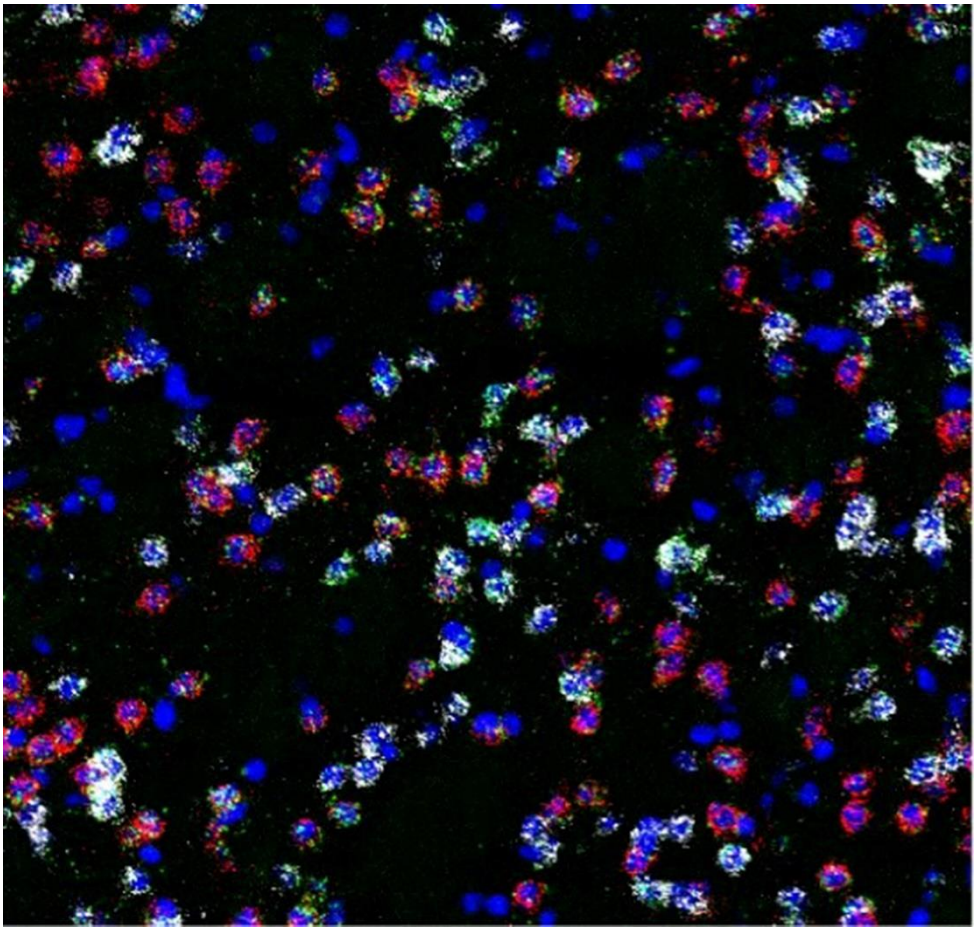
Abstracts of papers presented
at the 2025 Cold Spring Harbor Asia Conference

FRANCIS CRICK SYMPOSIUM IN NEUROSCIENCE: DEVELOPMENT, PLASTICITY & FUNCTION OF NEURAL CIRCUITS

September 8–September 12, 2025



Cold Spring Harbor Conferences Asia
Cold Spring Harbor Laboratory

Abstracts of papers presented
at the 2025 Cold Spring Harbor Asia Conference

FRANCIS CRICK SYMPOSIUM IN NEUROSCIENCE: DEVELOPMENT, PLASTICITY & FUNCTION OF NEURAL CIRCUITS

September 8–September 12, 2025

Arranged by

Joseph Gleeson, *University of California, San Diego*
Yukiko Goda, *Okinawa Institute of Science and Technology*
Bo Li, *Westlake University*
Anne Schaefer, *Max Planck Institute for Biology of Aging*
Song-Hai Shi, *Tsinghua University*

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FRANCIS CRICK SYMPOSIUM IN NEUROSCIENCE:
DEVELOPMENT, PLASTICITY & FUNCTION OF NEURAL CIRCUITS
Monday, September 8 – Friday, September 12, 2025

Monday	7:00 pm	**Keynote Speaker** **1** Circuit Assembly and mapping
Tuesday	9:00 am	**2** Stem Cells and Brain Development
Tuesday	2:00 pm	**Poster Session**
Tuesday	3:00 pm	*Chinese Tea and Beer Tasting*
Tuesday	7:00 pm	**3** Neuronal Development and Diversity
Wednesday	9:00 am	**4** Synapses and Local Circuit Milieu
Wednesday	1:30 pm	*Visit to Old Suzhou**
Wednesday	7:00 pm	**5** Learning and Memory
Thursday	9:00 am	**6** Brain Development and Brain Disorders
Thursday	2:00 pm	**7** Neural Circuits and Behavior
Thursday	5:00 pm	*Cocktails and Banquet*
Friday	9:00 am	**8** Neurobiology of Brain Disorders and Aging

Oral presentation sessions are located in the CSHA Auditorium
Poster session and Chinese Tea & Beer Tasting are in the Lake Front Hall.
Cocktail social hour is held outside in the Suz Garden.
Old Suzhou visits depart from the CSHA lobby
**optional tour requires additional fee.*

Meal locations and times are as follows:
Lunch: Main Cafeteria 12:00pm - 1:30pm
Dinner: Main Cafeteria 6:00pm - 7:30pm
Banquet: Suz Garden 7:00pm

More information will be available at CSHA office.
(Map at the end of this abstract book)

PROGRAM

MONDAY, September 8—7:00 PM

SESSION 1 CIRCUIT ASSEMBLY AND MAPPING

Chairpersons: **Bo Li,** Westlake University, Hangzhou, China
Song-Hai Shi, Tsinghua University, Beijing, China

KEYNOTE SPEAKER

Wiring specificity of neural circuits
Liqun Luo, Cheng Lyu, Zhuoran Li, Daniel Pederick, Ellen Gingrich, Uree Chon [35'+10']
Presenter affiliation: Stanford University, Stanford, California. 1

Activity-dependent remodeling of neuronal circuits during development
Takeshi Imai [20'+10']
Presenter affiliation: Kyushu University, Fukuoka, Japan. 2

Mesoscale mapping of the nervous system—From brain to body
Guoqiang Bi [20'+10']
Presenter affiliation: University of Science and Technology of China, Hefei, China; CAS Shenzhen Institute of Advanced Technology, Shenzhen, China. 3

Functional imaging of developing brain in mice and non-human primates
Jiejun Zhu, Dongming He, Mengzhu Sun, Hanming Zheng, Xiaoli Lin, Weizhen He, Lijie Zhang, Zihao Chen, Jin Yang, Chengqi Lin, Yun Shi, Lei Sun, Zhihai Qiu [10'+5']
Presenter affiliation: Guangdong Institute of Intelligence Science and Technology, Zhuhai, China. 4

SESSION 2 STEM CELLS AND BRAIN DEVELOPMENT

Chairpersons: **Yukiko Gotoh,** University of Tokyo, Tokyo, Japan
Fiona Doetsch, University of Basel, Basel, Switzerland

Temporal regulation of neural stem-progenitor cell fate during neocortical development
Yukiko Gotoh [20'+10']
Presenter affiliation: The University of Tokyo, Tokyo, Japan. 5

Regulation and heterogeneity of adult neural stem cells
Fiona Doetsch [20'+10']
Presenter affiliation: University of Basel, Basel, Switzerland. 6

Non-epithelial radial glial cells maintain the production of GABAergic inhibitory neurons and glial cells in the developing human brain
Longzhong Jia, Xiaohan Li, Yiming Yan, Linhe Xu, Weichao wang, Lianyan Li, Da Mi [10'+5']
Presenter affiliation: Tsinghua University, Beijing, China. 7

FEZ1 deletion disrupts human cortical brain development by altering the ratio of outer radial glial cell subpopulations
John Jia En Chua
Presenter affiliation: National University of Singapore, Yong Loo Lin School of Medicine, Singapore. 8

Break

Achieving singularity in olfactory receptor gene expression
Stavros Lomvardas [20'+10']
Presenter affiliation: Columbia University, New York, New York. 9

Oncogenic fusions converge on shared mechanisms in initiating astroblastoma
Wei Shi [10'+5']
Presenter affiliation: Chinese Institute for Brain Research, Beijing, China. 10

Premature cortical inhibitory neurogenesis in Down syndrome
Jingwen Ding, Chang N. Kim, Tomasz J. Nowakowski, Alex A. Pollen [10'+5']
Presenter affiliation: University of California San Francisco, San Francisco, California. 11

Teneurin isoformic coding in fly olfactory circuit assembly
Jiahang Ju, Tongchao Li
Presenter affiliation: Zhejiang University, Hangzhou, China. 12

Neuronal pruning and its implications in human neurodevelopmental disorders
Fengwei Yu
Presenter affiliation: Temasek Life Sciences Laboratory, Singapore. 13

TUESDAY, September 9—2:00 PM

POSTER SESSION

Stress novelty influences cholinergic involvement in hippocampal early gene activation
Alexey P. Bolshakov, Angelina K. Deryabina, Alexandra A. Fedulova, Alena A. Koryagina, Yulia V. Dobryakova
Presenter affiliation: Institute of Higher Nervous Activity and Neurophysiology, Moscow, Russia. 14

The role of calcium signaling during neural development in bipolar disorder
Melis Çelik, Mahnaz Nikpour, Vika Telle, Bingqing He, Berta Marcó de La Cruz, Parvaneh Nikpour, Lina Jonsson, Carl M. Sellgren, Mikael Landén, Erik Smedler
Presenter affiliation: University of Gothenburg, Gothenburg, Sweden. 15

Probiotic intervention restores microglial surveillance and synaptic architecture in the aging cortex
Chia-Chien E. Chen, Hyo Gun Lee, Ju Lu, Yi Zuo
Presenter affiliation: Duke Kunshan University, Suzhou, China; University of California Santa Cruz, Santa Cruz, California. 16

Mapping ultrasound-driven neural activity at millisecond resolution via voltage sensors
Congmin Chen, Langzhou Liu, Yong Wu, Lei Sun
Presenter affiliation: The Hong Kong Polytechnic University, Hong Kong, China. 17

Multimodal single-cell analyses reveal molecular markers of neuronal senescence in human drug-resistant epilepsy
Jiadong Chen
Presenter affiliation: Zhejiang University, Hangzhou, China. 18

How does the brain learn? A dynamical systems perspective
Zihao Chen, Zhihai Qiu, Lei Sun
Presenter affiliation: Guangdong Institute of Intelligence Science and Technology, Zhuhai, China; The Hongkong Polytechnic University, Hong Kong, China. 19

Pathway-specific hippocampal plasticity gated by theta-frequency medial septal cholinergic activity
Yulia V. Dobryakova, Tinna A. Korotkova, Alexey P. Bolshakov, Vladimir A. Markevich
Presenter affiliation: Institute of Higher Nervous Activity and Neurophysiology of the Russian Academy of Science, Moscow, Russia. 20

Identifying electrophysiological biomarkers in hiPSC-derived neurons from ADHD patients
Lorenzo D. Dodi, Sreedhar S. Kumar, Edna Grünblatt, Andreas Hierlemann
Presenter affiliation: ETH Zürich, Basel, Switzerland. 21

Dissecting the cellular effects of schizophrenia risk variants: 3q29 deletion and duplication in neurodevelopment and function
Bingqing He, Lovisa Lettius, Melis Çelik, Fredrik H. Sterky, Jennifer G. Mulle, Erik Smedler
Presenter affiliation: University of Gothenburg, Gothenburg, Sweden. 22

Temporal and Notch identity determine layer targeting and synapse location in the fly visual system
Isabel Holguera, Yen-Chung Chen, Yu-Chieh David Chen, Félix Simon, Adelia G. Gaffney, Rodas D. Joseph, Sergio Córdoba, Claude Desplan
Presenter affiliation: New York University, New York, New York; Institut Jacques Monod, Paris, France. 23

ERK-dependent amyloidogenesis drives hydrocephalus across etiologies
Lingling Ge, Zhichao Wang, Qingfeng Li, Wei Huang
Presenter affiliation: Chinese Academy of Sciences, Center for Excellence in Brain Science and Intelligence Technology, Shanghai, China. 24

Targeted mechanogenetics to modulate specific deep neural circuits
Xuandi Hou, Jianing Jing, Zhuohan Shi, Lei Sun
Presenter affiliation: The Hong Kong Polytechnic University, Hong Kong, China. 25

RACK1 as a dynamic platform for protein (de)phosphorylation and translational control in neurons
Peter M. Kolosov, Polina A. Fortygina, Nikita S. Biziaev, Ekaterina Y. Shuvalova, Elena Z. Alkalaeva
Presenter affiliation: Engelhardt Institute of Molecular Biology, The Russian Academy of Sciences, Moscow, Russia; Institute of Higher Nervous Activity and Neurophysiology, The Russian Academy of Sciences, Moscow, Russia. 26

Relation between valence and locomotor performance using well established optogenetic tools in *Drosophila melanogaster*
Nicole M. Lee, Adam Claridge-Chang
Presenter affiliation: Duke-NUS Medical School, Singapore. 27

Non-invasive targeted vector delivery for spatially precise and flexible sonogenetics
Danni Li, Quanxiang Xian, Yizhou Jiang, Lei Sun
Presenter affiliation: The Hong Kong Polytechnic University, KowLoon, Hong Kong, China. 28

An RNA-RNA interaction required for GABA signaling in *Drosophila*
Ryan Loker, Claude Desplan
Presenter affiliation: New York University, New York, New York. 29

Fluorescent barcode vectors for mesoscopic circuit mapping
Daichi Moriyasu, Satoshi Fujimoto, Fuyuki Kamizono, Saha Biswanath, Yuki Ishida, Hikari Takeshima, Akiya Watakabe, Itaru Imayoshi, Takeshi Imai
Presenter affiliation: Kyushu University, Fukuoka, Japan. 30

Alzheimer's disease associated PS1 mutation disrupt cortical development through NOTCH signaling
Jiyong Peng, Jiong Shi
Presenter affiliation: University of Science and Technology of China, Hefei, China. 31

Mechanisms of pial astrocyte specification in the developing mouse cerebral cortex
Masatake Sasaki, Koji Oishi, Yukiko Gotoh
Presenter affiliation: The University of Tokyo, Tokyo, Japan. 32

Perinatal opioid exposure disrupts immune signalling in the prefrontal cortex and dorsal hippocampus of juvenile rats
Rinjani Soengkoeng, Arshman S. Sahid, Abigail Marcus, Adam K. Walker, Ju-Lee Oei, Kelly Clemens, Meredith Ward
Presenter affiliation: University of New South Wales, Sydney, Australia. 33

3D in vitro modelling of 1q21.1 duplication syndrome
Aleksandra T. Spasova, Tianqi Wang, Yasir A. Syed
Presenter affiliation: Cardiff University, Cardiff, United Kingdom. 34

Sonogenetics and neuroscience utility
Lei Sun
Presenter affiliation: the Hong Kong Polytechnic University, Hung Hom, Hong Kong, China. 35

Dopamine D1 receptors in the medial prefrontal cortex regulate methamphetamine seeking
Yiwen Sun, Sai Shi
Presenter affiliation: Shanghai Jiao Tong University School of Medicine, Shanghai, China. 36

Investigating the cellular microarchitecture of squid optic lobe
Rudi Tong, Vasileios Glykos, Lada Dolezalova, Samuel Reiter, Yukiko Goda
Presenter affiliation: Okinawa Institute of Science and Technology, Okinawa, Japan. 37

Efficiency to inefficiency—EEG patterns of healthy ageing in response to a cognitive challenge
Lily Mei G. Mckechnie, Jon Garner, Shaoyu Wang
Presenter affiliation: Charles Sturt University, Orange, Australia. 38

Using patient-induced pluripotent stem cells derived cortical organoids to investigate human neuronal phenotypes in 3q29 deletion and duplication syndrome
Tiangi Wang, Kubra Trabzonlu, Yasir Ahmed Syed
Presenter affiliation: Cardiff University, Cardiff, United Kingdom. 39

DCX knockout ferret reveals a neurogenic mechanism in cortical development
Wei Wang, Xiaoqun Wang
Presenter affiliation: Institute of Biophysics, Chinese Academy of Sciences, Beijing, China. 40

Methyl gallate attenuates post-stroke emotional and cognitive symptoms by promoting hippocampal neurogenesis via PI3K/GSK3 and AMPK signaling
Limin Xu, Feifei Chen, Haimei Fan, Longxing Zhang, Fei Yang, Jianyu Pang, Baolong Pan, Xuemei Wu
Presenter affiliation: The Chinese University of Hong Kong, Shenzhen, China. 41

Miniaturized and accessible functional ultrasound imaging system for freely moving mice
Jin Yang, Erlei Zhou, Lei Sun, Wentao Wu, Zhihai Qiu
Presenter affiliation: Guangdong Institute of Intelligence Science and Technology, Zhuhai, China; The Hong Kong Polytechnic University, Hong Kong, China. 42

A vagal liver-brain circuit for inflammation sensing and response
Na Zhang, Zhiyin Lam, Sarah, Xinwei Luo
Presenter affiliation: Agency for Science, Technology and Research (ASTAR), Singapore. 43

Inhibitory-neuron-specific sonogenetics for microcircuit modulation
Dongshuai Zhao, Lei Sun
Presenter affiliation: The Hong Kong Polytechnic University, Hong Kong, China. 44

TMTC3 stabilizes LAMB1 to maintain basement membrane integrity in cortical development
Shufang Zhao, Xiaoxu Yang, Harry Vong, Adnan Halim, Guoliang Chai, Joseph G. Gleeson
Presenter affiliation: Xuanwu Hospital Capital Medical University, National Center for Neurological Disorders, Beijing, Beijing, China. 45

Cytoskeletal coordination in neuronal development—Nesprin-2-mediated nuclear translocation via microtubule motors and IgSF11-guided axon projection
Chuying Zhou, Chunyun Jia, Mineko Kengaku, Liming Tan
Presenter affiliation: Shenzhen Key Laboratory of Neuropsychiatric Modulation, Shenzhen, China; CAS Key Laboratory of Brain Connectome and Manipulation, Shenzhen, China; Guangdong Provincial Key Laboratory of Brain Connectome and Behavior, Shenzhen, China. 46

TUESDAY, September 9—3:00 PM

Chinese Tea and Beer Tasting

TUESDAY, September 9—7:00 PM

SESSION 3 NEURONAL DEVELOPMENT AND DIVERSITY

Chairpersons: **Song-Hai Shi,** School of Life Sciences, Tsinghua University, Beijing, China
Claude Desplan, New York University, New York, New York, USA

Evolutionary emergence of striatal GABAergic interneuron types in mammals modulates behavioral flexibility
Song-Hai Shi [20'+10']
Presenter affiliation: School of Life Sciences, Tsinghua University, Beijing, China. 47

Cortical inhibitory neurons exhibit expanding and contracting modes of diversification.
Lynette Lim [20'+10']
Presenter affiliation: VIB, Leuven, Belgium; KUL, Leuven, Belgium. 48

Deterministic and stochastic generation of neuronal diversity
Claude Desplan, Raghu Rajesh, Yen-Chung Chen, Khaled Ben Elkadhi, Asif Bakshi, Neset Ozel [20'+10']
Presenter affiliation: New York University, New York, New York; NYU Abu Dhabi, Abu Dhabi, United Arab Emirates. 49

Pyramidal neurons provide both instructive and permissive signaling to specific interneuron subtypes
Min Dai, Gord Fishell [10'+5']
Presenter affiliation: Harvard Medical School, Boston, Massachusetts. 50

Mitotic bookmarking in brain development
Yan Song [10'+5']
Presenter affiliation: Peking University, Beijing, China. 51

