates PD pathology by impairing the glymphatic system in an OXT-dependent manner. Both OXT supplementation and exercise, which boosts endogenous OXT, represent promising therapeutic strategies for rescuing glymphatic function and mitigating disease progression in PD.

33

Inhibitory-Neuron-Specific Sonogenetics for Microcircuit Modulation

Dongshuai Zhao[1#], Quanxiang Xian[1#], Yong Wu[1], Langzhou Liu[1], Congmin Chen[1], Yizhou Jiang[1], Zihao Chen[1], Binghao Zhao[1], Min Su[1], Jianing Jing[1], Xiaohui Huang[1], Zhuohan Shi[1], Xinyi Zhao[1], Lei Sun[1]*

[1] Department of Biomedical Engineering, The Hong Kong Polytechnic University, Hong Kong SAR, 999077

A method to modulate neural microcircuits by precisely controlling inhibitory neurons would be a highly-desirable neuroscientific tool, but their relatively sparse distribution hampers development. Ultrasound neuromodulation through sonogenetics has emerged as a promising minimally-invasive approach, combining superior spatial scalability with cell-type specificity. In this study, we develop an inhibitory-neuron-specific sonogenetics method to effectively alter neuronal balance in microcircuits. Sonogenetic activation of somatostatin-expressing interneurons (SST-INs) in the microcircuits of primary motor cortex (M1) induced significant enhancements in calcium transients and c-Fos expression. Pyramidal neurons' calcium activity within microcircuits was suppressed by the induced SST-IN inhibitory dynamics, with sustained inhibition below baseline lasting over 20 seconds. Furthermore, in a Parkinson's disease mouse model, this approach effectively eliminated microcircuit hyperactivity and mitigated behavioral deficits. Basic biosafety evaluation showed no overt adverse effects. This work establishes a novel, cell-type-specific, and volume-targetable approach for precise microcircuit manipulation, offering potential for basic research and therapeutic development.

34

Deciphering the role of LRP2 endocytic activity in human neural crest specification

Alessia Petrella, Sher Min Mak, Izabela Kowalczyk, Henrike Scherrer, Narasimha Swamy Telugu, Sebastian Diecke, Stefanie Grosswendt, and Annette Hammes

Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany

The low-density lipoprotein receptor-related protein 2 (LRP2) is an essential endocytic receptor localized at the apical surface of the neuroepithelium during embryogenesis, playing a significant role in signal transduction and scaffolding at the base of the primary cilium. Pathogenic variants of LRP2 cause Donnai-Barrow syndrome (DBS) in humans, an autosomal recessive congenital disorder characterized by craniofacial anomalies and forebrain malformations. LRP2-deficient mouse models recapitulate many DBS phenotypes, offering a valuable tool for understanding their underlying mechanisms.
Previous work from our group has shown that LRP2 is essential for folate uptake through its interaction with the folate receptor FOLR1 in the neural plate, a process crucial for neural tube morphogenesis. We have also recently discovered that LRP2 deficiency disrupts neural plate border (NPB) specification and patterning.
The NPB, a transient but crucial stem cell niche, gives rise to neural crest cells, neuroepithelial cells, and non-neural ectoderm, contributing to multiple structures in the head. Loss of LRP2 not only leads to defects in NPB patterning, but also to neural tube closure anomalies and aberrant migration dynamics of cranial neural crest (CNC) cells, highlighting the importance of this endocytic receptor for establishing and maintaining proper NPB stem cell niche character.
We aim to further uncover the molecular mechanisms by which LRP2 regulates tissue patterning and morphogenesis in the NPB, as well as its influence on CNC cell fate. In vivo studies will employ high and super-resolution microscopy to explore the effect of LRP2 on cytoskeletal rearrangements as well as on cilia morphology and length. We will also investigate potential alterations in CNC cell migration in control and LRP2-deficient mice utilizing a Sox10cre/wt; Ai14fx/fx; Lrp2+/- reporter mouse line.
In vitro studies using human induced pluripotent stem cells (hiPSCs) differentiated into NPB and neural crest cells, will further explore the impact of pathogenic LRP2 variants and will provide insights into the transferability of the mechanisms identified in our animal models.

In addition, these human cell culture models will be used to unravel the influence of folate supplementation during early human neural tube development.

35

LRP2, the endocytic receptor deficiency: as a model for congenital brain disorders in hiPSC-derived neuronal cultures

Gökçe Nihan Yıldırım[1], Isabel Weig[1], Izabela Kowalczyk[1], Lea Nell Marcks[1], Sebastian Diecke[1], Agnieszka Rybak-Wolf[1], Valérie Dupé[2], and Annette Hammes[1]

[1] Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany
[2] Institut de Génétique & Développement de Rennes, Rennes, France

Pathogenic gene variants affecting the Sonic Hedgehog (SHH) signaling pathway lead to congenital brain malformations like holoprosencephaly (HPE). A poorly understood feature of these anomalies is their highly variable penetrance. Modifier genes contribute to this variability by conferring resilience or susceptibility to disease. In our earlier studies, we used mouse models of HPE with genetic deletion of LDL receptor-related protein 2 (LRP2), a SHH co-receptor. *Lrp2-/-* mutants of the C57BL/6N strain showed full penetrance for HPE, while those on the FVB/N strain were fully rescued. Transcriptome analyses identified strain-specific genes as candidate modifiers, including Unc-51 Like Kinase 4 (ULK4) and Pituitary Tumor-Transforming Gene 1 Protein (PTTG1), which we found to positively regulate SHH signaling and are novel components of primary cilia (Mecklenburg et al., 2021).
To further investigate SHH regulation in human brain development, we differentiated human-induced pluripotent stem cells (hiPSCs) from various donors and *Lrp2-/-* pathogenic mutants into dorsal and ventral neuronal precursor cells (d/vNPCs) and forebrain organoids. We found aberrant early cell specification and patterning in the neuronal cultures of *Lrp2-/-* pathogenic mutants. We concluded that different hiPSC lines and clones display variability in terms of susceptibility to disease-relevant disturbances. In addition, transcriptomic and morphological investigations of changes in primary cilia upon LRP2 loss. Our ongoing work aims to clarify how the loss of LRP2 influences SHH signalling and beyond in hNPCs and forebrain organoids.
To understand the insights of the human forebrain patterning during development, and therefore the variability in congenital diseases, hiPSC-derived NPCs and forebrain organoids are being used. Functional assays to elucidate the mechanism of modifiers on

SHH pathway regulation in the human context will help clarify the phenotypic variability of human neurodevelopmental disorders.

36

TENM4 is a major component of tethers required for touch sensation

Letizia Dalmasso[1], Angela Tzu-Lun Huang[1], Mohammed A. Khallaf[1], Emilie Nello[1], Séverine Kunz[2], Lorena Suarez Artiles[3,4], Philipp Mertins[4] & Gary R. Lewin[1,5]

[1] Molecular Physiology of Somatic Sensation, Max-Delbrück Center for Molecular Medicine (MDC), Berlin, Germany.
[2] Electron Microscopy, MDC, Berlin, Germany.
[3] Translational Cardiology and Functional Genomics, MDC, Berlin, Germany.
[4] Proteomics, MDC, Berlin, Germany.
[5] Charité Universitätsmedizin Berlin, Berlin, Germany.

Our ability to perceive touch depends on specialized mechanosensory neurons in the skin that detect nanometer-scale mechanical displacements and convert them into electrical signals. Key molecular components of this mechanotransduction process have recently been identified, including the mechanosensitive ion channels Piezo2 and Elkin1. However, the precise mechanisms by which mechanical forces are transmitted to and gate these channels remain poorly understood. We hypothesize that activation of these channels in response to tactile stimuli requires mechanical coupling to the extracellular matrix (ECM) via specialized protein tethers. In sensory neurons cultured on laminin, these tethers appear as electron-dense extracellular filaments bridging the gap between neurites and the matrix. In our search for the molecular identity of these structures, we have narrowed the list of candidates to a small group of ECM-associated proteins, with the transmembrane protein TENM4 emerging as the most compelling candidate. We have previously demonstrated that TENM4 is critically required for normal touch sensation in vivo, possibly by participating in the formation of these extracellular tethers through interactions with other ECM proteins in the skin. In the present study, we show that TENM4 localizes specifically to the tether structures in cultured sensory neurons, as revealed by immunogold labeling combined with transmission electron microscopy (TEM). Additionally, using affinity purification followed by mass spectrometry, we identified multiple ECM proteins as potential TENM4 interactors in the skin. These findings support a model in which TENM4 plays a central role in tether assembly and function by linking mechanosensitive ion channels to the ECM, thereby enabling efficient force transmission during tactile stimulation.

37

Epigenetic modulation by guadecitabine and its impact on Natural Killer cell biology: perspectives for neuro-oncology.

Mariangela Petito[1,2], Melania Grottoli[1,2], Laura Damele[1], Adriana Agnese Amaro[1], Francesco Reggiani[1], Chiara Vitale[1,2], Gabriella Pietra[1,2], Ulrich Pfeffer[1]

[1] IRCCS Ospedale Policlinico San Martino, Genoa, Italy.
[2] Department of Experimental Medicine (DIMES), University of Genoa, Genoa, Italy.