Modelling neurodevelopmental disorders in flies and mammalian systems
Hongyan Wang [10'+5']
Presenter affiliation: Duke-NUS Medical School, Singapore. 52

WEDNESDAY, September 10—9:00 AM

SESSION 4 SYNAPSES AND LOCAL CIRCUIT MILIEU

Chairpersons: **Yukiko Goda,** Okinawa Institute of Science and Technology, Okinawa, Japan
Shujia Zhu, Southern University of Science and Technology, Shenzhen, China

Insights into synapse function through astrocyte links
Yukiko Goda [20'+10']
Presenter affiliation: Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan. 53

Decoding synaptic signaling dynamics underlying plasticity
Ryohei Yasuda [20'+10']
Presenter affiliation: Max Planck Florida Institute for Neuroscience, Jupiter, Florida. 54

Native NMDA receptors in the brain—From atomic structure to synaptic physiology
Shujia Zhu [20'+10']
Presenter affiliation: Southern University of Science and Technology, Shenzhen, China. 55

Break

Neuronal morphology maintenance involves membrane phospholipid asymmetry and extracellular vesicle biogenesis
Andrew D. Chisholm [10'+5']
Presenter affiliation: UC San Diego, La Jolla, California. 56

Proteomic landscape of neuronal nuclei in a mouse long-term potentiation model with ultra-low input
Mo Hu, Yuan Yuan, Ye Tian [10'+5']
Presenter affiliation: Changping Laboratory, Beijing, China. 57

Astrocyte morphogenesis requires self-recognition
John H. Lee, Alina Sergeeva, Göran Ahlsen, Seetha Mannepalli, Fabiana Bahna, Kerry Goodman, Runzheu Xu, Baljit Khakh, Joshua Weiner, Lawrence Shapiro, Barry Honig, Lawrence Zipursky [10'+5']
Presenter affiliation: University of California Los Angeles, Los Angeles, California. 58

CD47's regulation of memory processes
Jiaoyang Wo, Yunlong Liu, Xiaoyu Chen, Sheena A. Josselyn, Paul W. Frankland [10'+5']
Presenter affiliation: University of Toronto, Toronto, Canada; The Hospital for Sick Children, Toronto, Canada. 59

WEDNESDAY, September 10—1:30 PM

Visit to Old Suzhou

WEDNESDAY, September 10—7:00 PM

SESSION 5 LEARNING AND MEMORY

Chairpersons: **Paul Frankland,** Hospital for Sick Children, University of Toronto, Toronto, Canada
Yasunori Hayashi, Kyoto University Graduate School of Medicine, Kyoto, Japan

Roles of offline neuronal activity in the consolidation of spatial memory engrams
Yasunori Hayashi [20'+10']
Presenter affiliation: Kyoto University Graduate School of Medicine, Kyoto, Japan. 60

Developmental critical periods for episodic memory
Paul Frankland [20'+10']
Presenter affiliation: Hospital for Sick Children, Toronto, Canada; University of Toronto, Toronto, Canada. 61

Area-specific cell plasticity by vision-dependent spatial cellular gradients
Xiang Gao, Chuying Zhou, Liming Tan
Presenter affiliation: Brain Cognition and Brain Disease Institute, Shenzhen, China. 62

Remodeling synaptic connections via engineered neuron-astrocyte interactions
Shin Heun Kim, Woojin Won, Gyu Hyun Kim, Yeon Hee Kook, Seungkyu Son, Songhee Choi, Dong Yeop Kang, Mingu G. Park, Young-Jin Choi, Seong Su Won, Juhee Shin, Yong Jeong, Kea Joo Lee†, C. Justin Lee, Sangkyu Lee [10'+5']
Presenter affiliation: Institute for Basic Science (IBS), Daejeon, South Korea. 63

Regulation of adult neural stem cell establishment by FGF binding protein 3
Caiwei Li, Daichi Kawaguchi, Yukiko Gotoh [10'+5']
Presenter affiliation: The University of Tokyo, Tokyo, Japan. 64

Acute negative experiences promote memory generalization and mood vulnerability via a shared mPFC-BNST ensemble and molecular signature
Xin Cheng, Yan Zhao, Yubo Hu, Panwu Zhao, Jiale Chen, Xiaomeng Bai, Yi Chen, Deshan Kong, Shuyu Zheng, Yuena Zheng, Yumeng Wang, Yanni Zeng, Wei-Jye Lin, Xiaojing Ye [10'+5']
Presenter affiliation: Sun Yat-sen University, Guangzhou, China. 65

SESSION 6 BRAIN DEVELOPMENT AND BRAIN DISORDERS

Chairpersons: **Joseph Gleeson,** University of California, San Diego, La Jolla, California, USA
Chongyuan Luo, University of California, Los Angeles, Los Angeles, California, USA

TMTC3 stabilizes LAMB1 to maintain basement membrane integrity in cortical development
Shufang Zhao, Xiaoxu Yang, Harry Vong, Adnan Halim, Guoliang Chai, Joseph G. Gleeson [20'+10']
Presenter affiliation: University of California San Diego, La Jolla, California. 66

Cerebral organoids—Growing human brain tissue from stem cells to study development and disease
Juergen A. Knoblich [20'+10']
Presenter affiliation: Austrian Academy of Science, Vienna, Austria; Medical University of Vienna, Vienna, Austria. 67

Dissecting human brain development and neuropsychiatric disorders with single-cell and spatial 3D-multiomics
Matthew G. Heffel, Heng Xu, Katherine W. Eyring, Cuining Liu, Oier Pastor Alonso, Nasser Elhajjaoui, Xinzhe Li, Bogdan Pasaniuc, Jason Ernst, Eran Mukamel, Quan Zhu, Bogdan Bintu, Daniel H. Geschwind, Mercedes F. Paredes, Chongyuan Luo [20'+10']
Presenter affiliation: University of California Los Angeles, Los Angeles, California. 68

Break

Looking at glioblastoma through the lens of developmental and circuit neurobiology
Hongjun Song [20'+10']
Presenter affiliation: University of Pennsylvania, Philadelphia, Pennsylvania. 69

Pathological aggression in mice after repeated experience of aggression—Changes in brain immunity
Anastasia Mutovina [10'+5']
Presenter affiliation: Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia; Novosibirsk State University, Novosibirsk, Russia. 70

The distinct functions of autophagy genes FIP200 and ATG14 in postnatal brain development
Chenran Wang [10'+5']
Presenter affiliation: The Ohio State University, Columbus, Ohio. 71

Spatially targeted suppression of seizures using sonogenetics
Quanxiang Xian, Danni Li, Lei Sun [10'+5']
Presenter affiliation: The Hong Kong Polytechnic University, Hong Kong, China. 72

Social-isolation induced binge-like eating modulates anxiety and depression behaviors via a hypothalamic projecting circuit
Jing Cui, Jie Yu, Yuchu Liu, Ji-an Wei, Li Zhang [10'+5']
Presenter affiliation: GHM Institute of CNS Regeneration, Guangzhou, China. 73

THURSDAY, September 11—2:00 PM

SESSION 7 NEURAL CIRCUITS AND BEHAVIOR

Chairpersons: **Yang Dan,** Shenzhen Medical Academy of Research and Translation, Shenzhen, China
Bo Li, Westlake University, Hangzhou, China

What is sleep for?
Yang Dan [20'+10']
Presenter affiliation: Shenzhen Medical Academy of Research and Translation, Shenzhen, China. 74

Illuminating causal links between neural circuit activity and behavior
Michael Hausser [20'+10']
Presenter affiliation: HKU, Hong Kong, China; UCL, London, United Kingdom. 75

Dissecting the neural circuitry underlying motivated behaviors
Bo Li [20'+10']
Presenter affiliation: Westlake University, Hangzhou, China. 76

Break

Modulation of PMv^{DAT} cells during aggression development persistently alters social behaviour through changes in an upstream social brain circuit
Laura Heikkinen, Debora Masini, Liam Moran, Nancy Xu, Christian Broberger [10'+5']
Presenter affiliation: Stockholm University, Stockholm, Sweden; Karolinska Institutet, Stockholm, Sweden. 77

DeepEcoHAB—A machine learning based system for high-throughput behavioral phenotyping of social groups of laboratory animals
Marcin A. Lipiec, Konrad Danielewski, Jadwiga Zymer, Ewelina Knapska [10'+5']
Presenter affiliation: Nencki Institute of Experimental Biology, Warsaw, Poland. 78

All-optical interrogation of neural and astrocytic regulation of hunting behavior in larval zebrafish
Guangnan Tian, Gewei Yan, Thomas Ka Chung Lam, Kenny Kang Yuen Chen, Kai Fu, Jianan Y. Qu, Julie L. Semmelhack [10'+5']
Presenter affiliation: The Hong Kong University of Science and Technology, Hong Kong S.A.R., China. 79

Robust and ultrabright chemical labeling enables rapid neural connectivity profiling in large tissue samples
Shilin Zhong, Xiaoting Zhang, Xinwei Gao, Qingchun Guo, Minmin Luo, Rui Lin [10'+5']
Presenter affiliation: National Institute of Biological Sciences (NIBS), Beijing, Beijing, China. 80

THURSDAY, September 11—5:00 PM

COCKTAILS and BANQUET

SESSION 8 NEUROBIOLOGY OF BRAIN DISORDERS AND AGING

Chairpersons: **Joseph Ecker,** The Salk Institute, La Jolla, California, USA
Hannah Monyer, Heidelberg University and German Cancer Research Center (DKFZ), Heidelberg, Germany

GABAergic projection neurons—Powerful but vulnerable
Hannah Monyer [20'+10']
Presenter affiliation: Medical Faculty of Heidelberg University and German Cancer Research Center (DKFZ), Heidelberg, Germany. 81

Epigenomic remodeling in the aging mouse brain
Qiurui Zeng, Wei Tian, Amit Klein, Anna Bartlett, Hanqing Liu, Joseph Nery, Rosa G. Castanon, Chumo Chen, Yuru Song, Wenliang Wang, Wubin Ding, Huaming Chen, William Owens, Zhanghao Wu, Maria L. Amaral, Bing Ren, Margarita Behrens, Joseph R. Ecker [20'+10']
Presenter affiliation: The Salk Institute, La Jolla, California. 82

Patho-mechanism of eIF3F-associated neurodevelopmental disorder MRT67
Dan Su, Fajin Li, Luhao Yang, Dieter A. Wolf [10'+5']
Presenter affiliation: Technical University of Munich, Munich, Germany. 83

Break

Retrograde signaling as a trigger for TDP-43 mislocalization and motor neuron degeneration in ALS
Ruilei Cheng, Tal G. Pery, Eran Perlson [10'+5']
Presenter affiliation: Tel Aviv University, Tel Aviv, Israel. 84

Multiscale heterogeneous brain morphology of autism with hierarchical Bayesian regression normative modeling
Makliya Mamat, Yiyong Chen, Lin Li [10'+5']
Presenter affiliation: School of Basic Medical Sciences, Health Science Center, Ningbo, China. 85

Human models on a chip—Dissecting E/I balance in syndromic autism spectrum disorders
Maria Pascual-Garcia, Carolina Nunes, Philipp Hornauer, Linda Kaupp, Elias Hefti, Eva Harde, Stormy Chamberlain, Marcos R. Costa, Andreas Hierlemann, Manuel Schröter [10'+5']
Presenter affiliation: ETH Zurich, Basel, Switzerland. 86

A single-nucleus atlas of genetic and epigenetic landscapes in Parkinson's disease midbrain
Xinran Song, Anna Diacofotaki, Bernard Thienpont [10'+5']
Presenter affiliation: KU Leuven, Leuven, Belgium. 87

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WIRING SPECIFICITY OF NEURAL CIRCUITS

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Developing brains use a limited number of cell-surface proteins to instruct connection specificity of a much larger number of neurons and synapses. How is this feat achieved? How do different cell-surface proteins work together to assemble a functional circuit? To address these questions, I will first describe our work using the fly olfactory circuit as a model. I will then discuss functions of homologs of cell-surface proteins we identified in the fly olfactory circuits in instructing wiring specificity of neural circuits in the mouse brain.

ACTIVITY-DEPENDENT REMODELING OF NEURONAL CIRCUITS DURING DEVELOPMENT

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During development, neuronal connectivity is remodeled to form the functional neuronal circuits. In general, neuronal activity is critical for establishing some connections and eliminating others. However, the spatiotemporal regulation of synaptic formation versus elimination, and its critical role in circuit function, are not fully understood. In this study, we performed a comprehensive, high-resolution mapping of dendritic spines in layer 5 extratelencephalic-projecting (L5ET) neurons in the barrel cortex of mice. During the early stage of development (P14), dendritic spines were evenly distributed throughout the dendrites. During adolescence, spine density in the basal dendrites and apical tufts gradually decreased. However, spine density increased specifically in the middle compartment of the apical dendrites, where dendritic calcium spikes are generated. Dendritic compartment-specific spine formation was impaired in schizophrenia models, suggesting that reduced spine formation, rather than excessive elimination, underlies cortical dysfunction. These results suggest that the dendritic compartment-specific spine formation is critical for the maturation of nonlinear dendritic integration and cortical functions during adolescence.

MESOSCALE MAPPING OF THE NERVOUS SYSTEM: FROM BRAIN TO BODY

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The nervous system comprises myriad interconnected neurons forming intricate networks that span the brain and the entire body. Coordinated neuronal activity within these networks gives rise to perception, emotion, learning, and consciousness, while also regulating physiological functions. Over the past years, we have developed a high-speed, large-scale volumetric fluorescence microscopy technique—VISoR—capable of imaging an entire mouse brain at micron resolution within half and hour, and an rhesus monkey brain within 100 hours. This enables efficient mesoscale connectomic mapping and reveals unexpected trajectories and complex arborization patterns of individual thalamocortical axons. More recently, we extended this approach by developing a blockface-VISoR system and an optimized whole-body clearing pipeline, achieving uniform micron-resolution imaging of the entire mouse body. This enables comprehensive mapping of the body-wide architecture of the nervous and vascular system, revealing cross-segmental projection patterns of spinal sensory and motor neurons, organ-specific perivascular patterns of sympathetic nerves, and intricate fiber trajectories of vagus nerve axons.

FUNCTIONAL IMAGING OF DEVELOPING BRAIN IN MICE AND NON-HUMAN PRIMATES

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Despite substantial progress in structural and molecular profiling of the embryonic brain, in vivo functional investigations have remained limited by technical constraints. Functional ultrasound (fUS) imaging offers a unique combination of deep-tissue penetration, high spatiotemporal resolution, and sensitivity to hemodynamic changes, making it well suited for developmental neuroimaging. Here, we apply fUS to systematically monitor cerebral activity in mouse embryos from embryonic day (E) 12.5 to E18.5. Across this window, we identify dynamic resting-state signals and the emergence of low-frequency (delta-band) rhythms during late embryogenesis. We further demonstrate that fUS can detect neural responses to both deep brain stimulation and auditory stimuli in E18.5 embryos. Preliminarily extending this approach to cynomolgus macaque embryos, we observe auditory-evoked responses, highlighting the translational potential of fUS for fetal brain monitoring. Together, these findings establish fUS as a non-invasive modality for mapping functional brain development in vivo providing a framework for studying early neural activity and its perturbation in developmental disorders.

TEMPORAL REGULATION OF NEURAL STEM-PROGENITOR CELL FATE DURING NEOCORTICAL DEVELOPMENT

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A fundamental question in understanding tissue development is how resident stem cells or multipotent progenitors give rise to various cell types in the right numbers and in the right locations to achieve tissue organization. Neural stem/progenitor cells (NPCs) in the mammalian neocortex initially divide symmetrically, increasing their pool size (expansion phase). Subsequently, most apical NPCs begin to divide asymmetrically, giving rise to neuronal and then glial cells in a region- and developmental stage–dependent manner and with high precision (neurogenic and gliogenic phases, respectively). We have identified chromatin-based mechanisms, including those involving Polycomb group proteins, that underlie the developmental transitions of NPC fate. In this symposium, I would like to talk about another layer of temporal control of NPC fate which is coordinated by circadian rhythms and its relation to the chromatin-based mechanisms.

REGULATION AND HETEROGENEITY OF ADULT NEURAL STEM CELLS

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Neural stem cells reside in specialized niches in the adult mammalian brain and contribute to brain plasticity throughout life. The adult ventricular-subventricular zone (V-SVZ) adjacent to the lateral ventricles is the largest germinal niche in the adult mouse brain. Stem cells in the V-SVZ give rise to different subtypes of olfactory bulb interneurons, as well as to some glia. Importantly, adult V-SVZ neural stem cells are a heterogeneous population, with distinct molecular identities and fates, depending on their spatial location in the niche. They constantly integrate intrinsic and extrinsic signals to either maintain their quiescent state or to become activated to divide and generate progeny. However, the functional significance of this heterogeneity has remained elusive. I will present our recent findings highlighting the key role of physiological states, as well as of long-range signals, in regulating regionally distinct pools of adult neural stem cells for on-demand adaptive brain plasticity.

NON-EPITHELIAL RADIAL GLIAL CELLS MAINTAIN THE PRODUCTION OF GABAERGIC INHIBITORY NEURONS AND GLIAL CELLS IN THE DEVELOPING HUMAN BRAIN

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The human cerebrum has an extensive and diverse complement of inhibitory neurons (INs), which may contribute to the heightened cognitive capability of our species. However, the mechanisms underlying the generation of the vast repertoire of human INs remain elusive. We performed spatial and single-cell transcriptomics of human medial ganglionic eminence (hMGE), a pivotal source of INs destined for the cerebral cortex and subpallium, to build the developmental trajectories of MGE-derived cells throughout pregnancy. We identified spatiotemporally and molecularly segregated progenitor cell populations fated to produce distinct types of INs. Notably, we found a novel progenitor cell type in the hMGE subventricular zone (SVZ RGCs) with unique molecular and cellular features. We demonstrated that SVZ RGCs maintained the production of INs and glial cells throughout human brain development. Our findings reveal evolutionarily distinct features of IN generation and shed light on the unique mechanisms underlying human brain development.

FEZ1 DELETION DISRUPTS HUMAN CORTICAL BRAIN DEVELOPMENT BY ALTERING THE RATIO OF OUTER RADIAL GLIAL CELL SUBPOPULATIONS

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Abnormal neuronal networks arising from perturbations during early brain development contribute to neurodevelopmental disorders. Mutations and deletions of human Fasciculation and Elongation Protein Zeta 1 (FEZ1) are found in schizophrenia and Jacobsen syndrome patients. However, its roles in human brain development and manifestation of clinical pathological symptoms remain unknown. Here, using human cerebral organoids (hCOs), we observed that FEZ1 expression is turned on early during brain development and is expressed in neuroprogenitor subtypes and immature neurons. Deletion of FEZ1 disrupts expression of genes involved in neuronal and synaptic development. Moreover, FEZ1-null hCOs exhibited abnormal expansion of HOPX outer radial glia (oRG) at the expense of HOPX oRG. HOPX oRGs show higher cell mobility as compared to HOPX oRGs, which is accompanied by the ectopic localization of the neuroprogenitors to the outer layer of FEZ1-null hCOs. Abnormal encroachment of TBR2 intermediate progenitors into CTIP2 deep layer neurons indicated that cortical layer formation is disrupted in FEZ1-null hCOs. Collectively, our findings highlight the involvement of FEZ1 in early cortical brain development and how it contributes to neurodevelopmental disorders.

ACHIEVING SINGULARITY IN OLFACTORY RECEPTOR GENE EXPRESSION

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The sense of smell in mammals relies on the stable and singular expression of one out of 1000 olfactory receptor (OR) expression in mature olfactory neurons. Transcriptional singularity is mediated by an intricate network of interchromosomal genomic interactions that silence most OR genes and activate 5-10 OR alleles during neuronal differentiation. Subsequently, an RNA-mediated symmetry breaking process transforms the polygenic OR transcription into monogenic and monoallelic, whereas a protein-mediated feedback stabilizes this singular choice for the life of the neuron. Here, we present new data on the molecular mechanisms that enable interchromosomal interactions to occur with stability and specificity and the process by which the nascent OR mRNA signals for the transition to singularity.

ONCOGENIC FUSIONS CONVERGE ON SHARED MECHANISMS IN INITIATING ASTROBLASTOMA

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Chromosomal rearrangements and gene fusions are the initial events in the development of many cancers. Astroblastoma (ABM), a challenging brain cancer of unknown cellular origin, is associated with diverse in-frame gene fusions, including MN1:BEND2 and MN1:CXXC5. However, it remains unclear if these gene fusions contribute to tumorigenesis. Here, we show that the two ABM-associated fusions converge on similar molecular activities and initiate malignancy specifically in mouse ventral telencephalon neural progenitors. BEND2 and CXXC5 recognized similar DNA motifs, suggesting a convergence on downstream gene regulation. Expressing MN1:BEND2 in ventral telencephalon neural progenitors results in aberrant cell proliferation, impaired differentiation, a perivascular occupancy pattern of cells reminiscent of ABM, and acquisition of an ABM-associated transcriptional signature. In contrast, MN1:BEND2 expression in dorsal telencephalon neural progenitors leads to extensive cell death. This cell-type specific malignancy is dependent on Olig2 expression. Mechanistically, both ABM-associated fusion proteins (MN1:BEND2 and MN1:CXXC5) induce overlapping transcriptional responses, including the activation of a therapeutically targetable PDGFRα pathway. Collectively, our data suggest that distinct ABM-associated fusions upregulate shared transcriptional networks, thereby disrupting the normal development of ventral telencephalon neural progenitors and leading to oncogenic transformation. These findings uncover new avenues for targeted ABM treatment.

PREMATURE CORTICAL INHIBITORY NEUROGENESIS IN DOWN SYNDROME

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Down syndrome (DS), caused by trisomy 21 (TS21), is the most common chromosomal abnormality and cause of intellectual disability worldwide. The phenotypic features of the DS brain originate during prenatal life, which remains poorly understood. Hallmarks of DS brains during prenatal development have revealed differential abundance in both glial and neural cells originated from altered fate specification of radial glial (RG) progenitor cells. We performed high-throughput single-cell lineage tracing in human primary organotypic slice cultures from the midgestation to map the clonal output of cortical progenitor cells. We uncovered premature emergence of cortical-born inhibitory neurons coinciding with deep-layer excitatory neurogenesis and impaired RG lineage progression. Differential gene expression analysis combined with clonal information highlighted elevated inflammatory response in RG, connecting genomic abnormalities inherent to DS and RG lineage decisions.

TENEURIN ISOFORMIC CODING IN FLY OLFACTORY CIRCUIT ASSEMBLY

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Teneurins are type II single span transmembrane proteins. Previous studies showed that Teneurins instruct synaptic partner matching in fly olfactory system and mouse hippocampus through promoting homophilic adhesion in an evolutionarily conserved manner. We recently identified different isoforms of one of the two Teneurin fly homologs Ten-a (isoform A and B), generated through alternatively splicing, are differentially expressed between matching partners, ORN and PN, in the fly olfactory circuit. A and B isoforms, although differing with their intracellular domain, exhibit different binding capacity to the same extracellular domain (ECD) of Ten-a in trans in vitro. Only isoform A binds to the extracellular domain of Ten-a. Interestingly, isoform A is selectively expressed in one side of the matching partners, while B isoform is expressed in both sides. To test the ability of Ten-a isoform A and B to promote synaptic matching *in vivo*, we overexpressed different combinations of A and /or B in ORN and PN types that normally do not form connection in a Ten-a null mutant background. We observed that the synaptic matching only occurs when at least one side expressed isoform A. Finally, single cell MARCM clone analysis shows that there is a correlation between expression of the A+B and cell autonomous function of Ten-a in correct targeting of ORN axon or PN dendrite. This data suggests that A or A+B functions as the receptor to regulate correct targeting.

NEURONAL PRUNING AND ITS IMPLICATIONS IN HUMAN NEURODEVELOPMENTAL DISORDERS

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Pruning that selectively eliminates unnecessary or exuberant neuronal processes without causing death of the parental neurons is a crucial step for sculpting the nervous system during development. In Drosophila, dendritic arborization neurons ddaCs selectively prune their larval dendrites during metamorphosis. Dendrite pruning resembles pathological neurite degeneration in neurological disorders, and therefore serves as an excellent model for unraveling the mechanisms of neurodegeneration (Yu and Schuldiner, Curr. Opin. Neurobiol. 2014). My lab has initially identified a novel genetic pathway composed of Sox14 and Mical, which acts downstream of ecdysone receptor EcR and epigenetic factors Brm/CBP to promote dendrite pruning (Kirilly, Nature Neuroscience. 2009; Kirilly, Neuron 2011). This novel pathway determines the developmental timing of dendrite pruning. We then identified ubiquitin-proteasomal and endo-lysosomal pathways required for dendrite pruning (Wong, PLOS Biololgy 2013; Zhang, Developmental Cell 2014; Zong, PLOS Biology 2018). We have further investigated the roles of microtubule orientation and microtubule disassembly pathways during dendrite pruning (Wang Y., eLife 2019; Tang Q., EMBO J. 2020; Bu S., Cell Reports 2022).

More recently, we are focusing on the role of the transcription factor CncC in regulating proteasome degradation machinery during dendrite pruning. Fly CncC is the sole homolog of mammalian Nrf1 and Nrf2 which regulate protein and Redox homeostasis, respectively. We found that ecdysone signaling is required for the the cytoplasmic-to-nuclear translocation of CncC. CncC regulates dendrite pruning independently of its canonical antioxidant response pathway, instead, through proteasomal degradation pathway, suggesting that CncC behaves as an Nrf1 homolog during dendrite pruning (Chew LY., Cell Reports 2021). Moreover, the metabolic regulator AMPK and the insulin-TOR pathway act upstream to activate CncC during dendrite pruning (Chew, et al., Development 2022). Acting downstream of AMPK and the insulin-TOR pathway, autophagy is also required to regulate dendrite-specific pruning of ddaC sensory neurons. Impaired autophagy causes the formation of ubiquitinated protein aggregates in ddaC neurons, dependent on the autophagic receptor Ref(2)P. Importantly, autophagy is required to activate the transcription factor CncC, thereby promoting dendrite pruning. Conversely, CncC also indirectly affects autophagic activity via proteasomal degradation, as impaired CncC results in the inhibition of autophagy through sequestration of Atg8a into ubiquitinated protein aggregates. Thus, our study reveals an interplay between autophagy and CncC in neuronal pruning (Tan et al., PNAS 2024).

Human orthologs of various pruning genes we identified in Drosophila are found to be mutated in a number of human developmental disorders, like autism and microcephaly. We will update our recent studies on those human orthologs and their interesting roles in human cortical development and brain diseases.

STRESS NOVELTY INFLUENCES CHOLINERGIC INVOLVEMENT IN HIPPOCAMPAL EARLY GENE ACTIVATION

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The stress response is the body universal reaction to environmental changes, involving both nonspecific components (activation of the sympatho-adrenal and hypothalamic-pituitary-adrenal axes) and specific ones, depending on the stressor (activation of distinct brain regions and signaling systems). Changes in early gene expression (e.g., egr1, fos, npas4) reflect neuronal activity, but the induction mechanisms remain poorly understood.
Stress activates multiple signaling systems, including noradrenergic, serotonergic, and cholinergic pathways. While noradrenaline's role in early gene expression is established, the contribution of the cholinergic system, particularly muscarinic receptors, remains unclear. This study aimed to analyze the involvement of metabotropic muscarinic receptors in regulating gene transcription during acute stress induced by forced swimming.
Two experimental series were conducted. In the first, Wistar rats were subjected to 15-minute forced swimming after saline or scopolamine (muscarinic antagonist) injection. The second series involved 4 days of brief swimming (5 min/day), followed by a 15-minute session on day 5. Brains were collected 45 minutes post-stress, and gene expression was analyzed in the dorsal and ventral hippocampi by qPCR.
Results showed that acute swimming stress increased fos and npas4 expression in both hippocampal regions and egr1 in the ventral hippocampus. Scopolamine suppressed stress-induced npas4 and egr1 but not fos expression. In the second series, stress again upregulated fos and egr1, but scopolamine had no effect on egr1, unlike in the first series.
In conclusion, acute stress induces early gene expression in the hippocampus, but muscarinic receptor blockade only suppresses these changes in novel stress contexts, suggesting cholinergic involvement depends on stressor novelty.
Funding: The study was supported by the Russian Science Foundation, grant no. 25-15-00291.

THE ROLE OF CALCIUM SIGNALING DURING NEURAL DEVELOPMENT IN BIPOLAR DISORDER

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Bipolar disorder (BD) is a serious psychiatric condition characterized by episodes of depression and mania, affecting millions of people worldwide. While BD is highly heritable, its biological mechanisms remain poorly understood. Studies have identified *CACNA1C*, a calcium channel gene crucial for brain development and neural function, as a major genetic risk factor for BD. The strongest association is with a SNP located in the third intronic region of the gene, but its effects on brain development are still unclear.

To investigate this, we use an integrative approach combining patient-derived stem cells, CRISPR-engineered isogenic lines, and advanced 3D brain organoids and assembloids. Our findings reveal that the *CACNA1C* risk variant increases neural progenitor proliferation ($Ki67^+$) and impairs neuronal differentiation ($MAP2^+$). These results might suggest that the *CACNA1C* variant disrupts the timing of neural progenitor development and neuronal maturation, which may contribute to the neurodevelopmental origins of BD. By leveraging these innovative human-specific models, we aim to unravel the pathogenesis of BD and lay the groundwork for targeted therapeutic strategies. In future work, we plan to bridge molecular alterations in individual cells with network-level activity by utilizing advanced tools such as single-cell RNA sequencing, optogenetics, and multielectrode arrays.

PROBIOTIC INTERVENTION RESTORES MICROGLIAL SURVEILLANCE AND SYNAPTIC ARCHITECTURE IN THE AGING CORTEX

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Cortical plasticity declines with age, in parallel with shifts in microglial morphology and reductions in dendritic spine density and turnover at excitatory synapses. Given emerging evidence linking gut microbiota to brain aging and immune regulation, we tested whether microbiome modulation could restore structural and functional properties of cortical circuits by administering a three-week probiotic intervention to aged mice. Treated animals exhibited improved performance in whisker-based texture discrimination and novel object recognition tasks, consistent with levels observed in younger animals. Two-photon in vivo imaging revealed enhanced dendritic spine turnover and increased microglial process motility, indicating restored surveillance dynamics. Morphometric analyses in fixed tissue also revealed a structural restoration in spine density and morphology toward a more youthful profile in aged mice treated with probiotics. Microglial morphology also reverted toward a highly ramified, homeostatic state in both barrel and mPFC. These findings demonstrate that microbiome-based interventions can potentially reverse age-related deficits in cortical microglia and excitatory circuits, offering a non-invasive strategy to mitigate structural decline in the aging brain. Together, they establish an experimental framework for mechanistically linking microbiome manipulation with cortical circuit remodeling in an aging model.

MAPPING ULTRASOUND-DRIVEN NEURAL ACTIVITY AT MILLISECOND RESOLUTION VIA VOLTAGE SENSORS

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Ultrasound neuromodulation has emerged as a powerful tool for noninvasive brain stimulation, offering unparalleled spatial precision and deep tissue penetration. However, conventional monitoring techniques, such as calcium imaging and electrophysiology, face critical limitations—either insufficient temporal resolution to resolve fast neural dynamics or incompatibility with ultrasound environments. To overcome these challenges, we leveraged genetically encoded voltage indicators (GEVIs) to achieve millisecond-scale optical mapping of ultrasound-evoked neural activity. Our results show that voltage imaging reliably captures ultrasound-triggered action potentials, uncovering stimulus-dependent neuronal firing patterns. By combining voltage imaging with focused ultrasound stimulation, we established a closed-loop platform capable of simultaneous precise neural circuit manipulation and sub-millisecond electrophysiological readout in vitro. This integrated methodology not only advances the mechanistic understanding of ultrasound neuromodulation but also provides a versatile framework for studying neural circuit dynamics with high spatiotemporal precision.

MULTIMODAL SINGLE-CELL ANALYSES REVEAL MOLECULAR MARKERS OF NEURONAL SENESCENCE IN HUMAN DRUG-RESISTANT EPILEPSY

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The histopathological neurons in the brain tissue of drug-resistant epilepsy exhibit aberrant cytoarchitecture and imbalanced synaptic circuit function. However, the gene expression changes of these neurons remain unknown, making it difficult for the diagnosis or to dissect the mechanism of drug-resistant epilepsy. By integrating whole-cell patch clamp recording and single-cell RNA sequencing approaches, we identified a transcriptionally distinct subset of cortical pyramidal neurons. These neurons highly expressed genes *CDKN1A* (P21), *CCL2* and *NFKBIA* that associated with mTOR pathway, inflammatory response and cellular senescence. We confirmed the expression of senescent marker genes in a subpopulation of cortical pyramidal neurons with enlarged soma size in the brain tissue of drug-resistant epilepsy. We further revealed the expression of senescent cell markers P21, P53, COX2, γ-H2AX, β-Gal and reduction of nuclear integrity marker Lamin B1 in histopathological neurons in the brain tissue of drug-resistant epilepsy patients with different pathologies, but not in control brain tissue with no history of epilepsy. Additionally, chronic, but not acute, epileptic seizures induced senescent markers expression in cortical neurons in mouse models of drug-resistant epilepsy. These results provide important molecular markers for histopathological neurons and new insights into the pathophysiological mechanisms of drug-resistant epilepsy.

HOW DOES THE BRAIN LEARN? A DYNAMICAL SYSTEMS PERSPECTIVE

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The brain is a highly complex and dynamically evolving system, whose operational states undergo continuous reorganization during behavior and learning. However, defining, quantifying, and interpreting the evolution of brain states remains a central challenge in neuroscience. In this study, we investigate this question using a behavioral paradigm in which mice learn to obtain water via a pulley-based task. By combining widefield fluorescence imaging with functional ultrasound (fUS), we achieve high spatiotemporal resolution recordings of neural population activity and hemodynamic responses across both cortical and subcortical regions. Focusing on the neural dynamics during the learning process, we develop a theoretical model grounded in the principle of free energy minimization, which effectively accounts for our experimental observations. Furthermore, we examine whether the observed patterns of neural plasticity during learning conform to an underlying, unified brain dynamic principle. By quantitatively characterizing the evolution of brain states, this study provides experimental evidence and a theoretical framework to further our understanding of how the brain learns.

PATHWAY-SPECIFIC HIPPOCAMPAL PLASTICITY GATED BY THETA-FREQUENCY MEDIAL SEPTAL CHOLINERGIC ACTIVITY

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The medial septal area (MSA) is a heterogeneous structure containing multiple neuron types. MSA plays a crucial role in modulating hippocampal function through cholinergic signaling, yet how physiologically patterned MSA activity shapes synaptic plasticity in hippocampal-entorhinal cortex (EC) circuits remains unclear. We investigated in vivo the effects of theta-frequency MSA stimulation (4 Hz and 7 Hz) on synaptic transmission at two hippocampal pathways: the lateral perforant path (LPP) to dental gyrus (DG) and the temporoammonic path (TAP) to CA1. The experiments were conducted using adult male Wistar rats (250–350 g). In the first part of the experiment, all rats were divided into 4 groups: (1) lateral perforant path (LPP)-DG-7Hz, fEPSPs were recorded from the DG in response to LPP stimulation, with long-term changes induced by MSA stimulation at 7 Hz; (2) LPP-DG-4Hz, fEPSPs were recorded from the DG in response to the LPP stimulation with long-term changes induced by MSA stimulation at 4 Hz; (3) LPP-CA1-7Hz, fEPSPs were recorded from the hippocampal CA1 area in response to the LPP stimulation, with long-term changes induced by MSA stimulation at 7 Hz; (4) LPP-CA1-4Hz, fEPSPs were recorded from the hippocampal CA1 area in response to the LPP stimulation, with long-term changes induced by MSA stimulation at 4 Hz. Using in vivo electrophysiology in urethane-anesthetized rats, we found that prolonged 7 Hz MSA stimulation selectively induced synaptic potentiation at LPP-DG synapses, while 4 Hz stimulation had no effect. In contrast, neither 4 Hz nor 7 Hz stimulation altered synaptic efficacy at TAP-CA1 synapses. Analysis of paired-pulse ratio (PPR) revealed frequency-specific modulation of short-term plasticity, with 7 Hz stimulation reducing PPR in LPP-DG synapses during the early phase of synaptic potentiation. Given that only prolonged 7 Hz MSA stimulation modulated hippocampal synaptic plasticity, we next investigated whether the observed synaptic changes at LPP-DG synapses depended on cholinergic MSA neurons. We examined how cholinergic dysfunction induced by intraseptal injection of 192IgG-saporin affects synaptic potentiation at LPP-DG synapses in vivo. We found that synaptic changes were cholinergically dependent, as demonstrated by its abolition following cholinergic depletion. Our results suggest that the septohippocampal system employs frequency-tuned cholinergic signaling to gate cortical-hippocampal communication, with 7 Hz oscillations preferentially enhancing efficacy of transmission in LPP-DG synapses. This work was supported by the Russian Science Foundation [grant number 24-25-00221].

IDENTIFYING ELECTROPHYSIOLOGICAL BIOMARKERS IN hiPSC-DERIVED NEURONS FROM ADHD PATIENTS

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Attention-deficit hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders globally, affecting about 5.3% of children and 2.5% of adults. Psychostimulants are the primary treatment for ADHD, but, despite their potential, around 30% of children and over 50% of adults respond inadequately, with side effects often leading to poor medication adherence.

Currently, there are no predictive markers to identify non-responders early, resulting in unnecessary drug exposure. Traditional ADHD research methods, such as animal models and neuroimaging, have limitations. Animal models struggle to represent complex human mental disorders, and neuroimaging lacks the resolution to obtain single-cell data at high temporal resolution, which are crucial for detailed functional characterization.

This study aims at electrophysiologically characterizing neuronal disease phenotypes in patient-derived cells at high spatiotemporal resolution. By combining human stem cell biology with advanced CMOS-based high-density microelectrode array (HD-MEA) technology, we strive to achieve new levels of physiological relevance and detail. Human induced pluripotent stem cell (iPSC)-derived neurons retain donors' genetic signatures, and HD-MEA technology allows for non-invasive, longitudinal recordings of network activity at high resolution, from subcellular compartments to entire networks, using 26,400 electrodes at a 17.5 µm pitch.

We optimized culturing protocols to enhance cell adhesion and physiological stability, enabling long-term maintenance and stable network activity on HD-MEAs for several months. Recordings from multiple control and patient-derived cell lines showed differences in the temporal development of network dynamics, consistent with cortical developmental delays observed in ADHD. Variations in synchrony development between these cell lines further support these findings.

Developing a systematic feature extraction pipeline will enable the identification and quantification of additional multiscale electrophysiological attributes to capture more fine-grained temporal and spatial characteristics of network activity. An expanded feature set will facilitate the identification of the most informative biomarkers for distinguishing between control and patient-derived cell lines, which improves classification and phenotyping.

Our platform offers the potential to perform data-driven phenotypic screening and will aid medication assessment and the development of personalized treatment strategies. Tailoring interventions to patient-specific neurophysiological profiles may improve treatment outcomes, reduce societal burden, and contribute to discovering novel ADHD treatments.