INTRODUCTION: Epigenetic dysregulation is an emerging key hallmark of cancer progression and metastasis that reduces immunogenicity and impairs recognition by the host immune system, thereby providing tumor cells with additional immune evasion pathways. Hypomethylating agents, such as guadecitabine, can increase tumor susceptibility to immunotherapy and modulate immune cell activity. Guadecitabine (SGI-110) is a next-generation prodrug composed of the hypomethylating dinucleotide decitabine (DAC) and deoxyguanosine, which is resistant to degradation by cytidine deaminase. Natural killer cells (NK) play a crucial role in cancer immunosurveillance, as they can directly attack and destroy tumor cells, leading to target cell lysis. In some types of hematological malignancies, decitabine induces antitumor immunity mediated by NK cells through increased production of IFN-γ. Although the effects of these epigenetic drugs have been more extensively studied in hematological malignancies, their impact on immune cell function and their clinical applicability in solid tumors remain poorly understood.
OBJECTIVE: To evaluate the effects of guadecitabine on the phenotype and function of natural killer cells and to explore its potential implications for immunotherapy in neuro-oncology.
MATERIALS and METHODS: To investigate the effects of guadecitabine on NK cell function, we isolated various subsets of mature circulating NK cells as well as NK cells derived from human umbilical cord blood hematopoietic stem cells (HSCs). NK cells derived from human peripheral blood mononuclear cells (PBMCs) were treated with the drug for two days in the presence of IL-2, while NK cells derived from hematopoietic stem cells (HSCs) were obtained after 30 days of culture with SCF, FLT3-L, IL-7, and

IL-15, either in the absence or presence of a single stimulation with the drug. We also performed gene expression analysis to obtain an overview of the transcriptional state.
RESULTS: The results obtained indicate that, during in vitro culture, the expression of KIR and CD16 was induced in mature NK cells treated with guadecitabine. Functionally, NK cells derived from PBMCs exposed to SGI-110 showed increased degranulation potential and upregulation of key genes involved in activation processes, suggesting enhanced functional activity following drug exposure. In hematopoietic stem cells, a single administration of the prodrug promoted differentiation toward $CD56^{+}$ NK cells. These cells were characterized by increased cytotoxicity, higher expression of KIR and CD16 receptors, and the ability to produce greater amounts of IFN-γ.

CONCLUSIONS and FUTURE PERSPECTIVES: Pharmacological modulation of tumor epigenetics by guadecitabine may not only affect tumor growth and vulnerability but also directly enhance immune system function. The results suggest that guadecitabine seems to be a promising epigenetic drug for the development of novel therapeutic protocols, including in neuro-oncology for tumors with highly immunosuppressive microenvironments.

38

Cognitive modes underlying attentional control deficits in schizophrenia: a functional magnetic resonance imaging (fMRI) study

Ava Momeni[1,2], Paul Metzak[3], Madeleine Evora[1,2], Aida Momeni[1,2], Amber Lu[1,2], Helen Hsiao[4], Todd S. Woodward[1,2]

[1] BC Mental Health and Addictions Research Institute, Vancouver, Canada
[2] Department of Psychiatry, Faculty of Medicine, University of British Columbia, Vancouver, Canada
[3] Department of Psychiatry, Hotchkiss Brain Institute, University of Calgary, Calgary, Canada
[4] Faculty of Medicine, University of British Columbia, Vancouver, Canada

The current functional magnetic resonance imaging (fMRI) study examined the anatomical and functional patterns associated with attentional control deficits in schizophrenia. Participants completed a trivalent task switching paradigm in which they alternatively completed three tasks (i.e., identify colour, parity, or letter case), while viewing stimuli with one (univalent), two (bivalent), or three (trivalent) dimension(s). This required participants to counteract their attentional capture by biasing their attention away from up to two task-irrelevant dimensions. Three cognitive modes emerged via constrained principal component analysis (CPCA): (1) Multiple Demand (MD), (2) Language (LAN), and (3) Default Mode B (DM-B). Relative to neurotypical participants, patients with schizophrenia showed impaired task performance, muted but sustained activation of the MD mode, hypoactivation of the LAN mode, and hyperdeactivation of the DM-B mode. Moreover, the muted and sustained activation of the MD mode was associated with higher scores on hallucinations and delusions, while the hypoactivation of the LAN mode and the hyperdeactivation of the DM-B mode were associated with higher scores on poverty of speech and flattened affect. Thus, attentional biasing impairments in schizophrenia may reflect reduced engagement of task-positive MD and LAN modes and excessive suppression of task-negative DM-B mode, with mode-specific associations to symptom severity.

39

Dysfunctional myelin exacerbates tau pathology and behaviour in a mouse model of tauopathy

Lina Komarek[1,2*], Andrew Octavian Sasmita[1,3*], Erinne Cherisse Ong[1], Anne-Fleur Wildenburg[1], Shuying Mao[1], Sophie Siems[1], Vasiliki-Ilya Gargareta[1], Veronika Hantakova[1], Constanze Depp[1], and Klaus-Armin Nave[1]

[1] Department of Neurogenetics, Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany
[2] Georg-August University, School of Science, Göttingen, Germany
[3] Department of Anatomy and Neuroscience, University College Cork, Cork, Ireland

Myelin is essential for rapid signal transmission and axonal support. Age-related myelin decline has been implicated in the progression of amyloid pathology in Alzheimer's disease (AD). We recently demonstrated that myelin dysfunction drives amyloidosis in AD mouse models, but its potential contribution to phosphorylated tau pathology has remained unclear. To address this, we combined a genetic mouse model of late-onset myelin dysfunction (Plp-/Y) with a tauopathy model (PS19). Double-mutant mice exhibited markedly worsened behavioral phenotypes compared with single mutants, most prominently in anxiety-related measures, short-term memory, and motor function. These mice also showed an increased burden of phosphorylated tau, particularly in white-matter tracts, accompanied by elevated microgliosis. Correlation analyses indicated that gliosis was more strongly associated with behavioral impairment than phosphorylated tau load. Moreover, we observed increased numbers of p-tau–positive neurons in the spinal cord, correlating with motor deficits. Notably, even Plp-/Y single mutants—i.e., without the human tau transgene—displayed p-tau inclusions, a phenomenon absent in 5xFAD mice, a widely used amyloidosis model. This suggests that myelin dysfunction is sufficient to induce neuronal p-tau inclusions in this model, whereas amyloidosis alone (in 5xFAD mice) is not. In contrast, amyloid pathology in 5xFAD mice further exacerbated p-tau accumulation when combined with Plp-/Y mutations. Together, these findings indicate that myelin dysfunction promotes the generation and propagation of phosphorylated tau pathology, which is linked to axonal injury, gliosis, and behavioral decline. Thus, myelin dysfunction in the aging brain emerges as a potential risk factor not only for amyloidosis but also for tauopathy, as seen in Alzheimer's disease and frontotemporal dementia.

40

Accelerated brain aging in those with major depression and benzodiazepine use in older but not younger patients receiving repetitive transcranial magnetic stimulation

Sunavsky, Adam[1]; Marzbani, Hengameh[1]; Blumberger, Daniel[2]; Downar, Jonathan[2]; Vila-Rodriguez, Fidel[1]

[1] University of British Columbia
[2] University of Toronto

Machine learning (ML) techniques have utilized neuroimaging to measure 'brain age' in major depressive disorder (MDD). However, most studies offer a cross-sectional approach and do not assess change after treatment such as repetitive transcranial magnetic stimulation (rTMS). Here, we assess the brain-predicted age difference (brain-PAD; the difference between chronological age and estimated brain age), in a large cohort (N = 655) of those with MDD pooled from three clinical trials, before and after rTMS treatment, using two different ML algorithms. We tested for age-group differences (<60 vs. ≥60) in brain-PAD and determined whether brain-PAD decreased following rTMS or was associated with treatment response while controlling for clinical and demographic variables. Finally, we tested whether treatment response could predict brain-PAD. Brain-PAD was higher in the older (M = 2.46, 95% CI [0.58, 4.34]) relative to younger (M = −1.78, 95% CI [−2.60, −0.95]; p < 0.001) age group while controlling for chronological age; this finding was replicated using p < 0.10. Contrary to our hypothesis, younger MDD patients had an average brain-PAD change of +0.41 years after rTMS treatment (within subjects analysis); this finding was not replicated. Clinically, benzodiazepine use was associated with older appearing brains in the older age group and with lower rTMS response in the younger age group. This study highlights differential aging trajectories in younger relative to older MDD populations and the impact that benzodiazepines may have on accelerated brain aging and lower rTMS response.

41

Model of analysis of gait parameters and embodiment in exoskeleton control: a pilot study

M. Aguiar[1,2], D. Matos[3], A. Perrotta[4], M. Pais-Vieira[1], C. Pais-Vieira[5]

[1] Department of Medical Sciences, University of Aveiro,
[2] Graduate School of Neurosciences, LMU,
[3] ID+, Polytechnic Institute of Cavado and Ave,
[4] Department of Informatics and Engineering, University of Coimbra;
[5] Centre for Interdisciplinary Research in Health, Catholic University of Porto

Real-time tactile feedback during exoskeleton operation has been previously used for neurorehabilitation purposes, particularly through the delivery of tactile stimuli to the forearms in spinal cord injury patients when the soles of the exoskeleton touch the ground. It is not known, however, if delivering this type of feedback in control subjects affects exoskeleton operation and embodiment.

This project aims to determine if tactile feedback delivered to the forearm (FFF) affected gait parameters and embodiment. For this, gait parameters were extracted from a video. Gait parameters, users' preference and self- reported embodiment parameters (Usability, Perceptibility, Acceptability, Functionality, Fatigue, Stress, Energy Expenditure, and Attention) were then compared, in a small sample of control subjects (N=7) performing in an exoskeleton benchmarking scenario with and without tactile feedback. After each run, a brief interview was also conducted to determine if users preferred controlling the exoskeleton with or without real-time tactile feedback.

During the interviews 80% (4/5) of the subjects reported that using the tactile feedback facilitated exoskeleton control. Analysis of global embodiment index revelled embodiment also increased for 80% (4/5) of participants, supporting the results of the brief interviews. While most gait parameters didn't significantly change between conditions, WR decreased for 80% (4/5) of participants in the FFF condition. Additionally, step time asymmetry was lower in the FFF condition when compared with the control, becoming close to zero.

These results support the notion that subjects prefer controlling an exoskeleton that delivers real-time FFF even though no significant differences were present in gait and embodiment parameters. Future studies, with larger samples and additional neuropsychological measurements, will be critical to determine to which extent FFF affects exoskeleton control.

42

Pharmacological Modulation of Reward and Aversion Discounting by Amphetamine and Psilocybin in Rats

Adeoye A. Ewedemi, Marcus W. Meinhardt, Rainer Spanagel, Wolfgang H. Sommer

Institute of Psychopharmacology, Central Institute of Mental Health Mannheim, University of Heidelberg, Germany

Reward and aversion discounting capture fundamental components of value-based decision-making under uncertainty and delay. Although psychostimulants and psychedelics are known to alter valuation and cognitive flexibility, their effects on these two discounting processes have not been fully understood in animal models. Here, we investigated the effects of amphetamine and psilocybin on reward and aversion discounting in rats using established behavioural paradigms.

In the first experiment, rats were administered amphetamine (0.25mg/kg or 0.5mg/kg) or saline 30 minutes prior to performing reward and aversion discounting sessions assessing sensitivity to delayed reward (0.2% Saccharin solution) or delayed negative (foot shock) outcomes. In the second experiment, rats completed the same tasks following administration of psilocybin (2mg/kg) or saline under matched experimental conditions. Discounting behaviour was assessed using choice preferences across delay conditions, and discounting parameters indexing sensitivity to delayed outcomes were quantified.

Amphetamine significantly reduced reward discounting and produced similar effects on aversion discounting. In contrast, psilocybin showed no changes across both reward and aversion discounting. These findings demonstrate that distinct classes of psychoactive compounds differentially influence reward- and aversion-related decision making behaviour in rats.

Overall, this work emphasizes the importance of dissociating reward and aversion-related decision making in preclinical models and provides a behavioural framework for connecting pharmacological manipulation to underlying neural valuation processes.

43

From Cells to Circuits: Molecular and Morphological Characterization of NDNF Interneurons in the Murine Basolateral Amygdala and Their Role in Fear Regulation

Maria Fernanda Frutos Marquez[1,2], Ylenia Mastrodicasa[1,2], Rüdiger Klein[1,3]

[1] Max Planck Institute for Biological Intelligence, Department of Molecules – Signaling – Development, Martinsried, Germany
[2] Graduate School of Systemic Neurosciences, Ludwig-Maximilians-Universität, Munich, Germany
[3] Lead contact

The murine basolateral amygdala (BLA) consists of roughly 80% excitatory neurons and 20% inhibitory interneurons (INs), with their precise coordination playing a key role in regulating aversive memory consolidation, emotional processing, and motivation-related behaviors. While numerous studies have deepened our understanding of excitatory BLA circuits, our knowledge of inhibitory microcircuits, and the molecular mechanisms regulating excitatory activity, remains limited. Within the BLA's inhibitory population, neurogliaform cells (NGFCs) are known to regulate the precise timing of excitatory firing. In this study, we characterize neuron-derived neurotrophic factor (NDNF)-expressing neurons in the BLA (BLANdnf). Using high-resolution RNA-fluorescence in situ hybridization (RNA-FISH) with multiplexed Hybridization Chain Reaction™ (HCR), we found that BLANdnf neurons represent a sparse population without segregation along the anteroposterior, mediolateral, or dorsoventral axes of the BLA. Additionally, we see that BLANdnf are predominantly GABAergic, with 93.5% co-expressing Gad1. BLANdnf also expressed significant levels of key NGFC markers, such as Reln, Lamp5, and Npy, while expressing low levels of other main interneuron class markers, Sst and Pvalb. These results confirmed that BLANdnf represent a distinct interneuron population in the murine BLA. Finally, 3D morphological reconstruction revealed BLANdnf neurons to have small, round somata and dense dendritic arborizations, consistent with NGFC morphology. These findings help us understand how BLANdnf neurons form networks with principal neurons through their NGFC-like morphology, opening further studies for their regulatory role in BLA behaviors.

44

Establishment of in vitro models to explore mechanisms of hypoxia resistance in the naked mole-rat brain

Paraskevi Kakouri[1], Alice Rossi[1], Vladimir Jovanović[2], Oliver Popp[1], Katja Nowick[2], Gary R. Lewin[1]

[1] Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), 13125 Berlin-Buch, Germany
[2] Institut für Biologie, Freie Universität Berlin, Berlin, Germany

Naked mole-rats (NM-Rs) show extraordinary tolerance to hypoxia in vivo, yet the cellular and molecular mechanisms underlying this trait remain poorly understood. To address this, I established and characterized induced neuronal and primary astrocytic NM-R cultures for comparative studies with mouse. Functional experiments revealed increased survival in both NM-R cell types upon oxygen deprivation, with the effect being more pronounced in astrocytes. Preliminary proteomic profiling under normoxia and hypoxia revealed divergent responses: while mouse astrocytes mounted a broad HIF-1α–driven upregulation of glycolysis, translation, proteasome and stress pathways, the NM-R astrocytic proteome remained remarkably stable and displayed only selective changes. These included differential modulation of proteostasis machinery, as well as differences in calcium handling and secretory pathways. Complementary work in NM-R fibroblasts identified OPA1 as a potential orchestrator of hypoxia resilience via mitochondrial adaptation. NM-Rs not only express higher levels of this protein but also harbor a unique isoform predicted to stabilize mitochondrial cristae junctions and potentially delay initiation of cell death under hypoxic conditions. Together, these findings highlight cellular and mitochondrial strategies contributing to NM-R hypoxia resilience and point to potential targets for conditions associated with oxygen deprivation.

45

OnTrack: An intraoperative evaluation of tractography

J. H. Reilly[1, 2], S. Vellmer[1, 3], P. Vajkoczy[1], T. Picht[1, 2, 3], J. Onken[1]

[1] Department of Neurosurgery, Charité - Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Charitéplatz 1, 10117 Berlin, Germany
[2] Einstein Center for Neurosciences, Charité - Universitätsmedizin Berlin, Corporate Member of Freie Universität zu Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Charitéplatz 1, 10117 Berlin, Germany
[3] Cluster of Excellence: "Matters of Activity. Image Space Material", Humboldt University, Unter den Linden 6, 10099 Berlin, Germany

Background. Tractography allows the visualisation of white matter tracts revealing their spatial relation to pathologies for surgical planning and risk stratification. In neurosurgical practice, diffusion tensor imaging (DTI)-based tractography is often used due to its availability. Alternative approaches developed to account for complex fibre arrangements, such as fibre orientation distributions (FOD)-based tractography, may improve accuracy. Both DTI- and FOD-based approaches traditionally use anatomical landmarks to define seeding regions. However, tractography can also be created using functionally defined cortical areas from which to propagate streamlines. It remains uncertain which tractography method has the highest fidelity to the underlying anatomy due to the lack of a ground truth.

Methods. 22 patients undergoing surgery for tumours estimated to lie within 15mm of the corticospinal tract (CST) where prospectively recruited. Intraoperatively, direct subcortical stimulation of the corticospinal tract was used and motor evoked potentials recorded in the face, back, limbs, feet, and hands yielding positive and negative stimulation points. Navigated ultrasound was used to correct for brain shift. Postoperatively, eighteen different tractography renditions of the CST were created per patient. For each tractography method, distances between the stimulation points and tractography were measured providing information about missing and false-positive parts of tractography. A clinical motor assessment was performed pre-operatively, at discharge, and at 3-month follow-up.

Results. After surgery, there was no significant worsening of NIHSS and KPS scores at three months follow-up ($p>.05$). A total of 82 stimulation points and 288 tractographies were included in the final analysis. High accuracy was achieved by FOD-based tractography. Anatomical- and function-based tractography achieved comparable

accuracy scores. After applying a geometric optimisation criterium to all tractography methods, the accuracy scores were improved indicating the necessity to apply refinement criteria to tractography.

Conclusion. This study found that geometrically refined tractography had the highest correspondence to the surgically-relevant intraoperatively identified corticospinal tract. A quantified evaluation of tractography after brain-shift correction may inform the selection of tractography methods to support surgical planning and risk stratification and may improve the balance between extent of resection and preservation of function.

46

The Role of MAST2 in Neurodevelopment and Disease

Tabea Sophie Wabnitz[1*], Alexandra Catalina Vilceanu[1*], David Anthony Keays[1, 2]

[1] Graduate School of Systemic Neurosciences (GSN), Ludwig-Maximilians-Universität (LMU) München, Germany
[2] Department of Physiology, Development and Neuroscience, University of Cambridge, UK

Neurodevelopment is a very intricate and highly regulated series of cellular events. Thus, it is susceptible to errors that can lead to a wide range of conditions referred to as neurodevelopmental disorders. This study investigates a novel gene thought to be implicated in neurodevelopmental disorders: MAST2. MAST2 is part of the microtubule-associated serine-threonine kinase (MAST) family of proteins. MAST kinases are thought to play a role in brain development, as recent research shows mutations in MAST1, MAST3, or MAST4 can lead to neurodevelopmental disorders such as the mega-corpus callosum syndrome or developmental epilepsy. Despite being discovered more than 30 years ago, little is known about the function of MAST2, or whether it, too, might be involved in neurodevelopmental disorders. Mast2 has been shown to be highly expressed in the developing mouse cortex, especially in mature neurons rather than in neuronal progenitors. This pattern is consistent with a gene involved in brain development.