DISSECTING THE CELLULAR EFFECTS OF SCHIZOPHRENIA RISK VARIANTS: 3Q29 DELETION AND DUPLICATION IN NEURODEVELOPMENT AND FUNCTION

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Schizophrenia is a severe psychiatric disorder with high heritability (~80%), yet its diagnosis and treatment remain limited due to insufficient mechanistic understanding. Recent genomic studies have identified rare copy number variants that confer substantial disease risk, providing unprecedented opportunities to investigate molecular and cellular mechanisms underlying schizophrenia pathogenesis. The 3q29 locus has emerged as particularly significant: its deletion confers one of the highest known genetic risks for schizophrenia (>40-fold increased risk), while its duplication is associated with autism spectrum disorders and intellectual disability. This reciprocal dosage sensitivity suggests that precise gene expression levels within this 22-gene region are critical for normal neurodevelopment and function.

Thus, in this study, we aim to investigate the cellular mechanisms underlying 3q29 dosage sensitivity by using human pluripotent stem cells-based model. We address three fundamental questions: (1) Whether 3q29 deletion and duplication affect cortical neuron differentiation and synaptic transmission? (2) Do these variants alter neuronal maturation and function through glial cells, including astrocytes and oligodendrocytes? (3) Which of the 22 genes within the locus are primary drivers of observed phenotypes and potential therapeutic targets?

Currently, we have generated isogenic 3q29 deletion and duplication lines in human embryonic stem cells. Cortical neurons are differentiated using Ngn2-induction for rapid synaptic formation and directed differentiation protocols for comprehensive neurodevelopmental characterization. Current analyses focus on differentiation efficiency, neuronal morphology, synaptogenesis, spontaneous network activity, and mitochondrial metabolism—cellular processes frequently disrupted in psychiatric disorders.

In summary, this study will provide mechanistic insights into how 3q29 dosage alterations contribute to neurodevelopmental dysfunction and may identify cellular phenotypes that could serve as biomarkers or therapeutic targets. By bridging genetic risk to cellular mechanisms, our work will advance understanding of schizophrenia pathogenesis and inform precision medicine approaches for high-risk individuals carrying 3q29 variants.

TEMPORAL AND NOTCH IDENTITY DETERMINE LAYER TARGETING AND SYNAPSE LOCATION IN THE FLY VISUAL SYSTEM

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How specification mechanisms that generate neural diversity translate into specific neuronal targeting, connectivity, and function in the adult brain is not understood. In the *Drosophila* optic lobe, the visual processing center of the brain, neural progenitors generate different neurons in a fixed order by temporal patterning. Then, Notch signaling in intermediate progenitors further diversifies neuronal progeny, with most of the Notch^{On} neurons being projection neurons and most of the Notch^{Off} neurons being local interneurons. By establishing the birth order of optic lobe neurons from the medulla region, we found that their temporal identity correlates with the depth of neuropil targeting in the adult brain, for both interneurons and projection neurons. We show that this temporal identity-dependent targeting of projection neurons unfolds early in development and is genetically determined. Furthermore, by leveraging the recently completed Electron Microscopy reconstruction of the adult fly brain (FlyWire dataset), we determined the synapse location of medulla neurons in the different optic lobe neuropils and find that it is significantly associated with both their temporal identity and Notch status. Moreover, by using functional models derived from the connectome, we show that medulla neurons with the same predicted function share similar neuropil synapse location, indicating that ensembles of neuropil layers encode specific visual functions that are linked to temporal origin. In conclusion, we show that temporal identity and Notch status of medulla neurons can predict their neuropil synapse location and visual function, linking their developmental patterning with their specific connectivity and functional features in the adult brain.

ERK-DEPENDENT AMYLOIDOGENESIS DRIVES HYDROCEPHALUS ACROSS ETIOLOGIES

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The choroid plexus, a specialized epithelial structure governing cerebrospinal fluid (CSF) production and homeostasis, represents an understudied therapeutic target in hydrocephalus pathogenesis. In Neurofibromatosis Type 1 (NF1), a prototypical RASopathy disorder, hydrocephalus represents a clinical challenge due to poorly defined cellular and molecular drivers. Here, we identified communicating hydrocephalus in both genetically engineered Nf1-knockout mice and NF1 patients. Through integrated multi-omics approaches, we uncovered an amyloidogenic pathogenesis wherein Nf1 deficiency activates RAS-MEK/ERK signaling to transform choroid plexus epithelial cells into amyloidogenic-reactive epithelial cells (ARECs), with β-amyloid overproduction initiating and accompanying tight junction disruption, motile cilia impairment, and secretory homeostasis dysregulation, ultimately driving ventriculomegaly. Therapeutic interventions targeting this pathway at distinct nodal points proved effective, with upstream MEK inhibition suppressing ERK activation and downstream γ-secretase inhibition blocking amyloidogenic processing. Remarkably, single-cell RNA profiling identified parallel AREC populations with similar ERK activation in post-hemorrhagic hydrocephalus, while CSF proteomics uncovered shared amyloidogenic biomarkers across species and disease categories. These findings redefine NF1-associated hydrocephalus as an MEK/ERK-driven proteinopathy and establish choroid plexus amyloidogenesis as a convergent mechanism spanning genetic and acquired hydrocephalus, thereby open new therapeutic possibilities by repurposing existing RASopathy and Alzheimer's-directed therapies for hydrocephalus across diverse etiologies.

TARGETED MECHANOGENETICS TO MODULATE SPECIFIC DEEP NEURAL CIRCUITS

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Sonogenetics utilizes ultrasound for targeted, cell-type-specific modulation of neuronal activity. However, manipulating specific circuits within complex brain areas poses significant challenges due to heterogenous cell types with varying inherent sonosensitivity. In this study, we present the novel approach of "mechanogenetics" - nanoparticle-actuated sonogenetics, localizing and amplifying ultrasonic effects in targeted brain regions through nanoparticles, and activating specific neural circuits through sonogenetics. By applying mechanogenetics in distinct brain regions, we observed increased electromyography signals from the mouse-activated motor cortex and increased neural firing in deeper brain areas. Importantly, this combined approach enabled us to modulate neuronal circuits controlling feeding behavior by specifically stimulating the mouse lateral hypothalamus (LH), with minimal disturbance in adjacent areas. The observed mechanogenetically-induced decreases in feeding behavior were, further, found to be mediated by LH-glutamatergic inputs to the ventral tegmental area. Furthermore, we provide comprehensive evidence of the biosafety of this approach, through examining neural integrity, inflammation, and cell apoptosis. Overall, mechanogenetics enables neuron-typespecific, spatially-targeted, temporally-precise, and repeatable activation of deep brain circuits for neuromodulation, thereby opening exciting avenues for ultrasound applications in neural circuit control and behavioral research.

RACK1 AS A DYNAMIC PLATFORM FOR PROTEIN (DE)PHOSPHORYLATION AND TRANSLATIONAL CONTROL IN NEURONS

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Rack1 is involved in various signaling cascades through its effects on protein translation and degradation. Of particular interest is the ability of RACK1 to function as a molecular platform to which both kinases and phosphatases, as well as target proteins, can be simultaneously recruited. This allows RACK1 to act as a switch that determines the fate of proteins: they are activated or degraded depending on modifications of RACK1 itself and associated enzymes. In neurons it is itself an active participant in the regulated local translation of proteins on ribosomes. We showed that RACK1 mRNA decreases in neurons in response to picrotoxin activation. This suggests that during processes of synaptic plasticity, the pre-existing pool of RACK1 is sufficient for function. However, the distribution of the RACK1 protein across neuronal compartments remains inadequately characterized. One fundamental question regarding RACK1 localization is: what RACK1 variants (nonphosphorylated/phosphorylated status) exist in each subcellular location, and what are their relative abundances? To address this, we applied an approach involving the mechanical separation of neuronal cells into neurite and cell body fractions. Cells are cultured on top of microporous membranes, which contain pores large enough to allow neurites to extend through to the underside. After growth on the membranes, cells was mechanically fractionated and analysis was performed using Phos-Tag PAGE. Subsequent western blotting and immunostaining with RACK1 antibodies revealed that the phosphorylation status of RACK1 differs between the soma and neurites. The non-phosphorylated form of RACK1 is significantly less abundant in the neurite fraction and is likely phosphorylated at different sites compared to the soma. Furthermore, 30-minute activation with NMDA leads to an increase in non-phosphorylated RACK1 in neurites, accompanied by a decrease in one specific phosphorylated RACK1 variant in neurites. Our initial hypothesis was that only the non-phosphorylated form of RACK1 resides on the ribosome. Additionally, a consensus in the scientific literature suggests that certain kinases, such as AMPK1/2 or PKR, can phosphorylate RACK1 in response to stimuli (e.g., neuronal activation), thereby displacing it from the ribosome and implicating it in the regulation of translation for various mRNA pools. In the search for the kinase responsible for RACK1 phosphorylation, we selected four primary candidates: PKCβII, PKR, HRI, and AMPK1/2 (T172D). At this stage, we cannot confirm phosphorylation of RACK1 by PKR or PKCβII. Our PKR preparation successfully phosphorylated eIF2α, but not RACK1, leading us to conclude that PKR is not involved in RACK1 phosphorylation.

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RELATION BETWEEN VALENCE AND LOCOMOTOR PERFORMANCE USING WELL ESTABLISHED OPTOGENETIC TOOLS IN *DROSOPHILA MELANOGASTER*

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Animal survival depends on appropriate responses to sensory stimuli, guided by neural processes such as sensory perception, valence assignment, and decision-making. *Drosophila melanogaster* is a well-established model for studying locomotion and motor control, with over 60% of the human genome and 75% of human disease genes having functional homologs in fruit flies. In *Drosophila*, locomotor behaviors such as walking, turning, and climbing are generated by central pattern generators and further modulated by higher-order brain structures, including the mushroom body (MB) and central complex. While disease-modeling mutants (e.g., Parkinson's) exhibit clear motor deficits, the contribution of specific MB circuits, particularly MB output neurons (MBONs), to locomotor behavior remains underexplored. Traditionally, MBONs have been associated with encoding learned and innate valence, influencing whether stimuli are perceived as rewarding or aversive. However, recent findings suggest that activating specific MBONs can also modulate locomotor patterns independently of associative learning. This has led to an ongoing debate in the field, with some proposing that MBONs influence only directional turning rather than baseline locomotion. However, our preliminary data indicate that activation of certain MBON populations not only affects valence-related behavior but also induces direct and measurable changes in overall locomotor activity. Therefore, I hypothesize that valence in specific MBONs is modulated by fundamental aspects of locomotion and motor output. Using a comprehensive behavioral genetic screen, I assess the effects of activating and silencing MBONs on various aspects of locomotion using optogenetic modulation. In addition, my work utilizes a novel, multi-dimensional metric array to better capture the complexity of motor output beyond traditional movement measurements. This study will advance our understanding of the MBON locomotor circuitry and the mechanisms of how MBON inputs guide valence.

NON-INVASIVE TARGETED VECTOR DELIVERY FOR SPATIALLY PRECISE AND FLEXIBLE SONOGENETICS

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Sonogenetics is an emerging technology exploiting genetic specificity to enhance cellular responsiveness to ultrasound, thereby enabling the precise activation of specific neurons overexpressing mechanosensitive proteins. However, current applications typically rely on direct intracranial infusion for gene delivery, a highly invasive method associated with risks such as tissue damage, infection, and haemorrhage. In this study, we utilized the blood-brain barrier-permeable adeno-associated virus AAV-PHP.eB to achieve non-invasive overexpression of a mutant large-conductance mechanosensitive ion channel (MscL-G22S) in excitatory neurons of Emx1-Cre transgenic mice. To achieve spatial resolution of neuronal activation, we applied focused ultrasound (FUS), a non-invasive and millimeter-precise stimulation technique, to selectively activate brain regions expressing MscL-G22S. FUS stimulation induced significant c-Fos expression in the targeted regions without off-target activation. By integrating systemic, non-invasive delivery of genetic constructs with FUS-based activation of transduced neurons, this approach enables flexible neuromodulation and behavioral monitoring without the need for surgical intervention. Furthermore, targeted sonication of memory-related regions significantly enhanced spatial reference memory performance in Emx1-Cre mice. Immunohistochemical analyses revealed no significant activation of microglia or astrocytes, indicating that the approach does not elicit neuroinflammatory responses. Collectively, we establish a fully non-invasive and cell-type-specific in vivo sonogenetic platform for flexible and scalable neuromodulation, with broad implications for basic neuroscience and therapeutic applications.

AN RNA-RNA INTERACTION REQUIRED FOR GABA SIGNALING IN *DROSOPHILA*

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Post-transcriptional control of gene regulation is essential for development and function of the nervous system, yet the molecular interactions that can influence mRNA regulation remain incompletely understood. Here we describe an intermolecular RNA-RNA interaction (RRI) that is critical for GABA signaling in the *Drosophila* visual system, by promoting translation of the vesicular GABA transporter (VGAT). While VGAT is only present as protein in GABAergic neurons, it is transcribed pan-neuronally in the fly brain. We demonstrate that the spatial specificity of VGAT translation is dependent on another gene, CG14989, whose 3'UTR contains a 22 nucleotide sequence that is perfectly complementary to a region in the VGAT 3'UTR. The location of the CG14989 gene in the genome is next to the GABA Synthetase encoding GAD1 gene, and both are expressed exclusively in the GABAergic lineage. CG14989 expression is both necessary for VGAT translation in GABA neurons, and sufficient for induction of VGAT translation in non-GABA neurons when ectopically expressed. Furthermore, other gene pairs in the *Drosophila* genome contain similar complementary sequences, indicating that this mechanism is likely utilized more broadly outside of GABAergic neurons to control translation. In conclusion, our work shows that RRI interactions may be an underappreciated layer of gene regulation in the nervous system of *Drosophila*, and could potentially be utilized in other species including mammals.

FLUORESCENT BARCODE VECTORS FOR MESOSCOPIC CIRCUIT MAPPING

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Most brain functions result from the coordination across multiple brain areas. To understand information processing across brain areas, mesoscopic maps of neuronal connectivity have been extensively studied in the last decade. However, conventional whole brain imaging combined with tracer injection visualizes projections from only a single region per animal, requiring many animals to describe a comprehensive connectivity map. To overcome this limitation, we developed a multiplexed mesoscopic mapping tool of neuronal circuits called "fluorescent barcode vectors".
First, we developed adeno-associated virus vectors (AAV) expressing two of 7 different fluorescent proteins (XFPs), allowing the multiplexed labeling of neurons with up to 21 color combinations (fluorescent barcode vectors). Each XFP signal was detected in an all-or-none fashion after linear unmixing of fluorescence signals. We introduced the fluorescent barcode vectors into 10-20 cortical regions in mice, followed by fluorescence imaging of the brain slices spanning the entire brain. In the striatum and thalamus, the axonal projections were differentially visualized with multiple fluorescent barcodes, revealing a fine-scale and intermingled organization. To identify fluorescent barcode signals automatically, we then developed a machine learning-based “barcode reader” that enables pixel classification based on color and morphological information. Quantification of the barcode signals with the barcode reader revealed a comprehensive connectivity map from a single mouse brain.
Finally, we demonstrate its utility for studying the marmoset brain. Using this tool, we visualized the topographic axonal projections from the primary visual cortex to multiple brain regions including the lateral geniculate nucleus, pulvinar nucleus, and higher visual cortices.
Our fluorescent barcode vectors allow for a highly multiplexed mapping of mesoscopic projections. Using this tool, we can easily perform comprehensive mapping of the mouse brain under different life stages or disorder conditions, which help us to understand the dynamic nature of the connectome. Moreover, our tool facilitates mesoscopic mapping of the primate brain.

ALZHEIMER'S DISEASE ASSOCIATED PS1 MUTATION DISRUPT CORTICAL DEVELOPMENT THROUGH NOTCH SIGNALING

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Background:
Familial Alzheimer's disease (FAD) is caused by mutations in three genes: amyloid precursor protein (APP), presenilin 1 (PS1), and presenilin 2 (PS2). The most prevalent mutation occurs in PS1, which encodes the catalytic subunit of γ-secretase and alters APP cleavage. γ-secretase processes type 1 transmembrane proteins, including APP and NOTCH family proteins. Given the critical role of NOTCH signaling in cortical expansion during brain development, we investigated whether PS1 mutations affect brain development or cortical expansion in FAD patients.
Methods:
Peripheral blood mononuclear cells (PBMCs) from a PS1-P264L mutation carrier were reprogrammed into induced pluripotent stem cells (iPSCs). PS1-P264L and PS1-WT iPSCs were differentiated into cortical organoids and cortical neurons to model neural development and cortical expansion. Cell behavior was traced to assess proliferation, mitosis, differentiation, and neuronal output in neural stem cells (NSCs) and intermediate progenitors (IPs
Results:
PS1-P264L organoids were smaller than healthy controls. At early stages, mutant organoids exhibited fewer and thinner neural epithelial buds. The proportion of dividing NSCs and IPs was reduced, NSC markers expression were downregulated, and neuronal markers were upregulated. At later stages, NSCs were sparsely distributed throughout mutant organoids, whereas intact neural tubes persisted in controls.
PS1-P264L impaired NOTCH signaling in NSCs and NOTCH1-293T cells, reducing levels of the Notch1 intracellular domain (NICD) and target genes Hes1 and Hes5.Overexpression of NICD rescued cortical organoid size, NSC proliferation or maintenance, and neuronal output.
Conclusion:
PS1-P264L reduces cortical organoid size and disrupts neurodevelopment. Early deficits include impaired NSC/IP self-renewal; later stages show compromised NSC maintenance, neural tube integrity, and accelerated NSC depletion. NICD overexpression rescues these phenotypes, confirming that PS1-P264L impairs cortical expansion by disrupting NOTCH signaling through reduced NICD cleavage and downstream gene expression

MECHANISMS OF PIAL ASTROCYTE SPECIFICATION IN THE DEVELOPING MOUSE CEREBRAL CORTEX

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Astrocytes in the mouse cerebral cortex exhibit extensive heterogeneity. Pial astrocytes, located on the brain surface, exhibit distinct characteristics from other astrocytes; they attach to the pia mater, surround the brain, and preferentially express several genes such as Myoc. Despite these unique features, the mechanisms underlying their specification during brain development remain elusive. Given the close proximity of pial astrocytes to the meninges, we hypothesized that signals from the meninges induce pial astrocyte. We indeed found that co-culturing astrocytes with meningeal cells altered the morphology of astrocytes and upregulated the expression of several genes that are preferentially expressed in pial astrocytes. To identify the factors contributing to this fate specification, we focused on the BMP signaling pathway. Inhibition of Smad4, a key downstream effector of the BMP signaling pathway, reduced the proportion of pial astrocytes among L1 astrocytes. Furthermore, the Smad4-knockdown pial astrocytes showed a reduced adhesive area to the brain surface compared to control pial astrocytes. Together, these results suggest that BMPs released from the meninges play a crucial role in inducing pial astrocyte specification.

PERINATAL OPIOID EXPOSURE DISRUPTS IMMUNE SIGNALLING IN THE PREFRONTAL CORTEX AND DORSAL HIPPOCAMPUS OF JUVENILE RATS

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Perinatal opioid exposure (POE) impairs foetal brain development, but maternal dependence treatments with methadone or buprenorphine prolong infant exposure with unknown long-term consequences. Preclinical research links POE with white matter alterations and brain inflammation, however, it is unclear how these responses are activated, and how they differ across brain regions and opioids. Using an animal POE model, we investigated immune gene expression in regions controlling opioid-impaired functions: hippocampus (non-verbal memory) and prefrontal cortex (executive functioning). Both methadone and buprenorphine were predicted to increase inflammatory gene expression; a smaller increase was expected for buprenorphine, which is associated with improved neonatal outcomes.