In this study, we examined a MAST2 mutation identified in a patient suffering from developmental and epileptic encephalopathy, autism spectrum disorder, and intellectual disability: MAST2 L1191Wfs*92. This study aims to employ a mouse model (Mast2 L1196Wfs*92) to examine whether this mutation affects brain anatomy. To this end, we developed a neuroanatomical phenotyping pipeline for Nissl-stained adult mouse brain sections and performed an analysis on wild-type, heterozygous, and homozygous Mast2 L1196Wfs*92 mutant mice.

Consistent with the patient´s normal MRI, we could not detect gross neuroanatomical malformations in Mast2 mutant mice. However, we observed a slight reduction in cortical thickness and a potential distortion in cortical layer organization. Further, we detected a decrease in CA1 pyramidal layer thickness in Mast2 L1196Wfs*92 mice, although it did not reach statistical significance. Given the potentially small effect size of a Mast2 mutation on brain anatomy, further investigations are required to substantiate our findings.

47

Dynamic integration of dissociable value codes for reward and information in the human prefrontal cortex

Keyu Hu[1], Changlin Bai[1], Yi Yao[2], Haiyan Wu[1*]

[1] Centre for Cognitive and Brain Sciences and Department of Psychology, University of Macau, Macau, China
[2] Department of Functional Neurosurgery, Xiamen Humanity Hospital, Fujian Medical University, Fujian, China

Balancing the exploitation of known rewards with exploration for new information is a core challenge in decision neuroscience. While the prefrontal cortex is critical for this process, how its subregions dynamically cooperate to mediate this balance remains unclear. Here, we recorded intracranial stereo-electroencephalography (SEEG) from 17 human participants performing a gambling task that orthogonalized expected reward and information gain. Using reinforcement learning-based computational modeling, we confirmed that participants assigned intrinsic value to information. Time-frequency analyses identified functionally dissociable value representations: while ventromedial prefrontal cortex (vmPFC) activity encoded the integrated value of the current option, anterior cingulate cortex (ACC) activity tracked reward and information value in a distinct manner.

Network dynamics analysis revealed that undirected Theta synchronization (PLV) supported reward integration, whereas directed information flow (PSI) showed that the vmPFC initiates exploration by driving the ACC. Furthermore, Phase-Amplitude Coupling (PAC) identified specific gating mechanisms: a bottom-up striatal-cortical coupling encoded absolute motivational gain, while a top-down ACC Beta-Gamma coupling facilitated deliberation during high-conflict exploration. Crucially, state-space analysis of neural population dynamics confirmed a computational double dissociation: the vmPFC functioned as a value integrator where representations sharpened over time, while the ACC functioned as an information differentiator, with its neural state velocity precisely tracking the theoretical rate of information gain.

Collectvely, these results map a hierarchical architecture where exploration is initiated by value regions, gated by oscillatory control, and realized through a dynamic interplay between the integral of value and the derivative of information.

48

Metabolic modulation supports the neuroprotective effect of synaptic silencing during microglia neurotoxicity

Amr Elgez [1,2], Andrea Lewen[1], Lena Noack[1], Babak Khodaie[1], and Oliver Kann[1,2]

[1] Institute of Physiology and Pathophysiology, Heidelberg University, D-69120 Heidelberg, Germany
[2] Interdisciplinary Center for Neurosciences (IZN), Heidelberg University, D-69120 Heidelberg, Germany

Microglia, the resident CNS macrophages, maintain tissue homeostasis through continuous surveillance, synaptic remodeling, debris clearance and immune defence. Upon infection or injury, microglia are rapidly activated to efficiently limit the damage and restore homeostasis. However, dysregulated or chronic microglia activation is increasingly recognised as a key contributor to the aetiology and/or progression of several neurological disorders. Interestingly, different proinflammatory microglia phenotypes contribute variably to neuronal network dysfunction, with highly activated microglia triggering severe network disruption and neurodegeneration. Whether such disrupted network activity itself contributes to the neurodegenerative process remains unclear. Our hypothesis is that proinflammatory microglia drive pathological network activity, which in turn exacerbates neuronal metabolic and oxidative stress.

Rat organotypic hippocampal slice cultures (OHSCs), which permit chronic microglia activation and the experimental induction of neuronal network oscillations, were exposed to interferon-gamma (IFNγ) plus the TLR4 ligand lipopolysaccharide (LPS) or to the TLR7/8 ligand resiquimod (R848) plus LPS to evoke different levels of microglial activation and neuronal network dysfunction. A blocker cocktail (TTX, CNQX, D-AP5) was applied to inhibit neuronal action potentials and glutamatergic synaptic transmission during microglia activation, while glucose availability was varied to assess the contribution of metabolic support. Local field potential (LFP) recordings in CA3 region, biochemical assays—including LDH (cell death marker), nitric oxide (NO; Griess reaction) and proinflammatory cytokines TNF-α and IL-6 (ELISA)— and immunostaing were performed to evaluate neuroinflammatory responses.

LFP data revealed a significantly reduced fraction of slice cultures showing no activity in the presence of IFNγ+LPS+blocker cocktail compared to IFNγ+LPS. Interestingly, adding higher glucose concentration in the culture medium further reduced this fraction, and notably, a subset of slice cultures expressed gamma oscillations as an indication of preserved network activity. These results were further confirmed by the significant reduction in IL-6 and LDH activity in the collected culture medium and the wide preservation of parvalbumin-positive GABAergic interneurons, which are critical for the generation and maintenance of cortical network rhythms. Notably, these interneurons were entirely degenerated in slice cultures exposed to IFNγ+LPS. Applying the blocker cocktail plus glucose in slice cultures with R848+LPS-moderately activated microglia showed a significant decrease in slice cultures exhibiting bursts compared to R848+LPS. In both experimental paradigms, the blocker cocktail increased NO levels, indicating possible neuronal inhibitory signalling to microglia. However, TNF-α remained unchanged, implying that cytokine production is not uniformly modulated by metabolic modulation. Our findings suggest that targeting both metabolic deficit and excitatory drive may more effectively suppress neurotoxicity induced by proinflammatory microglia.

49

Repetitive mild brain injury affects microglia-mediated excitatory synapse removal differentially in male and female mice

Stephanie P Dzhuleva [#, 1, 2] ,Maryam Chahin [1, 2, 3], Julius Mutschler [#, 1, 2], Clara Dieterle [1, 2], Leidy Reyes Jimenez [1, 2], Srijan Raj Bhattarai [1, 2], Valerie Van Steenbergen [1, 2], Florence M Bareyre [4, 5, 6]

[1] Institute of Clinical Neuroimmunology, University Hospital, LMU Munich, Munich, Germany.
[2] Biomedical Center Munich (BMC), Faculty of Medicine, LMU Munich, Planegg-Martinsried, Germany.
[3] Graduate School of Systemic Neurosciences, LMU Munich, Planegg-Martinsried, Germany.
[4] Institute of Clinical Neuroimmunology, University Hospital, LMU Munich, Munich, Germany. florence.bareyre@med.uni-muenchen.de.
[5] Biomedical Center Munich (BMC), Faculty of Medicine, LMU Munich, Planegg-Martinsried, Germany. florence.bareyre@med.uni-muenchen.de.
[6] Munich Cluster of Systems Neurology (SyNergy), Munich, Germany. florence.bareyre@med.uni-muenchen.de.

Traumatic brain injury (TBI) is an acquired brain injury resulting in temporary or permanent brain damage ranging from mild to severe impairments, affecting millions of people worldwide. Neuroinflammatory response is a complex and hardly predictable manifestation of mild TBI (mTBI), influenced by a variety of factors such as sex, age or the presence of pre-existing conditions. In the case of repetitive mTBI, this successive brain damage may lead to persisting symptoms characterized by cognitive and motor deficits over time, with the possibility of progression into serious medical conditions, such as chronic traumatic encephalopathy (CTE). This is particularly translatable to contact sport players and people from special occupations that are more prone to sustaining multiple traumatic brain insults. The goal of this project was to examine the sex differences between male and female mice with regards to excitatory synapse engulfment by microglia upon TBI, either single or repeated (three times) with 48-hours interval between the hits. The study was conducted using 13 female and 13 male mice, distributed within three conditions – mice that have not experienced any brain trauma (SHAM), mice that have sustained a single brain injury (1 TBI) and mice that have undergone three repetitive hits (3 TBI). Results demonstrate that there is an increased probability of observing chronic dysfunctions as a consequence of repetitive mild brain injury by displaying increased microglia-mediated synaptic engulfment upon 3 TBI in mice. Furthermore, it is important to consider the sexual dimorphic character of these responses. Female microglia tend to have heightened phagocytic activity when compared

to male microglia upon TBI but also under normal physiological conditions, suggesting that sex-related differences in microglia phenotype are conserved in pathological conditions and that the reason for observing these differences is not sustaining a traumatic injury per se. However, studying these differences in brain structure and function between sexes already during development might explain how sex influences susceptibility and incidence of neurological disorders and potentially pave the way towards a better understanding on how to alleviate and prevent consequences of TBI.

50

Single-synapse glutamate imaging in the mouse brain in vivo

Chen Y, Kloos M, Zhang Y, Konnerth A

Institute of Neuroscience, Technical University of Munich, Munich, Germany

Glutamate is the primary excitatory neurotransmitter in the central nervous system and plays a critical role in sensory processing, learning, and memory. Traditional methods for monitoring glutamate levels in the brain include in vivo microdialysis and electrochemical electrodes; however, neither approach can resolve glutamate concentrations at the synaptic scale. Genetically encoded glutamate sensors enable cell-specific investigation of glutamate changes. However, all existing glutamate sensors suffer from a low signal-to-noise ratio and slow time course, making it impossible to probe glutamate transmission at single synapses in the brains of live animals. Here, we report the performance of the recently developed glutamate sensors iGluSnFR3 and iGluSnFR4 in mouse V1, demonstrating their fast speed and high fidelity in detecting synaptic glutamate signals. We selectively expressed iGluSnFR3 and iGluSnFR4 in functionally defined neurons in the mouse primary visual cortex (V1), cerebellum, and hippocampus using single-cell electroporation. In orientation-tuned L4 neurons in V1, iGluSnFR3 and iGluSnFR4 reported robust orientation-tuned (cortical origin) and non-tuned (thalamic origin) synaptic activity. In cerebellar Purkinje cells, iGluSnFR3 revealed distinct patterns of parallel and climbing fiber activity. In hippocampal CA1 pyramidal neurons, iGluSnFR3 uncovered a high prevalence of synaptic clustering on basal and apical dendrites.

51

Therapeutic Use of Psilocybin in Depression: A Systematic Review of Clinical Evidence

Filipe Reis Teodoro Andrade [1], Tobias Buchborn [1], Gabriel Thalheimer [3], Marcus Meinhardt [1], Samia Joca [2],and Rosa Maria Martins de Almeida [3]

[1] Department of Psychopharmacology, Central Institute of Mental Health (ZI) - Germany
[2] Department of Biomedicine - Forskning og uddannelse, Vest - Aarhus University - Denmark
[3] Department of Developmental and Personality Psychology, Laboratory of Experimental Psychology, Neuroscience, and Behavior (LPNeC), University Federal of Rio Grande do Sul (UFRGS), Brazil.

Major depressive disorder (MDD) is a significant public health concern, and current treatments often have limitations in effectiveness and adherence. Psilocybin, a psychedelic compound found in certain mushrooms, is being explored as a potential treatment for depression. It primarily acts through the serotonin 5-HT2A receptor but interacts with 5-HT1A and 5-HT2C receptors. Its precise mechanisms remain under investigation. Objectives: (1) To consolidate evidence on psilocybin's efficacy and safety for depression, and the role of 5HT2a (2) to identify limitations in the literature, and (3) to highlight areas needing further research. Methods: This systematic review follows PRISMA guidelines and analyzes 22 studies, including randomized controlled trials (RCTs) and open-label studies. The studies cover various populations, including individuals with treatment-resistant depression, different dosing regimens, and adjunctive therapies. Results: Psilocybin therapy shows substantial and rapid antidepressant effects, often after one or two sessions with psychological support. Improvements are sustained for weeks or months in many cases. Psilocybin is generally well-tolerated, with mild adverse effects such as anxiety during administration and transient headaches, which are manageable in controlled settings. Conclusions: Psilocybin demonstrates promise as a novel treatment for depression, especially for individuals unresponsive to conventional antidepressants. Further research is needed to refine dosing, explore long-term effects, and understand its mechanisms of action.

52

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

Ketrin Dimco*[1,2] , Rüstem Yilmaz[1] , Gentiane Krasniqi[4] , Carsten Hopf[4] , Philipp Koch[2] , Rosanna Parlato[1] , Jochen Weishaupt[1,3]

[1] Division of Neurodegenerative Disorders, Department of Neurology, Mannheim Center for Translational Neurosciences, Medical Faculty Mannheim of Heidelberg University, Mannheim, Germany
[2] Hector Institute of Translational Brain Research, Central Institute of Mental Health, University of Heidelberg/Medical Faculty Mannheim, Mannheim, Germany
[3] Department of Neurology, University Clinic Ulm, Ulm, Germany
[4] Center for Mass Spectrometry and Optical Spectroscopy, Technische Hochschule Mannheim, Mannheim, Germany

Homozygous mutations in myogenesis-regulating glycosidase (MYORG) gene lead to primary familial brain calcification (PFBC), characterized by bilateral intra- and perivascular calcifications in various brain regions. MYORG is a putative glycosidase in the endoplasmic reticulum (ER) potentially involved in post-translational modifications. This study aims to address the common pathomechanisms through which different MYORG mutations lead to PFBC, the cell-autonomous and non-cell autonomous function of MYORG in astrocytes, and the morphological, molecular and functional consequences of MYORG mutations. MYORG wild-type (WT) and missense mutant transgenes were overexpressed in HEK293T and immortalized astrocytes cells for western blot and immunostaining analysis. Cycloheximide chase assay was performed to determine MYORG protein stability and halflife, while MG132 treatment was utilized to investigate the degradation mechanism of these variants. Additionally, primary astrocyte cultures isolated from MYORG p.Ile658Thr mutant pups were analysed to delineate morphological and molecular changes induced by the mutation. Our data shows that mutant MYORG transgenes display decreased MYORG expression levels and impaired protein stability, with accelerated proteasomal degradation. Concurrently, mutant astrocytes showed impaired autophagic flux, elevated basal levels of p62/SQSTM1 and LC3B II, reduced AKT phosphorylation, and diminished activity of the mammalian target of rapamycin complex 1 (mTORC1) signalling pathway. Lastly, astrocytes carrying the MYORGI658T mutation exhibited lysosomal clearance deficits and decreased global N-glycosylation based on matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) analysis. Altogether, these insights into pathomechanisms provide a foundation for future therapeutic strategies targeting PFBC.

53

Microglia increase neuronal electrical activity along the hippocampal–cortical axis without altering theta or ripple events

Yan Zhou[1], Zhebing Ren[2], Fan Zeng[3], Wenqi Luo[4], Helmut Kettenmann[3,5], Xianyuan Xiang[3], Kaichuan Zhu[1,2]

[1]The Brain Cognition and Brain Disease Institute, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China
[2] Brain Science infrastructure, Brain Cognition and Brain Disease Institute, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences
[3] Bio-X International Institute, Faculty of Life and Health Sciences, Shenzhen University of Advanced Technology, Shenzhen, China
[4] Shenzhen University of Advanced Technology, Shenzhen, China
[5] Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany.

Microglia sense neuronal activity through a broad repertoire of receptors and, in turn, feed back to the neuronal network by synaptic pruning during development and plasticity. As a consequence, microglial activity may have a general impact on brain function such as rhythms of brain activity. To test this hypothesis, we performed longitudinal in vivo recording of electrical activity in adult mice before, during, and after reversible microglia depletion. Microglial cells were depleted by feeding mice for 14 days with PLX5622, a CSF1R inhibitor and the microglia loss was verified histologically. 14 days after the drug withdrawal microglial had repopulated these brain regions. In naïve, microglia-depleted and microglia-repopulated periods, local field potentials and single units were recorded simultaneously from dorsal CA1 regions of the hippocampus and neocortex using 64-channel silicon probe. Continuous locomotor tracking enabled the distinction between periods of locomotor activity and immobility. Across hippocampus and cortex, microglia depletion preserved the canonical locomotion-linked theta regime: running speed, theta frequency, and theta power were unchanged. During immobility, sharp-wave ripples were likewise stable, with no detectable differences in ripple rate, duration, ripple frequency, or amplitude. In contrast, microglia depletion resulted in a significant increase in spiking activity. Across 1,573 hippocampal neurons (1,033 pyramidal cells; 540 interneurons), firing rates were elevated during PLX5622 treatment relative to the microglia-repopulated period in both the running and immobility phases. Behaviorally, Y-maze alteration was unaffected, whereas novel object recognition discrimination increased during PLX5622 treatment. Together, these results suggest that microglia dampen

neuronal firing across hippocampal–cortical networks while leaving the large-scale oscillatory scaffold—theta rhythm during locomotion and ripples during immobility—remarkably intact, revealing a dissociation between rhythmic architecture and microglia-dependent control of excitability and cognition.

54

Decoding Hand Motor Cortex Activation from High-Density fNIRS Using a Standard GLM Framework

Tomas Codina 1,2 , Lilli Kießling 1,2 , Carlo Röpke 1,2 , Bilal Siddique 1,2 , Pavel Syarov 1,2 , Alexander von Lühmann 1,2

[1]Intelligent Biomedical Sensing (IBS) Lab, Technische Universität Berlin, 10587 Berlin, Germany
[2]BIFOLD – Berlin Institute for the Foundations of Learning and Data, 10587 Berlin, Germany

Functional near-infrared spectroscopy (fNIRS) is becoming increasingly popular in neuroscientific research due to its usability in naturalistic environments and its ability to detect hemodynamic brain responses similar to those measured by fMRI. However, the reliability of fNIRS devices is challenged by motion artifacts, physiological confounders, sparse channel spacing, and the general issue of spatiotemporal reconstruction. Here, we present our laboratory's approach to intelligent biomedical sensing and preliminary results from a multimodal, co-localized high-density fNIRS-EEG study in which cortical responses to hand movements were recorded. Participants performed a motor task using a single-hand joystick while receiving vibrotactile feedback. The high-density fNIRS setup was placed over the sensorimotor areas to enable channel-level analysis with high spatial sampling. To establish a robust baseline for further analysis, we first apply a standard statistical approach (General Linear Model) to estimate channel-level contrasts between the resting and button pressing conditions. Our preliminary findings show that motor activation can be detected using the GLM in channel space, demonstrating the feasibility of decoding hand movement using fNIRS, even in naturalistic environments. While this statistical approach is conservative and well established, it is important to use it as a reference point for developing more sophisticated machine-learning pipelines. All analyses were conducted using the Python-based Cedalion toolbox, developed by the Intelligent Biomedical Sensing Lab, for multimodal diffuse optical tomography (DOT) data analysis and visualisation. Cedalion provides state-of-the-art signal processing, modeling and quality control pipelines for modern neural data science. It is integrable within machine-learning ecosystems and employs standardised neuromaging data formats such as SNIRF and BIDS. The results demonstrate the value of using General Linear Models for complex tasks such as motor decoding from high-density fNIRS data. Our study highlights the importance of open and flexible software frameworks for advancing methodological development and cross-modal integration in naturalistic neuroscientific research.