Pregnant Sprague-Dawley rats were exposed to methadone (9mg/kg/day), buprenorphine (1 mg/kg/day) or saline using mini-osmotic pumps from early gestation until pup weaning. Pup brains (MET=16; BUP=17; VEH=18) were collected a week post-weaning, avoiding acute drug effects on neural physiology. RT-qPCR was used to assess expression of pro-inflammatory (TNFα, IL-6, IL-1β) and anti-inflammatory (IL-10) cytokines, as well as changes in JAK-STAT and MAPK signaling.
We found several gene expression differences across both brain regions indicating disrupted immune functioning. POE strongly downregulated IL-6 expression in the prefrontal cortex, $t(18)=7.08, p<.001, \eta 2=.74$, and dorsal hippocampus $t(17)=4.12, p=.001, \eta 2=.50$. In the latter region POE also upregulated expression of pro-inflammatory TNFα, $t(15)=-4.17, p=.001, \eta 2=.54$, without differences between opioid-exposed groups. There was no group change in anti-inflammatory IL-10 expression in either region. In the prefrontal cortex, expression of MAPK3 – which is activated by IL-6 – was down-regulated ($t(17)=2.85, p=.01, \eta 2=.32$), as was expression of the IL-6 receptor in exposed males, $t(18)=2.56, p=.02, \eta 2=.27$. Principal components analysis revealed relationships between TNFa and JAK-STAT/MAPK signaling gene expression in the dHPC, but no relationships between IL-6 expression and these mediators.

POE induces a complex pattern of cytokine and signal transduction factor expression in the prefrontal cortex and dorsal hippocampus of juvenile rats, revealing lasting neuro-immune impacts of both methadone and buprenorphine. Downregulated IL-6 expression – required for cellular maturation and immune defence – provides a possible mechanism for opioid-induced neurodevelopmental disruptions. POE may reduce MAPK cascade activation in the prefrontal cortex through IL-6 suppression, impacting cell development regulation, while elevated dorsal hippocampus TNFα levels suggest heightened risk of apoptosis and tissue damage. Through improved understanding of the molecular consequences of synthetic opioids, safer treatments for opioid-affected infants can be developed.

3D IN VITRO MODELLING OF 1Q21.1 DUPLICATION SYNDROME

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Copy Number Variants (CNVs) are known to significantly increase the risk of neurodevelopmental and neuropsychiatric disorders. Among these, CNVs at the 1q21.1 locus are linked to a wide range of clinical outcomes, including abnormal head size, congenital heart defects, skeletal anomalies, behavioural difficulties, and intellectual disabilities. While previous research has associated CNVs with impaired brain development due to synaptic dysfunction, the specific impact of 1q21.1 duplication on early human brain development remains largely unknown—an uncertainty largely attributed to its rarity.

To address this gap, we developed an in vitro cortical organoid model using induced pluripotent stem cells derived from individuals with the 1q21.1 duplication. Our research focused on neurodevelopmental trajectories, examining both the early proliferative phase—highlighting the ventricular zone (VZ), a key site of intense cell proliferation—and later stages of neuronal maturation.

Interestingly, while the overall morphology of the VZ appeared similar between control and 1q21.1 duplication organoids, we observed a reduction in the population of neuronal progenitor cells at both transcriptional and translational levels. Notably, organoids with the 1q21.1 duplication exhibited impaired neuronal maturation, characterized by a significant decrease in the expression of the mature neuronal marker MAP2 and an increase in synaptophysin levels, indicating synaptic dysfunction. Functional calcium imaging further revealed abnormal network activity, with a higher proportion of calcium-active cells and an increased frequency of calcium transients in duplication organoids compared to controls.

These findings highlight how 1q21.1 duplication disrupts progenitor cell dynamics, hinders neuronal maturation, and alters synaptic function, ultimately leading to aberrant neural network activity. This research sheds light on the underlying mechanisms of 1q21.1 duplication syndrome and its impact on early brain development.

SONOGENETICS AND NEUROSCIENCE UTILITY

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Noninvasive and accurate control of neuronal activity in deep brain is critical for probing brain function and treating brain dysfunctions. Transcranial low-intensity ultrasound is a promising neuromodulation modality, with the advantages of non-invasiveness, deep penetration and high spatiotemporal accuracy. However, the underlying mechanism of ultrasound neuromodulation remains largely unclear and controversial, hindering the development of efficacious treatments. In this talk, the current understanding of the mechanism of transcranial ultrasound, especially the role of mechanosensitive proteins, will be discussed, as well as the common research methodologies and possible complications. However, conventional ultrasound's spatial resolution is diffraction-limited and low-precision. Here, I will present our latest strategy to achieve precise ultrasound neuromodulation by sonogenetics with cell-type selectivity and spatial accuracy.

DOPAMINE D1 RECEPTORS IN THE MEDIAL PREFRONTAL CORTEX REGULATE METHAMPHETAMINE SEEKING

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[Background] While addiction research has extensively investigated ventral tegmental area (VTA) projections to the striatum, the functional role of dopamine (DA) in other VTA target regions remains incompletely characterized. The medial prefrontal cortex (mPFC) appears critical for cue-induced drug seeking, which commonly drives relapse in substance use disorder (SUD) patients. DA release in the mPFC has been implicated in associative learning and stimulus discrimination. Here, we aim to elucidate how mPFC DA release contributes to the recognition of drug-related stimuli and thereby facilitates drug seeking.
[Methods] Mice were trained on a methamphetamine self-administration procedure. DA sensors were used to monitor DA release dynamics in the prelimbic cortex (PL) in response to methamphetamine-associated cues. DA receptor expression changes following drug exposure were quantified by qPCR. Through detection of c-Fos protein expression and photometry signal increases, we confirmed the activation of PL dopamine receptor 1 (D1R)-expressing neurons by drug cues. We then investigated the role of these neurons in drug-seeking using chemogenetic approaches or local D1R antagonism.
[Results] Drug cues induced phasic DA release in PL. D1R expression was upregulated following methamphetamine self-administration. PL D1R-expressing neurons, rather than D2R-expressing neurons, were activated by drug cues. Both local D1R antagonist administration and chemogenetic inhibition of PL D1R-expressing neurons significantly suppressed cue-induced drug seeking. Neural circuits centered on these D1R-expressing neurons underlying drug-seeking behavior were identified via viral tracing and c-Fos mapping.
[Conclusions] Dopamine release in the prelimbic cortex (PL) is essential for cue-induced methamphetamine seeking. Pharmacological blockage of prefrontal D1 receptors may provide an optional strategy for treating SUD.

INVESTIGATING THE CELLULAR MICROARCHITECTURE OF SQUID OPTIC LOBE

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Coleoid cephalopods (squids, octopus, cuttlefish), with their complex visual system and exhibiting sophisticated visually-guided behaviour, have in recent years become a popular subject in the field of comparative neuroscience. However, little is known about the morphological, electrical, biochemical, and genetic properties of the cells comprising the optic lobe (OL). We have set out to build a comprehensive cell atlas of the OL of the bigfin reef squid *Sepioteuthis lessoniana*. We have developed an acute slice preparation of the optic lobe which allows us to combine electrophysiological recordings, morphological reconstructions, and single-cell RNA sequencing.

The OL consists of an outer, three-layered cortex, subdivided into the outer and inner granular layers (OGL, IGL) containing mostly cell bodies surrounding the plexiform layer which contains the neuropil. The Medulla forms the inner part of the OL and cell bodies are clustered in islands with a tree-like branching pattern.

Morphological reconstructions of neurons in the superficial Medulla of the OL revealed multiple novel cell types. The projection pattern of neurites is highly stereotyped: From the soma, neurites extend out parallel to the OL surface to exit the local cell body island, at which point they either project towards or away from the OL surface but rarely in both directions. Neurons with processes oriented towards the surface generally span six to eight neighbouring cell body islands whereas neurons projecting deeper into the Medulla span one to two. This suggests a columnar organization of the squid optic lobe whereby feedforward information flows from the OL surface to the deep Medulla converging at each step in a roughly 4:1 ratio. This organization is further corroborated by the tree-like organization of cell bodies within the Medulla and is akin to the architecture of convolutional neural networks.

Neurons in the IGL exhibit distinct branching patterns within the plexiform layer, with (putative) dendritic branches confined within defined horizontally organized layers. This suggests a layered organization of synaptic inputs in the OL cortex, akin to the inner plexiform layer of the mammalian retina. Like neurons in the Medulla, IGL neurons project into the Medulla in a columnar fashion.

In future work, we will identify the electrophysiological properties of these cell types and perform transcriptomics analysis to identify neurotransmitter and receptor compositions.

EFFICIENCY TO INEFFICIENCY: EEG PATTERNS OF HEALTHY AGEING IN RESPONSE TO A COGNITIVE CHALLENGE

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Background: Early detection of age-related neurodegenerative diseases (ARND) remains a significant challenge due to the masking effects of cognitive reserve (CR). CR enables compensatory neural mechanisms to sustain cognitive functioning with age-related structural demise, camouflaging cognitive decline. Identifying initial deviation from healthy ageing, before clinical symptoms emerge, could be key to early intervention. Electroencephalography (EEG) offers a non-invasive approach to characterise neural activity patterns in healthy ageing and detect early markers of neurodegenerative pathology in the brain.
Objective: This study aimed: (1) to establish a standardised EEG protocol incorporating cognitive challenge in characterising age-related neural responses in the form of EEG patterns, and (2) to measure cognitive reserve (CR) and correlate it to the EEG patterns.
Methods: Using a lightweight, mobile EEG device, the resting-state EEG measurements, both eyes open and closed, were recorded from 23 healthy adults before and after a cognitive challenge. Data were processed using EEGLAB and other standard analytical tools. CR was quantified using the Cognitive Reserve Index (CRI) questionnaire, incorporating lifestyle proxies.
Results: The EEG measurements found changes in neural activity across all age groups following a cognitive challenge. Old adults demonstrated widespread cortical activation in all frequency bands and over-recruitment of the prefrontal cortex. Young adults showed increased neural efficiency by engaging distinct, independent neural networks with reduced power output. The middle-aged adults showcased the transition period between neural efficiency and inefficiency, occurring in healthy ageing. The neural efficiency decreases with age as a component of the healthy ageing process. This study also found that higher CR scores were associated with differences in post-cognitive challenge neural activity, though the direction and significance of these effects varied across individuals.
Conclusion: The findings provide initial support for the feasibility of a standard EEG protocol in detecting age-related differences in neural response to cognitive demand. The identified EEG patterns may reflect functional changes in healthy ageing, offering a potential framework for detecting early compensatory or pathological changes. This finding advances our broader initiative integrating EEG with structural MRI and blood-based biomarkers to improve early ARND detection.

USING PATIENT-INDUCED PLURIPOTENT STEM CELLS DERIVED CORTICAL ORGANOIDS TO INVESTIGATE HUMAN NEURONAL PHENOTYPES IN 3Q29 DELETION AND DUPLICATION SYNDROME

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Copy number variations (CNVs) in the human genome are closely linked to a range of neurodevelopmental disorders. Among these, copy number variations (CNVs) in the 3q29 region are particularly noteworthy, as they are frequently observed in individuals with neurodevelopmental conditions. Notably, the 3q29 deletion is among the strongest genetic risk factors for schizophrenia (SCZ), carrying an odds ratio of over 40. Additionally, it is strongly associated with other psychiatric disorders, including intellectual disability (ID), autism spectrum disorder (ASD), and bipolar disorder.

While 3q29 deletions are known to disrupt transcriptional networks and metabolic dysfunction, the precise mechanisms connecting these genetic variations to neurophysiological deficits remain poorly understood. To explore the impact of 3q29 CNVs on neuronal development, we generated induced pluripotent stem cells (iPSCs) from individuals carrying either the 3q29 deletion or duplication and differentiated them into functional cortical organoids.

Our research demonstrates that cortical organoids with 3q29 deletion and duplication display converging phenotypes across critical aspects of neural development, including cell proliferation, differentiation potential, neuronal maturation, synaptic density, and functional activity. Interestingly, both 3q29 deletion and duplication organoids exhibited hyperactivity. Additionally, proteomic analysis of 3q29 duplication organoids revealed significant changes in synaptic protein expression, pointing to specific pathways that could inform future therapeutic strategies.

These results shed light on the neuropathological mechanisms underlying 3q29-associated brain disorders and offer potential avenues for therapeutic strategies. By advancing our understanding of how 3q29 CNVs disrupt neural development, this study provides a foundation for addressing unmet needs in the treatment of neurodevelopmental and psychiatric conditions.

DCX KNOCKOUT FERRET REVEALS A NEUROGENIC MECHANISM IN CORTICAL DEVELOPMENT

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Lissencephaly is a rare brain malformation for which our understanding remains limited due to the absence of suitable animal models that accurately represent human phenotypes. Here, we establish doublecortin (DCX) knockout ferrets as a model that faithfully replicates key features of the disorder. We reveal the critical roles of DCX in neural progenitor cell proliferation and radial glial fiber extension, processes essential for normal cortical development. Utilizing single-nucleus RNA sequencing (snRNA-seq) and spatial transcriptomics, we provide a detailed atlas of the lissencephalic cortex, illustrating disrupted neuronal lamination and the specific interactions between inhibitory and excitatory neurons. These findings enhance our understanding of the cellular and molecular mechanisms underlying lissencephaly and highlight the potential of DCX knockout ferrets as a valuable tool for neurodevelopmental research, offering insights into both the pathology of lissencephaly and the general principles of brain development.

METHYL GALLATE ATTENUATES POST-STROKE EMOTIONAL AND COGNITIVE SYMPTOMS BY PROMOTING HIPPOCAMPAL NEUROGENESIS VIA PI3K/GSK3 AND AMPK SIGNALING

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Background: Post-stroke depression (PSD) lacks effective treatments. Restoring hippocampal neurogenesis is a promising strategy. Methyl gallate (MET) exhibits neuroprotective effects; however, its efficacy and mechanism in PSD recovery remain unknown. Single-cell data suggest PSD shares features with primary depression but has distinct synaptic and inflammatory profiles. Molecular docking indicated that MET binds AMPK/GSK3.
Methods: C57BL/6N male mice underwent MCAO surgery (45-min occlusion) and 2-week post-stroke restraint stress (6h/day). MET (35/100 mg/kg) or inhibitors (LY294002/compound C) were administered via gavage/i.p. Behavioral tests (splash, forced swim, open field, MWM, ORT) assessed depression/anxiety/cognition. Serum ACTH/corticosterone were quantified via ELISA. NSC cultures underwent hypoxia ± MET/LY294002. Immunofluorescence (BrdU/DCX/Ki67/Nestin/Sox2) and Western blot (pGSK3β/GSK3β/pAMPKα/AMPKα) were performed. Public single-cell data (GSE232936) were re-analyzed for hippocampal transcriptomics. MET docking targeted AMPK/GSK3 structures. Statistics used ANOVA with post-hoc tests or t-tests ($p<0.05$ significant).
Results: Single-cell analysis revealed PSD shares cellular/molecular features with primary depression but exhibits weaker synaptic plasticity and stronger inflammatory signals. Molecular docking indicated strong MET binding to AMPK/GSK3. MET significantly attenuated PSD-induced depressive/anxiety behaviors and reduced serum corticosterone/ACTH. Cognitive deficits (Morris water maze, object recognition task) were alleviated. Hippocampal NSPC proliferation/differentiation increased post-MET. MET enhanced AMPK activity while suppressing GSK3β. In vitro, hypoxia-impaired neural development was rescued by MET; this effect was blocked by PI3K inhibitor LY294002 or AMPK inhibitor compound C. Animal studies confirmed both inhibitors reduced MET's antidepressant efficacy.
Conclusions: MET promotes functional recovery in PSD by restoring hippocampal neurogenesis through the activation of the AMPK pathway and modulation of the PI3K/GSK3β signalling pathway. It indicates that PI3K, as well as AMPK-mediated adult neurogenesis, is restored by MET to improve brain functions in the PSD model.

MINIATURIZED AND ACCESSIBLE FUNCTIONAL ULTRASOUND IMAGING SYSTEM FOR FREELY MOVING MICE

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Functional ultrasound (fUS) imaging provides brain-wide activity maps with high spatiotemporal resolution and deep penetration, positioning it as a key technology for brain function, neural circuits, and even future non-invasive brain-computer interfaces (BCIs) research. Realizing this potential, particularly for those applications requiring long-term monitoring in naturalistic settings, critically depends on significant system miniaturization to overcome the cost and complexity limitations of current platforms. Addressing this challenge, we present **Mini-fUS**, a miniaturized, cost-effective fUS platform engineered for accessibility without compromising core performance for demanding neuroscience research. The system features a compact transmit-receiver, low-noise power supply, and high-speed data transfer, achieving pulse repetition frequencies up to 5-50 kHz with negligible jitter while the system dimension is only 30cm*19cm*11cm. Real-time GPU-accelerated beamforming and fast singular value decomposition (SVD) enable whole-brain activity mapping, demonstrated here in freely moving mice at up to 3.57 Hz with ~100 µm spatial resolution and 15 mm penetration depth. Validated through recordings of brain activity during sensory stimulation, anesthesia, and behavior, this design defines a practical hardware-software framework for fUS. By significantly improving accessibility and enabling robust monitoring in mobile subjects, this work advances the development of fUS for both fundamental research and future BCI technologies, while clarifying essential fUS operational principles, providing neuroscientists with a new and efficient research tool.

A VAGAL LIVER-BRAIN CIRCUIT FOR INFLAMMATION SENSING AND RESPONSE

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As a gateway between the gastrointestinal tract and the internal circulation, the mammalian liver is uniquely positioned to sense and mount both behavioural and immune responses to incoming immune challenges that enter the liver via the gut. This requires functional coordination of the nervous and immune systems. Although the existence of vagal sensory neurons innervating the liver portal vein is known, their sensory function and molecular characterization is relatively unexplored. We identified Phox2b^{+} vagal nerves predominantly innervate the hepatic portal vein. Through projection-based single-cell transcriptomics, we further revealed that these vagal sensory neurons express receptors for key immune mediators released by liver macrophages during inflammation or injury. Moreover, an inflammatory challenge administered directly to the liver via a portal vein catheter strongly activated this liver–brain vagal circuit. This activation led to marked modulation of sickness behaviours, including reduced food intake, decreased locomotor activity, and lowered energy expenditure, partially mediated by downstream brain regions such as the nucleus of the solitary tract (NTS), paraventricular nucleus (PVN), and parabrachial nucleus (PBN). Additionally, chemogenetic activation of this circuit increased Kupffer cell accumulation around the portal region, indicating its role in regulating peripheral immune responses. In summary, our findings define a molecularly and anatomically distinct neuroimmune feedback circuit by which peripheral immune signals from the liver are conveyed to the brain to regulate feeding behaviour and local immune responses.

INHIBITORY-NEURON-SPECIFIC SONOGENETICS FOR MICROCIRCUIT MODULATION

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Precise modulation of inhibitory neurons in neural microcircuits represents a critical frontier in neuroscience research and therapeutic development. However, achieving non-invasive, targeted, and volume-controllable manipulation remains a significant challenge. Ultrasound neuromodulation through sonogenetics has emerged as a promising non-invasive approach, combining superior spatial scalability with cell-type specificity. In this study, we developed an inhibitory-neuron-specific sonogenetics method to effectively alter neuronal balance in microcircuits. Sonogenetic activation of somatostatin interneurons (SST-INs) in primary motor cortex (M1) was found to induce significant enhancements in calcium transients and c-Fos expression. M1 pyramidal neuron (PYN) activity was suppressed by ultrasound stimulation, with sustained inhibition lasting over 20 seconds. Furthermore, in a Parkinson's disease mouse model, this approach effectively restored microcircuit balance and behavioral outcomes. Systematic biosafety evaluation showed no obvious adverse effects. This work establishes a novel non-invasive, cell-type-specific, and volume-targetable approach for precise microcircuit manipulation, offering potential for basic research and therapeutic development.

Keywords: Sonogenetics; Inhibitory neurons; Microcircuit modulation; Volume-controllable;

TMTC3 STABILIZES LAMB1 TO MAINTAIN BASEMENT MEMBRANE INTEGRITY IN CORTICAL DEVELOPMENT

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Transmembrane O-mannosyltransferase Targeting Cadherins 3 (TMTC3), initially characterized as an endoplasmic reticulum (ER)–localized O-mannosyltransferase acting on E-cadherin, has been implicated in congenital cobblestone lissencephaly (COB)— a neuronal migration disorder marked by excessive migration and overextension of neurons through the pial basement membrane. Mutations in LAMB1, encoding the beta 1 subunit of laminin, lead to nearly identical COB in humans. Here we show that TMTC3 is a critical regulator of laminin production, mediated through the C-terminal helix of TMTC3 binding to LAMB1 within the ER. A mild COB phenotype in TMTC3 mouse mutants was dramatically aggravated by LAMB1 haploinsufficiency. TMTC3 loss resulted in altered laminin within the pia basement membrane, LAMB1 aggregation and subsequent proteasome-mediated degradation, an effect rescued by expression of the TMTC3 C-terminal helix fragment. Structural predictions suggest that this C-terminal helix promotes folding of the LAMB1 monomer by shielding hydrophobic regions prior to laminin trimer assembly. Together, our findings uncover a previously unrecognized genetic and physical interaction between the TMTC3 C-terminal helix and LAMB1, independent of canonical O-mannosyltransferase activity, and establish a long-sought mechanism of laminin assembly in vertebrates.

CYTOSKELETAL COORDINATION IN NEURONAL DEVELOPMENT: NESPRIN-2-MEDIATED NUCLEAR TRANSLOCATION VIA MICROTUBULE MOTORS AND IgSF11-GUIDED AXON PROJECTION

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During brain development, post-mitotic neurons undergo somal migration and axon outgrowth to establish precise cellular positioning and intercellular connectivity, processes critical for constructing functional neural circuits that ultimately direct animal behaviors. Here, I present two complementary studies investigating cytoskeletal mechanisms underlying: (1) Nesprin-2-mediated nuclear translocation in cerebellar granule neurons during neuronal migration; (2) IgSF11-guided axon projection of primary visual cortex (V1) layer 2/3 (L2/3) pyramidal neurons.

Nuclear translocation is essential for neuronal migration, with the nucleus transported by microtubule motors via the LINC (Linker of Nucleoskeleton and Cytoskeleton) complex. Nesprin-2, a key LINC component, interacts with opposing motors—dynein and kinesin-1—to drive nuclear translocation. However, how bidirectional motors coordinate to achieve net forward nuclear transport remained unclear. Using live-cell time-lapse imaging, molecular inhibition, biochemical assays, and knockout mouse models, we demonstrated that Nesprin-2 functions as a bidirectional motor adaptor requiring both dynein and kinesin-1 for nuclear transport during neuronal migration. Nesprin-2 deletion causes granule neuron mis-localization during cerebellar development. Critically, we discovered that Nesprin-2 not only activates opposing motors for prolonged bidirectional movements but also couples the nucleus to anterograde-moving microtubules, resolving the paradox of how opposing motors achieve net forward nuclear displacement.

In the developing visual cortex, visual experience during critical periods upregulates expression of the cell adhesion molecule Immunoglobulin Superfamily Member 11 (IgSF11) in V1 L2/3 excitatory neurons. Intriguingly, IgSF11 expression correlates spatiotemporally with axon projection patterns of V1 L2/3 neurons, yet its role in guiding axon projection remains largely unknown. Our ongoing study investigates whether and how IgSF11 regulates axon projection development from V1 L2/3 pyramidal neurons and consequent visuospatial behavioral outcomes. Using IgSF11 knockout mouse models, visuospatial behavioral analyses, and examination of axon morphology and projection patterns in wildtype versus knockout mice, we aim to elucidate molecular, cellular, and circuit-level mechanisms through which IgSF11 regulates visual cortical circuit development and corresponding visuospatial behaviors.

Together, these studies reveal fundamental cytoskeletal coordination mechanisms during neuronal development, offering novel mechanistic insights into brain construction and providing potential therapeutic targets for diagnosing and treating neurodevelopmental diseases.

EVOLUTIONARY EMERGENCE OF STRIATAL GABAERGIC INTERNEURON TYPES IN MAMMALS MODULATES BEHAVIORAL FLEXIBILITY

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Unlike the dorsal pallium that exhibits drastic evolutionary changes in cell number, type, and organization across vertebrates, the ventral subpallium is considered to be highly conserved for motor, cognitive, and emotional control. Here, we report the evolutionary emergence of specific striatal interneuron types – Parvalbumin-expressing (PVALB)+ and Thyrotropin-releasing hormone-expressing (TRH)+ – from spatiotemporally and molecularly defined progenitors in mammals to modulate flexible behavior. In-depth comparative single-cell transcriptomics analyses revealed the existence of specific striatal GABAergic neurons in mice, but not lizards or salamanders. Spatial transcriptomics, lineage tracing, and genetic analyses delineated the unique progenitor origins and developmental trajectories of these interneurons specifically corresponding to PVALB+ and TRH+ interneurons in the striatum. Their disruption led to reduced synaptic inhibition, abnormal repetitive behavior, impaired sensorimotor gating, and heightened anxiety, core phenotypes reminiscent of obsessive-compulsive disorder. These findings suggest that the mammalian striatum acquires specialized GABAergic interneurons to enhance inhibitory synaptic modulation and behavioral flexibility.

CORTICAL INHIBITORY NEURONS EXHIBIT EXPANDING AND CONTRACTING MODES OF DIVERSIFICATION.

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The cerebral cortex relies on vastly different types of inhibitory neurons to compute. How this diversity emerges during development remains an open question. The rarity of individual inhibitory neuron types often leads to their underrepresentation in single-cell RNA sequencing (scRNAseq) datasets, limiting insights into their developmental trajectories. To address this problem, we enrich and integrate rare cell types across multiple datasets, and construct the Dev-SST-Atlas, a comprehensive resource containing mouse transcriptomic data of over 51,000 somatostatin-expressing (SST+) inhibitory neurons—the most diverse inhibitory cell class in the cortex. We identify three principal groups—Martinotti cells (MCs), non-Martinotti cells (nMCs), and long-range projecting neurons (LRPs)—each following distinct diversification trajectories. MCs commit early, with distinct embryonic and neonatal clusters that map directly to adult counterparts. In contrast, nMCs diversify gradually, with each developmental cluster giving rise to multiple adult cell types. LRPs follow a unique 'contracting' mode. Initially, two clusters are present until postnatal day 5 (P5), but by P7, one type is eliminated through programmed cell death, leaving a single surviving population. This transient LRP type is also found in the fetal human cortex, revealing an evolutionarily conserved feature of cortical development. Together, these findings highlight three distinct modes of SST+ neuron diversification—invariant, expanding, and contracting—offering a new framework to understand how the large repertoire of inhibitory neurons emerges and wire during development.

DETERMINISTIC AND STOCHASTIC GENERATION OF NEURONAL DIVERSITY

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In the Drosophila optic lobes, >250 types of neurons organized as 800 columns process the inputs from 800 unit-eyes. We study how this variety of neurons is generated during development and how connectivity among these neurons is regulated

The neural stem cells in the medulla sequentially express a series of temporal transcription factors (tTFs), producing at each temporal window different neurons that innervate each of the 800 columns. At each division, the neural stem cell produces an intermediate precursor that divides once, generating a NotchON and a NotchOFF neuron.

We used single-cell mRNA sequencing to identify the complete series of tTFs that specify most optic lobe neurons from birth to adulthood. Each tTF regulates the progression of the series by activating the next tTF and repressing the previous one. This allowed us to establish the temporal window of origin and birth order of each neuronal type in the medulla. These tTFs are sufficient to explain the generation of the entire neuronal diversity in this brain region by integrating temporal and spatial patterning as well as their Notch status.

However, because the cell cycle is slower than the transitions between temporal windows and the two are not synchronized, not all neuroblasts 'use' all temporal windows and thus only produce a stochastic subset of the total neural types that can be specified by this neuroblast, thus providing flexibility to the system.

We also discovered that the 250 distinct neurons in the Drosophila visual system can be defined by unique combinations of ~10 ‘terminal selector’ TFs that are continuously expressed in each neuron from birth to adulthood. Targeted modifications of this ‘selector’ code induce predictable conversions of cell fates between neurons that appear to be morphologically and transcriptionally complete. Using single nuclei ATAC-seq data, we showed that Cis-regulatory sequences link this ‘selector’ program to the upstream tTFs that specify neuronal fates. This provides a generalizable framework of how specific fates are initiated and maintained in postmitotic neurons.

PYRAMIDAL NEURONS PROVIDE BOTH INSTRUCTIVE AND PERMISSIVE SIGNALING TO SPECIFIC INTERNEURON SUBTYPES.

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The mammalian cerebral cortex comprises a complex neuronal network that maintains a delicate balance between excitatory neurons and inhibitory interneurons. Previous studies, including our own research, have shown that specific interneuron subtypes are closely associated with particular pyramidal neuron types, forming stereotyped local inhibitory microcircuits. However, the developmental processes that establish these precise networks are not well understood. Here we show that pyramidal neuron types are instrumental in driving the terminal differentiation and maintaining survival of specific associated interneuron subtypes. In a wild-type cortex, the relative abundance of different interneuron subtypes aligns precisely with the pyramidal neuron types with which they synaptically target. In Fezf2 mutant cortex, characterized by the absence of layer 5 pyramidal tract neurons and an expansion of layer 6 intratelencephalic neurons, we observed a corresponding decrease in associated layer 5b interneurons and an increase in layer 6 subtypes. Interestingly, these shifts in composition are achieved through mechanisms that are specific to different interneuron types. While SST interneurons adjust their abundance to the change in pyramidal neuron prevalence through the regulation of programmed cell death, parvalbumin interneurons alter their identity. These findings illustrate two key strategies by which the dynamic interplay between pyramidal neurons and interneurons allows local microcircuits to be sculpted precisely. These insights underscored the precise roles of extrinsic signals from pyramidal cells in the establishment of interneuron diversity and their subsequent integration into local cortical microcircuits.

MITOTIC BOOKMARKING IN BRAIN DEVELOPMENT

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In brain development, neural stem cells (NSCs) undergo asymmetric cell divisions to replicate themselves and meanwhile produce differentiating siblings. It remains obscure how NSCs preserve their self-renewing fate across mitosis, when characteristic gene expression programs and chromatin architecture are drastically altered. Even less is known how NSC-specific self-renewing fate memory is selectively erased in the differentiating daughter cells. Mitotic bookmarking has been posited as an important strategy for cells to faithfully propagate their fate memory though cell generations. However, the physiological significance and regulatory mechanisms of mitotic bookmarking in brain development remain unexplored. By developing a new pipeline for enriching mitotic versus interphase cells from developing brains, followed by low-input omics analysis, we recently identified TBP (TATA binding protein) as a crucial mitotic bookmarker for preserving NSC fate memory. Importantly, TBP achieves its mitotic retention through recruiting the chromatin remodeler EP400, which in turn increases local chromatin accessibility via depositing histone variant H2A.Z. Our unpublished work further reveals, during and immediately after NSC asymmetric cell divisions, how cell fate memory is differentially propagated to sibling daughter cells adopting distinct cell fates. Together, our latest discoveries unveil fundamental principles underlying the timely and precise preservation or erasure of cell fate memory in neural development, crucial for the construction of a brain with enormous diversity and precision.

MODELLING NEURODEVELOPMENTAL DISORDERS IN FLIES AND MAMMALIAN SYSTEMS

Hongyan Wang

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Variants in ARF1 have been identified in patients with neurodevelopmental disorders, including microcephaly, periventricular heterotopia, severe intellectual disability, and corpus callosum abnormalities. ARF1 is a Golgi-resident small GTPase that regulates the structures and functions of Golgi. We have recently demonstrated the function of Drosophila Arf1 in the reactivation of quiescent neural stem cells and asymmetric division of proliferative neural stem cells (Developmental Cell 2023; PNAS 2025), highlighting the critical function of Arf1 in brain development. Our study also revealed a new function of Drosophila Arf1 in regulating microtubule growth in quiescent neural stem cells during brain development. However, the role of mammalian ARF1 during brain development is not established. Our unpublished data suggested the important novel function of mammalian Arf1 in cortical development. We will discuss molecular mechanisms underlying mammalian Arf1-dependent cortical development.

INSIGHTS INTO SYNAPSE FUNCTION THROUGH ASTROCYTE LINKS

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Recent studies have highlighted the contribution of astrocyte network in shaping synaptic circuit activity in various brain areas with behavioral consequences. Yet, precisely how astrocytes interact with synapses and how they modify individual synaptic strengths, especially in local circuits, remains to be fully understood. We have sought to clarify the cellular organization and the molecular basis that shape tripartite synapses in the hippocampus by focusing on a class of cell adhesion proteins. The amyloid precursor protein (APP) has been intensely studied for its role in Alzheimer's disease, but its physiological function, especially in the developing nervous system remains unclear. In neurons, APP and its homologs, the amyloid precursor-like proteins (APLPs) are present at synapses where they have been suggested to serve an adhesive function and promote synaptic transmission and plasticity. Astrocytes also express APP although a role for astrocytic APP has not been fully explored. We have studied the expression and function of APP in rodent astrocytes in vitro and in vivo. Knocking down astrocytic APP compromises the development of astrocyte morphological complexity with potential consequences on synapse function. Our recent findings will be discussed.

DECODING SYNAPTIC SIGNALING DYNAMICS UNDERLYING PLASTICITY

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Learning and memory depend on activity-dependent changes in synaptic strength and structure, yet the molecular mechanisms orchestrating these changes remain incompletely understood. These processes are mediated by complex biochemical signaling networks involving hundreds of intracellular and extracellular molecules operating on fine spatial and temporal scales. To dissect these networks, we have developed innovative imaging and optogenetic tools that allow visualization and perturbation of protein activity at individual synapses in freely behaving animals. These approaches have revealed how specific signaling events drive synaptic plasticity, modulate circuit function, and contribute to behavior, providing a new framework for understanding information storage in the brain.

NATIVE NMDA RECEPTORS IN THE BRAIN: FROM ATOMIC STRUCTURE TO SYNAPTIC PHYSIOLOGY

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The cerebral cortex and hippocampus are critical brain regions for learning and memory, processes that rely on activity-induced synaptic plasticity mediated by N-methyl-D-aspartate receptors (NMDARs). However, the subunit assembly and molecular architecture of these endogenous NMDARs (eNMDARs) remain poorly understood. Using conformation- and subunit-specific antibodies, we purified eNMDARs from adult rat cerebral cortex and hippocampus and resolved three major eNMDAR subtypes of GluN1-N2A-N2B, GluN1-N2B, and GluN1-N2A receptors, at resolutions up to 4.2 Å. These subtypes exhibited a particle ratio of 9:7:4, indicating that approximately half of GluN2A and GluN2B subunits are incorporated into tri-heterotetrameric complexes (***Cell*** 2025). Our findings reveal the structural and functional complexity of eNMDARs and shed light on structure-based therapeutic design targeting these receptors *in vivo*. In the second part, I will present the structural basis for the diverse regulatory roles of magnesium (Mg^{2+}) in NMDAR function. Mg^{2+} is a key ion that enables NMDARs to act as coincidence detectors in excitatory synaptic transmission. Cryo-EM structures revealed three distinct Mg^{2+} binding sites on the GluN1-N2B receptors. Site I, located at the selectivity filter, features an asparagine ring that coordinates Mg^{2+} and mediates voltage-dependent Mg^{2+} block. Site II and III reside in the N-terminal domain (NTD) of GluN2B subunit, mediating GluN2B-specific allosteric potentiation and NTD-driven allosteric inhibition, respectively (***Neuron*** 2025). These findings elucidate the multifaceted mechanisms by which Mg^{2+} regulates NMDAR function and synaptic plasticity.

NEURONAL MORPHOLOGY MAINTENANCE INVOLVES MEMBRANE PHOSPHOLIPID ASYMMETRY AND EXTRACELLULAR VESICLE BIOGENESIS

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Mature neurons maintain their distinctive morphology throughout adult life, via mechanisms distinct from those involved in initial neurite outgrowth or guidance. Failure to maintain neuronal morphology can result in neurodegeneration or aberrant process outgrowth. Loss of function in C. elegans dip-2, a member of the Dip2 family of lipid metabolic regulators, results in progressive neurite overgrowth. Animals lacking sax-2, the C. elegans ortholog of Drosophila Furry and mammalian FRY, also lose the ability to maintain neuronal shape. Strikingly, combined loss of dip-2 and sax-2 results in dramatic failure to maintain neuronal morphology, severe behavioral deficits, and elevated release of neuronal extracellular vesicles (EVs) (Park et al., 2024). Using screens for suppressors of dip-2(0) sax-2(0) phenotypes we identified rare gain-of-function (gf) mutations affecting PAD-1, a member of the Dopey family of membrane trafficking regulators. C. elegans PAD-1 inhibits release of ectosome-type EVs and regulates the membrane phospholipid flippase TAT-5/ATP9A. The PAD-1(gf) missense alterations may influence lipid or membrane binding. Tissue-specific knockdown experiments are consistent with PAD-1(gf) acting cell autonomously. Our screen has identified multiple alteration of function mutations in TAT-5 that restore normal neuronal morphology and normal levels of EV release to dip-2(0) sax-2(0) double mutants. TAT-5 specifically flips phosphatidylethanolamine (PE), maintaining the asymmetric distribution of PE the cytofacial leaflet of the plasma membrane. We have identified several other suppressor loci that may function in the PAD-1/TAT-5 pathway or in parallel to control neuronal morphology. To monitor PE localization in vivo we are pursuing strategies to develop genetically encoded PE sensors. Our findings reveal the critical importance of phospholipid asymmetry and EV biogenesis in neuronal architecture.

Reference: Dopey-dependent regulation of extracellular vesicles maintains neuronal morphology. Park S, Noblett N, Pitts L, Colavita A, Wehman AM, Jin Y, Chisholm AD. Curr Biol. 2024 Nov 4; 34(21):4920-4933.e11. PMID: 39378880

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PROTEOMIC LANDSCAPE OF NEURONAL NUCLEI IN A MOUSE LONG-TERM POTENTIATION MODEL WITH ULTRA-LOW INPUT

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Long-term potentiation (LTP) is a fundamental cellular mechanism underlying synaptic plasticity and memory formation. Mass spectrometry-based proteomics enables comprehensive profiling of protein expression and post-translational modifications. Recent advances in high-resolution mass spectrometers (e.g., timsTOF Ultra and Orbitrap Astral series) allow for the analysis of single-cell level inputs (~100 pg total protein). In this study, we developed a trace-input proteomic workflow to characterize the protein landscape of hippocampal neuronal nuclei in a mouse LTP induction model.

Mouse nuclei were collected by FACS at various time points post-LTP induction, then processed using a one-pot bottom-up proteomic protocol for trace input. Peptides were analyzed by high-throughput liquid chromatography (Evosep One, 40 samples/day) coupled to a timsTOF Ultra 2 mass spectrometer (Bruker) operating in data-independent acquisition (diaPASEF) mode.

We quantified over 3,000 proteins from 500 nuclei, including 250 transcription factors and regulators. Robust quantification of ~1,000 proteins was achieved from as few as 5 nuclei. Notably, transcription factors CREB and c-FOS were regulated after LTP induction, with distinct dynamics at 3 and 90 minutes post-induction, consistent with immunofluorescence data. Even with ultra-low inputs of 5 nuclei, nuclear c-FOS only after 90 min post LTP induction was successfully detected. Encouraged by these results, we further optimized the workflow for single nucleus using a benchtop microfluidic device (ACX BOXmini SCP), quantifying ~500 proteins (n=15).