55

Attenuation of Ca^{2+} signaling by overexpression of PMCA2 affects the microglial response to pathological events

Fan Zeng1,2, Sagun Tiwari3, Alaa Chok3, Yan Zhou2, Yue Zhao2, Xuezhen Chen2, Yue Hao1, Kaichuan Zhu2, Helmut Kettenmann1,2,4, *,#, Xianyuan Xiang1,2,*,#

[1]Faculty of Life and Health Sciences, Shenzhen University of Advanced Technology, Shenzhen, China
[2]Shenzhen Key Laboratory of Immunomodulation for Neurological Diseases, The Brain Cognition and Brain Disease Institute, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China.
[3] University of Chinese Academy of Sciences, Beijing, China
[4] Max-Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany
*Correspondence: Xiang.xy@suat-sz.edu.cn; Kettenmann@suat-sz.edu.cn
*These authors contribute equally.
Correspondence: Xiang.xy@suat-sz.edu.cn; Kettenmann@suat-sz.edu.cn

Microglia strongly impact the pathologic course of brain diseases and injuries. Intracellular Ca2+ dynamics serve as central integrators, connecting microglial sensing capacity to their responses. We generated a mouse line with microglial overexpression of plasma membrane Ca2+-ATPase (PMCA)2, a central regulator of cytoplasmic Ca2+ homeostasis. This manipulation significantly attenuated ATP-evoked Ca2+ signals in vitro and spontaneous Ca2+ transients in vivo. Notably, in contrast to astrocytes, PMCA2 overexpression in microglia/macrophages did not affect animal behavior and survival. It had, however, a profound impact on microglial reactivity in pathological contexts, including reduced inflammatory responses following lipopolysaccharide challenge and diminished microglial proliferation at sites of acute injury. In an Alzheimer's disease model, PMCA2 overexpression attenuated the disease-associated microglial signature, reducing amyloid plaque burden and plaque-associated neuritic dystrophy. These findings highlight the importance of Ca2+-mediated signaling for modulating the microglial response to pathologic events. Attenuating microglial Ca2+ signaling by PMCA2 overexpression is a potential strategy to promote beneficial microglial phenotypes in brain inflammation or degeneration.

56

The Involvement of 5-HT_2A Receptors in Microglial Activation During Alcohol Abstinence

Gülcan Demir[1], Francesco Giannone[1], Tobias Buchborn[1], Rainer Spanagel[1], Anita C. Hansson[1]

[1]Central Institute of Mental Health, Mannheim, Germany, Interdisciplinary Center for Neurosciences, University of Heidelberg, Germany

Alcohol Use Disorder (AUD) is characterized by persistent neurobiological adaptations that promote relapse during abstinence. Chronic alcohol exposure disrupts serotonergic signalling and induces sustained neuroimmune activation; however, the temporal interaction between these systems during withdrawal remains poorly defined. In particular, adaptations of the 5-HT_2A receptor and microglial activation may represent converging mechanisms that shape vulnerability to relapse.
Using a chronic intermittent ethanol (CIE) exposure model in rats, we first characterized microglial activation across multiple abstinence time points. We then examined the temporal relationship between 5-HT_2A receptor function and microglial activity during alcohol abstinence. Functional 5-HT_2A receptor sensitivity was assessed using the serotonergic agonist quipazine, which elicits the head-twitch response (HTR), a validated behavioural proxy of postsynaptic 5-HT_2A activation. Behavioural and physiological responses, including HTR and tail vasoconstriction, were measured during early and protracted abstinence.
Based on evidence that microglia express functional 5-HT_2A receptors and serotonergic psychedelics exert immunomodulatory effects, we further investigated whether psilocybin, a classic 5-HT_2A agonist, modulates microglial activation during abstinence. We hypothesized that early abstinence is characterized by functional sensitization of 5-HT_2A receptors, reflected by exaggerated quipazine-induced behavioural and physiological responses accompanied by increased microglial activation and inflammatory signalling, whereas protracted abstinence shows partial normalization. Furthermore, we hypothesized that psilocybin attenuates abstinence-associated neuroinflammation by modulating serotonergic–microglial interactions.
Molecular analyses focused on the prefrontal cortex and the dorsomedial and dorsolateral striatum, key nodes of corticostriatal circuits that receive dense serotonergic innervation and are highly sensitive to alcohol-induced neuroimmune alterations. Together, this work aims to identify abstinence-dependent windows during which serotonergic–microglial interactions may drive relapse vulnerability in AUD.

57

Multimodal Correlates of Aggression Potential in Early-Stage Psychosis: Integrating Brain Structure, Polygenic Risk, and Social Adversity

Clara Weyer, MSc[1,2]; Clara Vetter, MSc[1,3,4]; Nora Penzel, PhD[1,5]; Unn Haukvik, MD, PhD[6,7]; Nikolaos Koutsouleris, MD[1,2,3,8,9*]; David Popovic, MD, PhD[1,3*]; and the PRONIA Consortium

[1]Department of Psychiatry and Psychotherapy, Ludwig-Maximilian-University, Munich, Germany
[2] Graduate School of Systemic Neurosciences, Ludwig-Maximilian-University, Munich, Germany
[3] Max Planck Institute of Psychiatry, Munich, Germany
[4] Munich Center of Machine Learning (MCML), Munich, Germany
[5] Department of Psychiatry, Mass General Brigham, Harvard Medical School, Boston, MA, USA
[6] NORMENT, Division of Mental Health and Addiction, Oslo University Hospital, Institute of Clinical Medicine, University of Oslo, Norway
[7] Centre for Research and Education in Forensic Psychiatry (SIFER), Oslo University Hospital, Institute of Clinical Medicine, University of Oslo, Oslo, Norway
[8] Munich-Augsburg site of the German Centre for Mental Health (DZPG)
[9]Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom

*Authors contributed equally

Aggression in mental disorders imposes a substantial clinical and societal burden across diagnostic boundaries and is shaped by the interplay of environmental factors and underlying neurobiological and genetic liabilities. Elucidating these multilevel associations may inform preventive risk stratification and early intervention. We used data from the prospective, multicentric European PRONIA project, comprising 402 minimally medicated participants (mean (SD) age = 25.34 (5.84); female sex = 49.5%) with clinical high-risk states for psychosis (n = 116), recent-onset depression (n = 146) or psychosis (n = 140). The multi-block sparse partial least squares (MB-SPLS) algorithm was employed within a nested cross-validation framework to detect multivariate associations between aggression potential (Excited Component of the PANSS), social adversity (childhood trauma, discrimination, bullying), brain structure (T1-weighted structural MRI, diffusion MRI) and polygenic risk. Post hoc analyses examined associations with personality and emotion recognition, sensitivity to overall illness severity, and longitudinal prediction of role and social functioning at 9-month follow-up, including interactions with coping and resilience. MB-SPLS linked higher aggression potential in recent-onset psychosis to greater social adversity, elevated polygenic risk, and a distinct neuroanatomical pattern characterized by ventricular enlargement, reduced fronto-parietal-temporal white matter volume, and widespread microstructural alterations of commissural, association, and limbic tracts. Higher aggression potential was further

associated with lower agreeableness, impaired emotion recognition, and predicted 9-month functioning, moderated by emotion-oriented coping and deficient resilience. These findings suggest that aggression potential represents a distinct, multidimensional vulnerability in early psychosis, characterized by white matter pathology in the context of genetic and environmental risk. Targeting modifiable components of this vulnerability, particularly social adversity, social cognition, coping styles, and resilience, may improve functional outcomes and support preventive strategies aimed at reducing the risk of aggressive behavior.

58

Aducanumab-mediated clearance of amyloid plaques decreases microglial activation and induces a peri-plaque microglial phenotype associated with antigen presentation

Lis de Weerd[1], Selina Hummel[2], Stephan A. Müller[1,7], Iñaki Paris[1], Thomas Sandmann[3], Marie Eichholtz[6], Robin Gröger[2], Amelie Englert[2], Stephan Wagner[2], Connie Ha[3], Sonnet S. Davis[3], Valerie Warkins[3], Dan Xia[3], Brigitte Nuscher[4], Anna Berghofer[1], Marvin Reich[1,†], Astrid Feiten[4], Kai Schlepckow[1], Michael Willem[4], Stefan F. Lichtenthaler[1,5,7], Joseph W. Lewcock[3], Kathryn M. Monroe[3], Matthias Brendel[1,2,5], Christian Haass[1,4,5]

[1] German Center for Neurodegenerative Diseases (DZNE), Munich, Germany.
[2] Department of Nuclear Medicine, University Hospital of Munich, Ludwig Maximilians University, Munich, Germany.
[3] Denali Therapeutics, Inc., South San Francisco, USA.
[4] Metabolic Biochemistry, Biomedical Center, Faculty of Medicine, Ludwig-Maximilians University (LMU), Munich, Germany.
[5] Munich Cluster for Systems Neurology (SyNergy), Munich, Germany.
[6] Biochemistry Master's Program, Gene Center Munich, Ludwig-Maximilians University (LMU), Munich, Germany.
[7] Neuroproteomics, School of Medicine and Health, Klinikum Rechts der Isar, Technical University of Munich (TUM), Munich, Germany.

Anti-amyloid β-peptide (Aβ) immunotherapy was developed to reduce amyloid plaque pathology and slow cognitive decline during progression of Alzheimer's disease. Efficient amyloid clearance has been proven in clinical trials testing anti-Aβ antibodies, by their impact on cognitive endpoints correlating with the extent of amyloid removal. However, treatment is associated with adverse side effects, such as oedema and haemorrhages, which are potentially linked to the induced immune response. To improve the safety profile of these molecules, it is imperative to understand the consequences of anti-Aβ antibody treatment on immune cell function. Here, we investigated the effects of long-term chronic anti-Aβ treatment on amyloid plaque pathology and microglial response in the APP-SAA triple knock-in mouse model with an intervention paradigm early during amyloidogenesis. Long-term treatment with anti-Aβ results in a robust and

dose-dependent lowering of amyloid plaque pathology, with a higher efficiency for reducing diffuse over dense-core plaque deposition. Analysis of the CSF proteome indicates a reduction of markers for neurodegeneration including Tau and α-Synuclein, as well as immune-cell-related proteins. Bulk RNA-seq revealed a dose-dependent attenuation of disease-associated microglial (DAM) and glycolytic gene expression, which is supported by a parallel decrease of glucose uptake and protein levels of Triggering Receptor Expressed on Myeloid cells 2 (Trem2) protein, a major immune receptor involved in DAM activation of microglia. In contrast, DAM activation around residual plaques remains high, regardless of treatment dose. In addition, microglia surrounding residual plaques display a dose-dependent increase in microglial clustering and a selective increase in antigen-presenting and immune signalling proteins. These findings demonstrate that chronic early intervention by an anti-amyloid immunotherapy leads to a dose-dependent decrease in plaque formation, which is associated with lower brain-wide microglial DAM activation and neurodegeneration. Microglia at residual plaques still display a combined DAM and antigen-presenting phenotype that suggests a continued treatment response.

59

Anterior olfactory nucleus: an intrinsically mechanosensitive relay for olfaction

*A. BALOMENOS, S. CHAKRABARTI, W. LUO, R. PROCE, G. IELACQUA, V. BEGAY-MUELLER, M. PAMPOLS PEREZ, H. HÖRNBERG, A. HAMMES, G. R. LEWIN;

[1] Max Delbrück Center for Molecular Medicine, 13125, Berlin, Germany

Mammalian olfaction depends on inhalation and the perception of smell may result from convergent processing of chemosensory (odors) and mechanosensory (airflow) information. Numerous studies highlight the putative role of mechanosensation in the olfactory system, but the relationship between the two is not well understood. Using single molecule Fluorescent in Situ Hybridisation (smFISH), we detected the expression of the mechanically gated ion channel Piezo2, in many cells of the olfactory system, including high levels of expression in neurons within the Anterior Olfactory Nucleus (AON). Expression of this mechanically gated ion channel in AON neurons was especially prominent in the first two post-natal weeks. Using mice in which Piezo2-Cre drives tdTomato expression in Piezo2 expressing cells, we found that Piezo2 fate mapped cells exist in the olfactory system, most prominently in the AON. These observations prompted us to ask whether robust mechanically activated currents (MA currents) can be found in CNS areas that process olfactory information. We established a primary culture protocol for AON neurons in order to examine whether AON neurons are mechanosensitive. Using single nucleus RNA sequencing we detected expression of Piezo2 and other mechanically gated ion channels in specific cell clusters from our neuronal cultures. Patch clamp electrophysiological recordings from AON neurons showed that mechanical stimulation via substrate deflection was able to generate robust MA currents. The proportion of tdTomato+ neurons (reading out Piezo2 promoter activity) with MA currents was significantly higher than tdTomato- neurons. We sought to understand the role of AON mechanosensitivity in vivo and using functional ultrasound imaging in anesthetized mice we observed activation of AON neurons upon airflow-driven mechanical stimulation. We established a gain-of-function approach by overexpressing Stoml3, a known modulator of mechanosensitivity in Piezo2+ cells within the AON, which was associated with impaired odor discrimination in mice without altering the AON structure. Future work will investigate the airflow-driven activation in this gain-of-function model.

60

Attenuating Microglial Ca^{2+} Signaling via PMCA2 Overexpression Promotes Glioma Progression

Alaa Chok[1,2,3], Fan Zeng[3], Sagun Tiwari[1,2,3], Helmut Kettenmann[3,4*], Xianyuan Xiang[3*]

[1] The Brain Cognition and Brain Disease Institute, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China
[2] University of Chinese Academy of Sciences, Beijing, China.
[3] Bio-X International Institute, Faculty of Life and Health Sciences, Shenzhen University of Advanced Technology, Shenzhen, China
[4] Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany.
*Equal contribution

Microglial cells and infiltrating macrophages are integral components of the glioma microenvironment and constitute a large component of the tumor mass. Many microglial and macrophage functions and communication pathways are controlled by intracellular Ca^{2+} signaling. To study the relevance of such pathways in tumor progression we used a transgenic mouse model with microglia-specific overexpression of the plasma membrane Ca^{2+}-ATPase 2 (PMCA2), a key Ca^{2+} extruder, which attenuates ATP-evoked and spontaneous Ca^{2+} transients thereby dampening Ca^{2+} signaling.

After intracranial implantation of GL261 glioma cells, mice with PMCA2-overexpressing brain macrophages/microglia (BAMs) developed a rapidly deteriorating disease course, with earlier body-weight loss and increased mortality rate before the predefined endpoint at day 21. At day 21, tumor volume was significantly larger in PMCA2 mice. Concomitantly, $Iba1^{+}$ coverage by BAMs within the tumor was increased, and GL261 cells displayed higher Ki67 positivity, indicating enhanced proliferative activity. To test whether microglia-derived cues promote tumor growth, we collected conditioned medium from cells of the microglial cell line BV2 overexpressing PMCA2 and found that it significantly increased GL261 proliferation as compared to control medium. Together, these findings establish that disruption of microglial Ca^{2+} homeostasis via PMCA2 overexpression, promotes a pro-tumorigenic phenotype of BAMs which fuels glioma progression.

61

Attenuation of Ca^{2+} signaling by overexpression of PMCA2 affects the microglial response to pathological events

Fan Zeng[1,2], Sagun Tiwari[3], Alaa Chok[3], Yan Zhou[2], Yue Zhao[2], Xuezhen Chen[2], Yue Hao[1], Kaichuan Zhu[2], Helmut Kettenmann[1,2,4, *,#], Xianyuan Xiang[1,2,*,#]

1 Faculty of Life and Health Sciences, Shenzhen University of Advanced Technology, Shenzhen, China
2 Shenzhen Key Laboratory of Immunomodulation for Neurological Diseases, The Brain Cognition and Brain Disease Institute, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China.
3 University of Chinese Academy of Sciences, Beijing, China
4 Max-Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany
*These authors contribute equally.

Microglia strongly impact the pathologic course of brain diseases and injuries. Intracellular Ca^{2+} dynamics serve as central integrators, connecting microglial sensing capacity to their responses. We generated a mouse line with microglial overexpression of plasma membrane Ca^{2+}-ATPase (PMCA)2, a central regulator of cytoplasmic Ca^{2+} homeostasis. This manipulation significantly attenuated ATP-evoked Ca^{2+} signals in vitro and spontaneous Ca^{2+} transients in vivo. Notably, in contrast to astrocytes, PMCA2 overexpression in microglia/macrophages did not affect animal behavior and survival. It had, however, a profound impact on microglial reactivity in pathological contexts, including reduced inflammatory responses following lipopolysaccharide challenge and diminished microglial proliferation at sites of acute injury. In an Alzheimer's disease model, PMCA2 overexpression attenuated the disease-associated microglial signature, reducing amyloid plaque burden and plaque-associated neuritic dystrophy. These findings highlight the importance of Ca^{2+}-mediated signaling for modulating the microglial response to pathologic events. Attenuating microglial Ca^{2+} signaling by PMCA2 overexpression is a potential strategy to promote beneficial microglial phenotypes in brain inflammation or degeneration.

62

Pathogenesis and Treatment of Small Artery Occlusive Cerebral Infarction

Xiao Hong [1]， Aoqier [*1,2]

[1] Inner Mongolia Medical University, Hohhot, Inner Mongolia 010110, China
[2] Inner Mongolia International Mongolian Hospital, Hohhot, Inner Mongolia 010065, China)

Stroke is the second leading cause of human mortality, with ischemic stroke being the most prevalent. Small artery occlusive cerebral infarction accounts for approximately 25% of all ischemic strokes. With the aging population and changes in lifestyle and dietary structure in China, its incidence, disability rate, and mortality have risen significantly, severely impacting patients' physical and mental health as well as their quality of life. In Mongolian medicine, small artery occlusive cerebral infarction falls under the category of "Grage disease." Mainstream medical treatment primarily focuses on pharmacological therapy and risk factor control. When combined with Mongolian medicine's syndrome differentiation and treatment, it can effectively reduce recurrence, disability, and mortality rates. This article provides a systematic review of the pathogenesis, current treatment status, and future prospects of small artery occlusive cerebral infarction. Results: The pathogenesis of small artery occlusive cerebral infarction is associated with lipohyalinosis, atherosclerosis, embolism, and other factors. Currently, Western medicine mainly employs pharmacological and non-pharmacological treatments, generally managing it within ischemic stroke prevention and treatment protocols, including intravenous thrombolysis, antiplatelet aggregation, anticoagulation, neuroprotection, and risk factor control. However, due to its relatively mild clinical symptoms, timely diagnosis and treatment are often delayed, leading to potential progression in some patients. Long-term use of single drugs may also cause certain adverse reactions, imposing a significant economic burden on patients. Mongolian medicine is an important component of China's traditional medicine, characterized by multi-target effects, holistic regulation, and minimal side effects. Clinically commonly used Mongolian medicines for ischemic stroke, such as Erdun Wurile, Zhenzhu Kangmai Pill, and Garudi-13, have shown favorable clinical efficacy. Conclusion: With the advancement of national policies on ethnic medicine and deepening brain science research, the integration of Mongolian and Western medicine holds promise as a

"Chinese solution" with ethnic characteristics in the prevention and management of cerebral small vessel disease. It offers a superior choice, particularly for elderly patients and those with multiple comorbidities.