Our findings demonstrate that next-generation mass spectrometers enable high-throughput proteomic analysis (40–100 samples/day) from extremely limited samples. This advancement in both sensitivity and throughput opens new avenues for neuroscience research, particularly in studying synaptic plasticity and memory formation. Single-nucleus proteomics may further provide insight into the heterogeneity of neuronal responses during LTP at the individual cell level.

ASTROCYTE MORPHOGENESIS REQUIRES SELF-RECOGNITION

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Self-recognition is a fundamental cellular process across evolution and forms the basis of neuronal self-avoidance. Clustered protocadherins (cPcdh), comprising a large family of isoform-specific homophilic recognition molecules, play a pivotal role in neuronal self-avoidance required for mammalian brain development. The probabilistic expression of different cPcdh isoforms confers unique identities upon neurons and forms the basis for neuronal processes to discriminate between self and non-self. Whether this self-recognition mechanism exists in astrocytes, the other predominant cell type of the brain, remains unknown. Here, we report that a specific isoform in the Pcdhγ family, γC3, is enriched in human and mouse astrocytes. Through genetic manipulation, we demonstrate that γC3 acts autonomously to regulate astrocyte morphogenesis in the mouse visual cortex. To determine if γC3 proteins act by promoting recognition between processes of the same astrocyte, we generated pairs of γC3 chimeric proteins capable of heterophilic binding to each other, but incapable of homophilic binding. Co-expressing complementary heterophilic binding isoform pairs in the same γC3 null astrocyte restored normal morphology. By contrast, chimeric γC3 proteins individually expressed in single γC3 null mutant astrocytes did not. These data establish that self-recognition mediated by γC3 contributes to astrocyte development in the mammalian brain.

CD47'S REGULATION OF MEMORY PROCESSES

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Microglia regulate synaptic connections through synaptic pruning, either eliminating or protecting synapses based on neuronal expression of “Eat Me” or “Don’t Eat Me” signals. While "Eat Me" signals are well-studied and known to promote memory forgetting by enhancing synapse elimination, the role of "Don't Eat Me" signals, such as CD47, which protect synapses, remains largely unexplored in the context of memory. In this study, we investigated the function of CD47 in memory processes using engram tagging, behavioral paradigms, and cellular analysis. Our findings reveal three major roles for CD47 in regulating memory. First, during the post-learning period, CD47 supports memory persistence and precision. Overexpression of CD47 in engram cells promotes memory persistence, demonstrated by slower fear memory extinction and resistance to forgetting. CD47 also enhances memory precision by improving the animal’s ability to discriminate between similar contexts even at remote delays. At the cellular level, CD47 promotes engram synaptic connectivity through increased spine density and enhances engram reactivation during memory recall. Second, even before learning occurs, CD47 increases dendritic spine density and enhances neuronal excitability, priming neurons to support future memory encoding. This experience-independent role of CD47 highlights its contribution to synaptic transmission and memory allocation. Third, CD47 overexpression reduces microglial phagocytic activity. Together, these findings indicate that CD47 plays a key role in both stabilizing memory traces and regulating engram excitability, offering new insights into how “Don’t Eat Me” signals influence memory processes. This work advances our understanding of microglia's contributions to cognitive function and opens new avenues for targeting synaptic stability in memory-related disorders.

ROLES OF OFFLINE NEURONAL ACTIVITY IN THE CONSOLIDATION OF SPATIAL MEMORY ENGRAMS

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While an animal is at rest, the brain exhibits a pattern of activity—referred to here as *offline neuronal activity*—that is distinct from the activity observed during wakefulness. At the cellular level, sequences of neuronal firing that occurred during the awake period are replayed. This replay likely strengthens selective synaptic inputs while weakening others through homeostatic depression, a process implicated in memory consolidation. However, the precise impact of such activity on memory content remains unclear. To address this, we performed imaging of both the hippocampus and the anterior cingulate cortex (ACC). We found that the ACC contains a distinct population of neurons that respond selectively to specific spatial contexts. These spatial context neurons require hippocampal activity for their initial formation, but once established, they become independent of it. Notably, c-Fos labeling revealed that these neurons preferentially become active in response to memory retrieval, suggesting that they represent memory engrams. Furthermore, online suppression of replay events prevented the formation of spatial context cells. Together, our findings reveal for the first time that a specific pattern of offline neuronal activity underlies the formation of a stable neural substrate for remote memory.

DEVELOPMENTAL CRITICAL PERIODS FOR EPISODIC MEMORY

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Memories for events (i.e., episodic memories) formed in early development differ from those in adulthood in at least two regards. First, these memories tend to be less precise (i.e., infantile generalization). Second, they tend to be rapidly forgotten (i.e., infantile amnesia). My talk will focus on the neurobiological mechanisms that account for these different operating characteristics of episodic memory in the developing brain. With respect to infantile generalization, our studies have shown that maturation of inhibitory hippocampal microcircuits is necessary for the formation of adult-like, precise memories for events. With respect to infantile amnesia, our studies have revealed that developmentally-regulated myelination of prefrontal cortical circuits is necessary for the formation of adult-like, enduring event memories. Similar to developing sensory systems—where cortical circuit refinement occurs during defined windows of heightened brain plasticity known as critical periods—our work suggests that similar refinement of hippocampal and prefrontal cortical circuits underlies the emergence of adult-like episodic memory function.

AREA-SPECIFIC CELL PLASTICITY BY VISION-DEPENDENT SPATIAL CELLULAR GRADIENTS

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Mammalian brain comprises distinct areas consisting of diverse cell type. Specialized circuits formed by area-specific cell types are initially established by genetic programs and further refined by postnatal experience. Yet how experience influences cell-type composition in distinct areas is unclear. Here we show that visual experience impacts area-specific cell-type composition and circuit architecture via configuration of spatial cellular gradients. Dark-rearing from eye-opening to critical period closure changes identity of glutamatergic cell types in diverse visual-processing areas at graded levels, which can be largely predicted by the level of reconfiguration of intercellular expression variation of multi-hundred genes along dorsal-ventral brain axis. Spatial co-expression analysis revealed that experience acts on top of area-specific genetic programs to suppress genetically encoded molecular gradients and promote experience-dependent yet less TF-dependent spatial molecular gradients. Majority of experience-regulated genes are associated with brain development and function, enriched in regulating neuronal morphogenesis and physiology, with many also linked to neurodevelopmental disorders. Changes in spatial morphogenic and ion-channel gradients by dark-rearing is mirrored by graded shifts in patterns of axon-projection specificity and spontaneous activity of V1 L2/3 pyramidal neurons along cortical depth, which can be attributed to key genes that are highly associated with neurodevelopmental disorders. Thus, experience alters area-specific cellular architecture by patterning continuous multi-modal spatial cellular heterogeneity.

REMODELING SYNAPTIC CONNECTIONS VIA ENGINEERED NEURON-ASTROCYTE INTERACTIONS

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Information flow through synapses in the central nervous system is regulated by both rapid electrochemical activity and slower structural remodeling. While remarkable technological advances have enabled precise control of synaptic activity, methods for structural remodeling of synaptic connections remain limited. Here, we present SynTrogo (Synthetic Trogocytosis), a synthetic molecular approach for targeted synapse elimination. Leveraging astrocytes' natural proximity to synapses and phagocytic capabilities, we engineered hippocampal CA3 neurons and CA1 astrocytes in adult mice by introducing a synthetic 'eat-me' signal and its complementary receptor, respectively. Physical binding of neurons and astrocytes triggered ‘Trogocytosis’, whereby astrocytes ingest synaptic components, leading to synapse elimination. Remarkably, the remaining synapses undergo substantial remodeling, exhibiting enlarged pre- and post-synaptic structures, reorganized synaptic components and organelles, and enhanced synaptic plasticity and memory. This study reveals how the brain adaptively reshapes neural circuits following astrocyte-mediated synapse pruning and provides a foundation for synapse editing with therapeutic potential for connectopathies.

REGULATION OF ADULT NEURAL STEM CELL ESTABLISHMENT BY FGF BINDING PROTEIN 3

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During embryonic development, neural stem cells (NSCs) primarily exist in a highly proliferative state, generating neurons and glial cells that shape the maturing brain. However, a distinct subpopulation of slowly dividing, quiescent NSCs emerges from late embryonic stage. A portion of these cells persists into adulthood as adult NSCs, contributing to adult neurogenesis and brain functions such as olfactory memory and innate behaviors. (Furutachi et al., 2015; Fuentealba et al., 2015) Despite sharing a similar environment with rapidly dividing NSCs, the mechanisms by which these slow-dividing embryonic NSCs are established and maintained remain poorly understood.

In this study, we identified FGFBP3, a fibroblast growth factor binding protein, as highly expressed in slowly dividing embryonic NSCs. Using *Fgfbp3* knockout (KO) mice, we observed a reduction in both slowly dividing embryonic NSCs and their subsequent postnatal NSCs in *Fgfbp3* KO brains. On the other hand, overexpression of *Fgfbp3* to embryonic NSCs increased the proportion of undifferentiated cells. These findings suggest that FGFBP3 contributes to the establishment or maintenance of the adult NSC lineage by preventing NSCs from differentiation, thereby preventing depletion.

Given that FGFBP3 is a binding protein for FGF, we therefore investigated whether its role in maintaining NSC stemness is FGF-dependent. Combining in situ hybridization and immunohistochemistry, we revealed a strong correlation between Fgfbp3 expression and phosphorylated ERK (pERK), a key downstream effector of FGF signaling. Notably, *Fgfbp3* KO brains exhibited reduced pERK levels in NSCs, particularly in the quiescent population, where *Fgfbp3* is highly expressed. Furthermore, utilizing *in vitro* primary NSCs, we found that FGFBP3 selectively maintained the stemness of *Fgfbp3*-overexpressing cells only in a condition where limited amount of FGF was available. Collectively, these data suggest that FGFBP3 enhances local FGF signaling in quiescent NSCs, potentially by relocating the limited endogenous FGF to *Fgfbp3*-expressing quiescent cells, thereby supporting long-term stem cell preservation.

ACUTE NEGATIVE EXPERIENCES PROMOTE MEMORY GENERALIZATION AND MOOD VULNERABILITY VIA A SHARED MPFC-BNST ENSEMBLE AND MOLECULAR SIGNATURE

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Epidemiological studies indicate that many individuals with depression experience acute, unpredictable stressful life events prior to onset, which act as critical triggers of depressive episodes, sometimes even occurring independently of chronic stress situation. While chronic stress paradigms in rodent models have been instrumental in advancing our understanding of depression pathophysiology, they may not fully capture the impact of discrete stressful events relevant to a substantial subset of clinical cases. This potential limitation limits our understanding of how stress-related memory process contribute to mood vulnerability. To address this gap, we developed behavioral paradigms that dissociate memory generalization from memory strengthening following acute negative experiences. We found that experiences inducing memory generalization, rather than memory strengthening, were selectively associated with depression-like behaviors. At the circuit level, we identified the medial prefrontal cortex (mPFC) to bed nucleus of the stria terminalis (BNST) projection as a key pathway engaged in both generalized memory processing and depression-like states. Single-cell calcium imaging revealed that $mPFC^{BNST}$ neuronal activity features during the memory generalization test was more predictive of depression-like outcomes than activity during the memory strength test. Moreover, there was significant overlap in the $mPFC^{BNST}$ neuronal ensembles encoding depression-like behaviors and generalization, but not with those encoding memory strength. To elucidate the molecular basis of this shared representation, we combined projection-specific transcriptomics, chromophore-assisted light inactivation, and shRNA-mediated knockdown. These approaches converged on actin cytoskeleton remodeling in the mPFC-BNST circuit as a key process selectively supporting the consolidation of generalized, but not strengthened, memories linked to depression-like behaviors. Together, our findings identify a previously unrecognized shared circuit and molecular mechanism linking memory generalization and mood vulnerability. This work provides a mechanistic framework for understanding vulnerability to depression and underscores the potential of targeting memory generalization as a therapeutic strategy.

TMTC3 STABILIZES LAMB1 TO MAINTAIN BASEMENT MEMBRANE INTEGRITY IN CORTICAL DEVELOPMENT

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Transmembrane O-mannosyltransferase Targeting Cadherins 3 (TMTC3), initially characterized as an endoplasmic reticulum (ER)–localized O-mannosyltransferase acting on E-cadherin, has been implicated in congenital cobblestone lissencephaly (COB)— a neuronal migration disorder marked by excessive migration and overextension of neurons through the pial basement membrane. Mutations in LAMB1, encoding the beta 1 subunit of laminin, lead to nearly identical COB in humans. Here we show that TMTC3 is a critical regulator of laminin production, mediated through the C-terminal helix of TMTC3 binding to LAMB1 within the ER. A mild COB phenotype in TMTC3 mouse mutants was dramatically aggravated by LAMB1 haploinsufficiency. TMTC3 loss resulted in altered laminin within the pia basement membrane, LAMB1 aggregation and subsequent proteasome-mediated degradation, an effect rescued by expression of the TMTC3 C-terminal helix fragment. Structural predictions suggest that this C-terminal helix promotes folding of the LAMB1 monomer by shielding hydrophobic regions prior to laminin trimer assembly. Together, our findings uncover a previously unrecognized genetic and physical interaction between the TMTC3 C-terminal helix and LAMB1, independent of canonical O-mannosyltransferase activity, and establish a long-sought mechanism of laminin assembly in vertebrates.

CEREBRAL ORGANOIDS: GROWING HUMAN BRAIN TISSUE FROM STEM CELLS TO STUDY DEVELOPMENT AND DISEASE

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The human brain is unique in its size and complexity. We have developed cerebral organoids, 3D cell cultures derived from patient stem cells that recapitulate the development of the human brain. Using this technology, we have identified developmental processes unique to humans, studied the mechanistic basis for brain diseases and reconstituted human neural network activity (Lancaster et al., Nature 2013; Esk et al., Science 2020; Eichmüller et al., Science 2022; Li et al., Nature 2023). Our efforts to recapitulate human specific processes of brain development and to replicate disease pathology on the circuit level will be presented. Specifically, I will cover our recent efforts to use electrophysiology and barcoded connectome analysis with single-cell resolution in order to determine and understand how neural network activity and network architecture change in patients that develop epilepsy.

DISSECTING HUMAN BRAIN DEVELOPMENT AND NEUROPSYCHIATRIC DISORDERS WITH SINGLE-CELL AND SPATIAL 3D-MULTIOMICS

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Our research program develops single-cell and spatial technologies to study the genetic and epigenomic basis of human diseases. Human brain development is guided by gene regulatory programs that define neurogenic regions and give rise to diverse brain structures. Excitatory neurons arise from the ventricular zone (VZ), while inhibitory interneurons and striatal medium spiny neurons (MSNs) originate from the ganglionic eminences (GEs). The molecular programs that regionalize GE subtypes (MGE, LGE, CGE) and cortical areas (e.g., frontal vs. temporal) remain poorly understood. We used single-nucleus methyl-3C sequencing (snm3C-seq) and chromatin+RNA MERFISH to profile DNA methylation and 3D genome architecture across GEs, striatum, hippocampus, and cortical regions spanning prenatal to adult stages. We reconstructed developmental trajectories and identified region- and stage-specific regulatory programs. In our effort to identify the single cell DNA methylation signatures of autism spectrum disorder (ASD), we profiled >60,000 nuclei using snmCT-seq across 26 ASD and 23 neurotypical donors and identified thousands of differentially methylated regions (DMRs), enriched in promoters and cell-type-specific regulatory elements. ASD-associated methylation signatures are moderate compared to age-related changes and were largely uncorrelated with gene expression. The age-related changes in CG and non-CG sequence contexts and age-by-ASD interactions highlight areas of future research interests.

LOOKING AT GLIOBLASTOMA THROUGH THE LENS OF DEVELOPMENTAL AND CIRCUIT NEUROBIOLOGY

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In contrast to many other cancers, little advances have been over the past two decade in treating glioblastoma (GBM), a deadly adult brain cancer, in part due to challenges in translating findings from preclinical models to the clinic. Current preclinical models of GBM cell lines and xenografts are not adequate to model patient tumor heterogeneity and tumor microenvironment. We developed a novel surgical resection-derived GBM tumor organoid model that retains tumor characteristic of individual patients at cellular, molecular, genetic and structural levels as well as tumor microenvironment. I will present our recent studies applying these organoids for translational and basic research, highlighting the spatial organization similarity between GBM and the developing human cortex as well as GBM neuronal circuitry connectome in the adult brain.

PATHOLOGICAL AGGRESSION IN MICE AFTER REPEATED EXPERIENCE OF AGGRESSION: CHANGES IN BRAIN IMMUNITY

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Prolonged experience of aggression is stressful for the body and leads not only to changes at the level of neurotransmitter systems of the brain and in the hypothalamic-pituitary-adrenal system but also modulates the immune profile. However, it is still unclear which mechanisms contribute to such changes. In this study we investigated the effect of prolonged experience of aggression in the sensory contact model, which forms an aggressive type of behavior, on the state of the brain immune system of male CD1 mice.
The mice that had won 30 consecutive encounters were then treated with LPS (500 mkg/kg) for 6 days to induce inflammation. Control mice did not go through any aggressive experience. The development of pathological aggression was assessed using encounters with immobilized male CD1 mice. Microglia were isolated from half of the mouse brain and purified using a 37%/70% Percoll gradient. Further, the cells were stained with microglia-specific monoclonal antibodies and analyzed using flow cytometry. From the other half of the brain, we isolated the hypothalamus and nucleus accumbens and conducted qPCR analysis.
After prolonged aggressive experience, 36% of mice exhibited pathological aggression: high levels of aggression to the free-moving male and high levels of aggression to a non-moving (anesthetized) conspecific. All mice who went through prolonged experience of aggression had lower immune cell (CD45+) population compared to the control. They also had a lower active microglia population (CD45hiCD11+). qPCR analysis did not find differences in proinflammatory (*Aif1, Il1b*) gene expression in the hypothalamus or nucleus accumbens between aggressors and control mice. LPS injections led to a decrease of resting microglia (P2RY12+CD45intCD11b+) in the control group, but not in aggressors. We also found no association between pathological aggression and microglial subtypes. Furthermore, qPCR gene expression analysis in the hypothalamus showed that LPS injections led to an increase in expression of *Aif1* (microglial activation gene) and *Il1b* (interleukin 1 beta) genes in both control and aggressive mice, but in the nucleus accumbens, only control mice had higher expression of these genes after LPS, which confirms low microglial activation in aggressive mice.
Overall, aggressive mice had lower inflammatory response to LPS injections compared to the control. Thus, long experience of aggression leads to a modified reaction of the brain's immune system in response to LPS-induced inflammation.
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THE DISTINCT FUNCTIONS OF AUTOPHAGY GENES FIP200 AND ATG14 IN POSTNATAL BRAIN DEVELOPMENT

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Autophagy is a highly conserved cellular process involving the package of cytoplasmic contents and organelles into double membrane structure and their subsequent delivery to lysosome for degradation. The process of autophagy can be divided into autophagy induction, phagophore nucleation, autophagosome elongation and closure, autophagosome-lysosome fusion, cargo degradation and materials recycle. For each step, different autophagy-related genes (Atg) are assembled to form large protein complexes for proper functions. Autophagy is implicated in normal development, aging, and diseases. In this study, we examined the functions of autophagy genes of Fip200 (autophagy induction), Atg14 (phagophore nucleation), and At16l1 (autophagosome elongation and closure) in postnatal brain development using a brain specific conditional knock out (cKO) system. We observed the accumulation of p62 puncta, which indicated autophagy block, in the cortex and the granule layer of the hippocampus of cKO mice but not in control mice. Interestingly, we noticed spongiform in brain regions of Fip200 cKO and Atg14 cKO mice, but not in Atg16l1 cKO mice. Similarly, our data revealed a significantly decreased number of NeuN+ neurons and Bcl11b+ neurons in Fip200 and Atg14 cKO mice, but not in Atg16l1 cKO mice. Further analysis of neonatal Fip200 cKO mice indicated that the decreased number of Bcl11b+ neuron was a result of postnatal neuronal death. We performed single nuclei RNA-sequencing experiments of cortical cells from control and mutant mice and our results revealed distinct transcriptomic changes in different populations of Fip200 and Atg14 cKO mice. Taken together, our data suggested early onset neurodegeneration in the young adult Fip200 cKO mice and Atg14 cKO mice, indicating distinct roles of autophagy genes in postnatal brain development.