63

Bi-directional Modulation of α-Synuclein Expression in SH-SY5Y Cells by circFKBP8(5S,6)-encoded Protein.

Jude Uzoechina[1,2,3] , Zhijun Zhang[1,2,4]

[1]Shenzhen Key Laboratory of Precision Diagnosis and Treatment of Depression, Department of Mental Health and Public Health, Faculty of Life and Health Sciences, Shenzhen University of Advanced Technology, The Brain Cognition and Brain Disease Institute of Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, Guangdong 518055, P.R. China.
[2]Department of Mental Health and Public Health, Faculty of Life and Health Sciences, Shenzhen University of Advanced Technology, Shenzhen, Guangdong 518055, P.R. China.
[3]University of Chinese Academy of Sciences, Beijing 100049, P. R. China
[4]Department of Neurology in Affiliated Zhongda Hospital and Jiangsu Provincial Medical Key Discipline, School of Medicine, Institute of Neuropsychiatry, Key Laboratory of Developmental Genes and Human Disease of Ministry of Education, Southeast University, Nanjing, Jiangsu 210009, P. R. China.

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease worldwide after Alzheimer's disease, and is characterized by the unusual aggregation of α-synuclein. Recent research indicates that circular RNA (circRNA)-encoded proteins play a role in neurodegenerative processes, although their exact function in PD remains unclear. This study investigated the expression and role of the circular RNA–encoded protein, cFKBP8, encoded by the circRNA, circFKBP8(5S,6), in a PD-relevant cellular model. An in vitro PD model was established by exposing SH-SY5Y cells to 1-methyl-4-phenylpyridinium (MPP^+), which induces neuronal stress. cFKBP8 and its circular RNA expression level were evaluated by immunofluorescence staining and quantitative real-time polymerase chain reaction (RT-qPCR), respectively. A significant increase in cFKBP8 expression was observed in MPP^+-treated cells compared to untreated controls. Gain- and loss-of-function experiments showed that cFKBP8 overexpression increased α-synuclein expression, whereas knockdown of cFKBP8 significantly lowered α-synuclein levels in SH-SY5Y cells. These findings suggest that cFKBP8 reacts to PD-like neurotoxic stress and can bidirectionally modulate α-synuclein expression in neuronal cells. This study identified cFKBP8 as a circRNA-encoded protein linked to PD and highlighted the emerging role of circRNA-encoded proteins in α-synuclein biology, with potential implications for developing biomarkers and therapeutic strategies for PD.

64

Modeling Cushing's Disease: Neuroendocrine Insights from the Mouse Pituitary

Sydney Reil[1], Marily Theodoropoulou[1]

[1]LMU Hospital, Department of Internal Medicine IV, Department of Endocrinology, Diabetes and Metabolism, Department of Nuclear Medicine

Cushing's disease is the most common form of endogenous hypercortisolism, caused by ACTH-secreting pituitary tumors. It is a severe condition characterized by clinical manifestations of cortisol excess, e.g. anxiety and depression, metabolic syndrome, hypertension, extreme fatigue, muscle weakness, and immune dysfunction. Research on the pathogenesis and treatment of Cushing's disease is imbedded by the lack of suitable animal models. Existing animal models, primarily developed in mice, are focused on inducing hypercortisolism, a simplification of Cushing's disease, and each model has significant limitations; some are aggressive, with a rapid onset and progression of symptoms, others produce hypercortisolism but fail to produce adenomas, or induce incomplete Cushing's phenotypes. Overall, current models deviate significantly from human Cushing's disease, potentially due to the absence of human-relevant genetic drivers.

Somatic variants in the USP8 gene, which encodes ubiquitin-specific protease 8, represent a major genetic driver of Cushing's disease and are present in approximately 40% of corticotroph tumors. To address the limitations of current models, we set out to create an inducible, transgenic mouse expressing a USP8 variant form frequently found in human corticotroph tumors. We had 13 control (10 female) and 12 variant (10 female) mice. Mice were sacrificed aged 52 weeks. Their pituitary glands were extracted, preserved, sliced, and analyzed via immunohistochemistry for ACTH. No detectable signs of ACTH neoplasia or hyperplasia were observed between the pituitaries of transgenic mice and those of control littermates. No significant differences were observed in the adrenal gland weights (P=0.39). The project is ongoing.

65

Role of Autoantibodies in Long-Term Outcome and Cognitive Function after Stroke

Haleh Khosravi[1,2], Elisa Sanchez-sendin[1,3], Daniel Berchtold[1,2], Andreas Meisel[1,2], Harald Prüß[1,3]

[1]Department of Experimental Neurology, Charité –Universitätsmedizin Berlin, Germany
[2]Center for Stroke Research Berlin, Germany
[3]German Center for Neurodegenerative Diseases.

Ischemic stroke induces a local inflammatory response that can promote auto-reactive immune responses against central nervous system (CNS) antigens. CNS-directed autoantibodies are frequently detected in stroke patients; however, their contribution to long-term neurological outcome and cognitive decline remains unclear. We investigated whether post stroke autoantibodies contribute to long-term outcome and cognitive function. For this purpose, plasma cells were isolated from a stroke-induced mouse model, and recombinant monoclonal antibodies were generated. Functional analyses are being performed to assess antibody effects on CNS cells and tissues. To distinguish the impact of pre-existing versus newly generated autoantibodies, recombinant candidate antibodies are administered either before or after experimental stroke induction in mice. The analysis focuses on multiple immunoglobulin isotypes targeting a range of CNS antigens, including neuronal, astrocytic, endothelial, and other brain-resident cell populations. This work aims to define the pathological role of CNS-directed autoantibodies in post-stroke cognitive decline and long-term neurological outcome. Understanding post-stroke humoral immune responses may reveal novel therapeutic targets to improve recovery after stroke.

66

Resolving simple and complex spikes in Purkinje cell populations in vivo with the voltage indicator ASAP7

Qiyuan Liang[1], Yukun A. Hao[2,3], Sungmoo Lee[2,3], Huiqi Zhang[1], Guangnan Tian[1], Michael Z. Lin[2,3] and Michael Häusser[1]

[1]School of Biomedical Sciences, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China
[2]Department of Neurobiology, Stanford University, Stanford, CA, USA
[3]Department of Bioengineering, Stanford University, Stanford, CA, USA

Purkinje cells (PCs) are the sole output of the cerebellar cortex and key to motor coordination and learning. They integrate massive sensorimotor information via parallel fibers to generate high-frequency simple spikes (SS), while simultaneously receiving potent "error signals" from climbing fibers that trigger low-frequency complex spikes (CS). The synchrony of complex spikes is a well-established mechanism for encoding motor errors and regulating downstream plasticity, but the spatiotemporal organization of SS remains poorly understood. This gap exists because calcium imaging lacks the temporal resolution to capture fast SS firing (60–100 Hz), while electrophysiology lacks the spatial information.

Here, we overcame these limitations by combining the latest negatively tuned genetically encoded voltage indicator (GEVI) ASAP7 with an Acousto-Optic Deflector (AOD)-based random-access two-photon microscope. This system enables inertia-free, multi-point scanning to achieve kilohertz sampling rates for recording voltage signals across populations of Purkinje cell somata in awake mice. The fast kinetics of ASAP7 enabled the first optical resolution of SS dynamics with single-spike fidelity. Our preliminary results demonstrate the potential of this toolkit to simultaneously map the spatial synchrony of both simple and complex spikes, offering a new window into the spatiotemporal codes underlying cerebellar computation.

67

Localized Release of Muscle-generated BDNF Regulates the Initial Formation of Postsynaptic Apparatus at Neuromuscular Synapses

Jinkai Zhang[1], Hiu-Lam Rachel Kwan[2], Chi Bun Chan[3] and Chi Wai Lee[1,4]

[1]Department of Biology, Faculty of Science, Hong Kong Baptist University, Hong Kong, China.
[2]School of Biomedical Sciences, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong, China.
[3]School of Biological Sciences, Faculty of Science, The University of Hong Kong, Hong Kong, China.
[4]Golden Meditech Centre for NeuroRegeneration Sciences, Hong Kong Baptist University, Hong Kong, China.

Growing evidence indicates that brain-derived neurotrophic factor (BDNF) is produced in contracting skeletal muscles and is secreted as a myokine that plays an important role in muscle metabolism. However, the involvement of muscle-generated BDNF and the regulation of its vesicular trafficking, localization, proteolytic processing, and spatially restricted release during the development of vertebrate neuromuscular junctions (NMJs) remain largely unknown. In this study, we first reported that BDNF is spatially associated with the actin-rich core domain of podosome-like structures (PLSs) at topologically complex acetylcholine receptor (AChR) clusters in cultured Xenopus muscle cells. The release of spatially localized BDNF is tightly controlled by activity-regulated mechanisms in a calcium-dependent manner. Live-cell time-lapse imaging further showed that BDNF-containing vesicles are transported to and captured at PLSs in both aneural and synaptic AChR clusters for spatially restricted release. Functionally, BDNF knockdown or furin-mediated endoproteolytic activity inhibition significantly suppresses aneural AChR cluster formation, which in turn affects synaptic AChR clustering induced by nerve innervation or agrin-coated beads. Lastly, skeletal muscle-specific BDNF knockout (MBKO) mice exhibit structural defects in the formation of aneural AChR clusters and their subsequent recruitment to nerve-induced synaptic AChR clusters during the initial stages of NMJ development in vivo. Together, this study demonstrated the regulatory roles of PLSs in the intracellular trafficking, spatial localization, and activity-dependent release of BDNF in muscle cells and revealed the involvement of muscle-

generated BDNF and its proteolytic conversion in regulating the initial formation of aneural and synaptic AChR clusters during early NMJ development in vitro and in vivo.