SPATIALLY TARGETED SUPPRESSION OF SEIZURES USING SONOGENETICS

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Background, Motivation and Objective

Epilepsy is a neurological disorder characterized by hyperexcitability in neural circuits. Sonogenetics, combining ultrasound with genetic targeting, offers a non-invasive approach for precise neural modulation. This study aims to utilize sonogenetics to selectively control inhibitory interneurons, leveraging cell-type-specific promoters for targeted expression of ultrasound-sensitive actuators, and to explore its therapeutic potential in epilepsy.

Statement of Contribution/Methods

We employed an inhibitory neuron promoter to express the ultrasound-sensitive ion channel selectively in inhibitory interneurons. Immunohistochemistry confirmed targeted expression, while in vivo fiber photometry recorded calcium dynamics during ultrasonic stimulation. EEG recording assays assessed seizure suppression in a kainic acid-induced epilepsy model. Spatial precision of ultrasound was exploited to compare single-region (hippocampus) versus dual-region (hippocampus + thalamus) interneuron inhibition.

Results/Discussion

Immunostaining confirmed the selective expression of mechanical ion channels in interneurons. Ultrasound stimulation effectively activated these neurons by enhancing calcium dynamics and eliciting distinct behavioral responses. In a subsequent kainic acid (KA)-induced seizure model, we demonstrated that modulating inhibitory neurons in the hippocampus, which is closely linked to seizure initiation, significantly suppresses seizure onset. Moreover, to evaluate the effectiveness of wide-field modulation through sonogenetics in managing seizures, we focused on both the hippocampus and thalamus, as both regions are crucial in seizure propagation, thereby improving seizure suppression. Our findings indicate that targeted modulation of the hippocampus during the initiation phase markedly inhibits the onset of seizures, underscoring its essential role as a key modulatory node. Moreover, simultaneous targeting of both the hippocampus and thalamus yields even greater suppression of seizures. The sonogenetics methodology offers a novel approach to modulating larger regions of the brain for seizure treatment.

Overall, the versatile modulation capabilities of sonogenetics signify a substantial advancement in our understanding of brain function and provide a new and effective framework for the treatment of neurological disorders, including epilepsy.

SOCIAL-ISOLATION INDUCED BINGE-LIKE EATING MODULATES ANXIETY AND DEPRESSION BEHAVIORS VIA A HYPOTHALAMIC PROJECTING CIRCUIT

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Chronic stress such as social isolation may distort eating behaviors and, in some cases, cause binge-like eating. The potential relationship between overeating and mental disorders, however, remains elusive. In the current study, we identify the over-eating phenotype in socially isolated mice compared to group house ones. Interestingly, a simple food rationing paradigm, in which socially isolated mice are provided with daily food volume equal to that of group housed mice, relieves both anxiety and depressive behaviors. The dissection of neural substrate underlying these behavioral phenotypes reveals the participation of a circuit from the ventromedial hypothalamic nuclei (VMH) to the calretinin (CR)-expressing neurons in the zona incerta (ZI), which further projects to the lateral periaqueductal gray (lPAG) for mediating anxiety and depressive behaviors. Further interrogation of molecular and cellular mechanisms finds microglial reactivation and neuroinflammation under the disruption of this VMH-ZI-lPAG circuit, and the body metabolic hormones induced by overeating direct the microglial polarization in VMH. In sum, this work identifies a previously unrecognized peripheral-central interplay, in which stress-induced overeating aggravates mental disorders, via hormone-mediated glial-neuron interaction.

WHAT IS SLEEP FOR?

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An important, unresolved question is why all animals sleep. We propose a “catecholamine hypothesis”, in which inactivation of catecholamine signaling may be a basic process underlying how sleep interacts with the cardiovascular, immune, and neuroendocrine systems. I will discuss recent studies testing this hypothesis.

ILLUMINATING CAUSAL LINKS BETWEEN NEURAL CIRCUIT ACTIVITY AND BEHAVIOUR

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Understanding the causal relationship between activity patterns in neural circuits and behavior is one of the fundamental questions in systems neuroscience. Addressing this problem requires the ability to perform rapid and targeted interventions in ongoing neuronal activity at cellular resolution and with millisecond precision. I will describe results of experiments using a powerful new "all-optical" strategy for interrogating neural circuits which combines simultaneous two-photon imaging and two-photon optogenetics. This enables the activity of functionally characterized and genetically defined ensembles of neurons to be manipulated with sufficient temporal and spatial resolution to enable physiological patterns of network activity to be reproduced. We have used this approach to identify the lower bound for perception of cortical activity, probe how brain state influences the role of cortex in perception, provide causal tests of the role of hippocampal place cells in spatial navigation, and examine how active dendrites mediate interactions between neighbouring cortical areas.

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DISSECTING THE NEURAL CIRCUITRY UNDERLYING MOTIVATED BEHAVIORS

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The amygdala and basal ganglia circuits have important roles in learning and executing behaviors motivated by either appetitive or aversive stimuli. How exactly these circuits contribute to the generation of divergent behavioral responses remains elusive. Our recent studies indicate that learning driven by reward or punishment induces distinct plastic changes in discrete circuits in the basal ganglia and the amygdala, and reveal how these learning-induced changes participate in guiding flexible behaviors. Interestingly, neurons in these circuits can also convey information about the nutritional properties of foods and the metabolic status of animals, and furthermore control energy utilization and weight gain. An emerging picture is that these circuits are used to regulate different aspects of motivated behaviors as well as energy homeostasis.

MODULATION OF PMv^{DAT} CELLS DURING AGGRESSION DEVELOPMENT PERSISTENTLY ALTERS SOCIAL BEHAVIOUR THROUGH CHANGES IN AN UPSTREAM SOCIAL BRAIN CIRCUIT.

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Intermale aggression in mice forms a core social behaviour displayed by the majority of male mice, but initially requires multiple exposures to novel mice to settle at a stable level, at which it is then maintained through adult life. This experience-dependent change suggests that the neural circuits responsible for aggression are innate, but also plastic and capable of further development in adulthood.
A population of dopamine transporter -expressing neurons in the ventral premammillary nucleus of the hypothalamus (PMv^{DAT+}) have previously been shown to be both necessary and sufficient for the expression of intermale aggression. Because these cells are extensively and reciprocally connected with other aggression-linked nuclei and their excitability differs significantly between aggressive and non-aggressive mice, we asked if activity of this population can drive the circuit-level changes required for aggression development.

Repeated resident/intruder (R/I) tests were performed in initially naïve male mice while we manipulated PMv^{DAT+} activity using either opto- or chemogenetics during or before the resident mouse's first encounters with an intruder. In the first experiments, we inhibited PMv^{DAT+} during three R/I trials, followed by three R/I trials with no inhibition. In this paradigm, development of intermale aggression was delayed by PMv^{DAT+} inhibition, but appeared during subsequent modulation-free trials.
In a second set of experiments, we stimulated PMv^{DAT+} while the subject mouse was alone in its home cage across three stimulation sessions, followed by three R/I trials without stimulation. Repeated stimulation of PMv^{DAT+} in a non-social context impaired the later development of a typical R/I aggression phenotype compared to control mice. Following the behaviour testing, we performed *ex vivo* electrophysiological recordings. These recordings revealed changes in both excitatory and inhibitory post-synaptic currents recorded from PMv^{DAT+}, while their excitability and other membrane properties remained similar between treatment and control groups.
These data suggest that while persistent change in network state and ensuing behaviour can be driven by brief modulation of a single neuronal population, the long-term effects are mediated through changes across multiple components of the circuit, some of which form feedback loops. Because the PMv has been previously shown to be reciprocally connected with multiple other regions within the neural circuits controlling aggression and social behaviours, we carried out a further experiment on cleared brains collected from mice after the asocial stimulation paradigm. Whole-brain immunostaining for neural activity markers was performed with the goal of identifying individual populations or brain regions which both provide input to PMv^{DAT+} and undergo plastic changes as a result of PMv^{DAT+} modulation.

DeepEcoHAB - A MACHINE LEARNING BASED SYSTEM FOR HIGH-THROUGHPUT BEHAVIORAL PHENOTYPING OF SOCIAL GROUPS OF LABORATORY ANIMALS

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DeepEcoHAB is a next-generation platform designed to overcome the limitations of traditional, low-throughput behavioral assays by enabling continuous, weeks-long monitoring of up to 12 mice with minimal experimenter intervention and AI-driven data analysis. The system integrates four core components:
1) EcoHAB platform: Four spacious cages interconnected by burrow-like tunnels outfitted with RFID antennas, allowing continuous tracking of each animal's identity, position, and activity in a semi-naturalistic environment. This validated setup permits mice to express their natural behaviors over extended periods - establishing social hierarchies, claiming territories, displaying social preferences or aversions, and competing for or sharing resources.
2) RFID data-acquisition hardware: A professionally redesigned RFID-DAQ board that supports 16 antenna channels, advanced HITAG S tags with anticollision capability, and four independent TTL inputs for precise synchronization with cameras, Bonsai workflows, wireless optogenetics, or electrophysiology. It functions as the central synchronizing clock within DeepEcoHAB or as a standalone module for third-party experiments.
3) DeepLabCut-powered pose estimation: A custom-trained, multi-animal model that simultaneously tracks 17 anatomical landmarks on each of 12 mice at high spatial and temporal resolution - without physical markers. Trained on over 2,500 labeled animals (more than 42,000 labels), this model dramatically outperforms existing commercial and open-source solutions in both precision and stability.
4) Integrated analytics suite: A bespoke software pipeline that merges RFID and video-based pose data, automatically classifies a wide repertoire of social and individual behaviors, and generates quantitative readouts for downstream analysis.
By combining automated, markerless tracking with robust RFID integration, DeepEcoHAB provides an objective, scalable alternative to classical behavioral assays, reducing variability from handling and observer bias. Its capacity to monitor 12 animals concurrently enables high-throughput analysis of social preferences and dominance hierarchies in both wild-type and transgenic mouse models.
Here, we present the current state of system development: (1) the advanced status of our pose-estimation and identity-reidentification protocols, (2) a comprehensive behavioral analysis based on RFID data using a conditional *Tsc2* knockout in *Vglut2*-positive cells as an autism model, and (3) the integration of DeepEcoHAB with wireless optogenetics, greatly expanding the system's experimental versatility.

ALL-OPTICAL INTERROGATION OF NEURAL AND ASTROCYTIC REGULATION OF HUNTING BEHAVIOR IN LARVAL ZEBRAFISH

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All-optical interrogation using high-resolution two-photon stimulation and imaging enables precise, simultaneous manipulation and recording of neuronal activity. To improve the fidelity of our all-optical platform, we developed an active pixel power control (APPC) method that dynamically adjusts laser power at each scan pixel. This approach minimized optogenetic crosstalk while preserving calcium signal quality, enhancing the accuracy of in vivo circuit-level analysis.

Leveraging this technique, we examined the neuronal and glial mechanisms underlying predation in larval zebrafish. Although binocular vision is crucial for predation in animals with two eyes, the neuronal mechanisms of binocular integration remain unclear. Using reversible eye occlusion and volumetric two-photon calcium imaging, we identified binocular prey-responsive neurons (bino-PRNs) in the pretectum, thalamus, and nucleus isthmi. These neurons exhibited offset receptive fields corresponding to natural hunting distances and showed increased activity during prey capture. Optogenetically evoked hunting movements also activated bino-PRNs, suggesting their role in integrating visual and motor signals to encode prey position in three-dimensional space.

While hunting-related neuronal circuits have been extensively studied, glial contributions remain elusive. We found that optogenetic stimulation of hindbrain radial astrocytes robustly elicited hunting behaviour within 200 ms. Calcium imaging further revealed astrocytic activation during hunting episodes, indicating that astrocytes may interact with hindbrain neurons to elicit predatory behavior.

ROBUST AND ULTRABRIGHT CHEMICAL LABELING ENABLES RAPID NEURAL CONNECTIVITY PROFILING IN LARGE TISSUE SAMPLES

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The comprehensive mapping of neuronal connections across entire nervous systems remains a fundamental challenge in neuroscience. Given that individual neurons in a specific brain area often manifest unique morphologies, genetic heterogeneities and distinct functions, recent efforts have been directed at more precisely studying the complete morphologies of individual neurons at the whole-brain level in mammalian model organisms. Here, we present LINCS (Label Individual Neurons with Chemical dyes and with controllable Sparseness), a robust chemical labeling system that combines *in vivo* biotinylation produced by an engineered, solubility-enhanced biotin ligase with rapid whole-mount staining. LINCS enables ultrabright, photostable, and cell-type-specific labeling of diverse tissue samples, ranging from entire mouse brains to whole adult mouse bodies. By integrating LINCS with tissue clearing and commercially available light-sheet microscopy, we established an efficient pipeline for rapid profiling of neuron projections in both the central and peripheral nervous systems. Implementation of sparse labeling strategies empowers the LINCS-based pipeline for precise morphological reconstruction of individual neurons across the mouse brain. LINCS and LINCS-based single neuron reconstruction pipeline should accelerate single-neuron connectivity mapping and broaden the application of single-neuron reconstruction technologies in neuroscience research across mammalian models.

GABAERGIC PROJECTION NEURONS – POWERFUL BUT VULNERABLE

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Cortical GABAergic neurons were long thought to be “interneurons”. They are the major source of inhibition in the adult brain and play a crucial role in controlling synchronized activity within the local network they are embedded in. More than a decade ago, we identified a population of cortical GABAergic population that are “projection neurons”. These cells exhibit an interesting wiring pattern in that they inhibit selectively GABAergic neurons in the target whereby they cause disinhibition at long distance. Ever since viral tracing has become a common tool in neuroscience, a remarkable number of so-far unknown GABAergic projection neurons have been discovered. They connect unilaterally or bidirectionally many cortical and subcortical brain areas, but their functional role has remained largely unknown. Interestingly, some subcortical GABAergic projections follow the same rule of connectivity. Thus, GABAergic projections of the medial septum inhibit selectively different types of GABAergic interneurons in most, if not all, regions of the hippocampal formation.
I will present a number of GABAergic projections that we study in our lab, including projections of the hippocampal formation, prefrontal cortex, and last but not least medial septum. I will discuss their role in information processing, but also their involvement in pathophysiological processes such as neurodegenerative diseases.

EPIGENOMIC REMODELING IN THE AGING MOUSE BRAIN

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Aging is a major risk factor for neurodegenerative diseases, yet underlying epigenetic mechanisms remain unclear. Here, we generated a comprehensive single-nucleus cell atlas of brain aging across multiple brain regions, comprising 132,551 single-cell methylomes and 72,666 joint chromatin conformation-methylome nuclei. Integration with companion transcriptomic and chromatin accessibility data yielded a cross-modality taxonomy of 36 major cell types. We observed that age-related methylation changes were more pronounced in non-neuronal cells. Transposable element methylation alone distinguished age groups, showing cell-type-specific genome-wide demethylation. Chromatin conformation analysis demonstrated age-related increases in TAD boundary strength with enhanced accessibility at CTCF binding sites. Spatial transcriptomics across 895,296 cells revealed regional heterogeneity during aging within identical cell types. Finally, we developed novel deep-learning models that accurately predict age-related gene expression changes using multi-modal epigenetic features, providing mechanistic insights into gene regulation. This dataset advances our understanding of brain aging and offers potential translational applications.

PATHO-MECHANISM OF eIF3F-ASSOCIATED NEURODEVELOPMENTAL DISORDER MRT67

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In mammalian cells, the eukaryotic translation initiation factor 3 (eIF3) complex, containing 13 subunits alphabetically named from a to j, is involved in all major aspects of mRNA translation, including translation initiation, elongation, termination, and ribosome recycling. Homozygous missense variants in the EIF3F gene, c.694T>G (p. Phe232Val), were recently discovered in children with the severe neurodevelopmental disorder MRT67. Main symptoms include intellectual disability, delayed speech development, and behavioral difficulties. However, the patho-mechanism of MRT67 remains unknown, and thus neither a specific disease hypothesis nor a therapeutic target has emerged. To address this gap, we are using iPSCs to construct a 3D brain organoid model for detailed molecular profiling. We are employing various advanced techniques such as single-cell mRNA sequencing, SeCLIP, and ribosome profiling to uncover the molecular mechanisms behind the disease-associated variant, especially investigating EIF3F's role in controlling specific mRNA translation during neuron development. We found that the hyperactivated WNT/BMP signaling in early disease organoids displays premature formation of choroid plexus and Cajal Retzius cells and early neurons but decreased pleiotrophin signaling in older disease organoids, which may underlie the observed depletion of mature neurons. Ultimately, we are hoping that gaining a detailed mechanistic understanding of these functions will help us to identify actionable targets within eIF3F-mediated differentiation pathways for therapeutic intervention.

RETROGRADE SIGNALING AS A TRIGGER FOR TDP-43 MISLOCALIZATION AND MOTOR NEURON DEGENERATION IN ALS

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder marked by progressive motor neuron (MN) loss and mislocalization of the RNA-binding protein TDP-43 from the nucleus to the cytoplasm. Although TDP-43 pathology is central to ALS, the mechanisms initiating its mislocalization remain poorly understood. Axonal transport dysfunction, particularly in the retrograde direction, has been implicated in ALS progression. Retrograde transport by dynein conveys injury and stress signals from distal axons to the soma and is essential for neuronal homeostasis.

We hypothesize that chronic stress at distal axons triggers aberrant retrograde signaling, leading to dynein-mediated mislocalization of TDP-43, axonal degeneration, and MN death. To investigate this, we utilize human induced pluripotent stem cell-derived motor neurons (iPSC-MNs) from two ALS models: one harboring the familial TDP-43^{M337V} mutation and one derived from a sporadic ALS case.

To identify retrograde signaling components altered in ALS, we employ Biotinylation by Antibody Recognition (BAR) to selectively label dynein-associated proteins in healthy and ALS MNs. These proteins are compared by mass spectrometry to uncover candidate retrograde cargoes. Candidate-dynein interactions will be validated using Proximity Ligation Assays (PLA), followed by immunofluorescence, western blotting, and live-cell imaging in iPSC-MNs, ALS mouse models, and patient biopsy samples. Functional studies using inhibitors or blocking peptides will assess how specific cargo disruptions impact TDP-43 localization, axonal integrity, and neuron viability.

This work aims to elucidate how retrograde transport dysfunction contributes to TDP-43 mislocalization and motor neuron degeneration in ALS, offering potential molecular targets for early therapeutic intervention.

Keywords: ALS, TDP-43, retrograde transport, dynein, iPSC-MNs, axonal degeneration

MULTISCALE HETEROGENEOUS BRAIN MORPHOLOGY OF AUTISM WITH HIERARCHICAL BAYESIAN REGRESSION NORMATIVE MODELING

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Autism Spectrum Disorder (ASD) is a neurodevelopmental condition with pronounced individual heterogeneity. Traditional case–control neuroimaging analyses can obscure subject-level variations, motivating normative-modeling methods that quantify each individual's deviation from typical brain organization. We applied a multiscale normative modeling framework to 1,344 structural MRI scans, constructing individual Morphometric INverse Divergence (MIND) networks that quantify the similarity of cortical morphology profiles across 308 brain regions. We summarized each region's similarity by its nodal degree. We then trained hierarchical Bayesian regression (HBR) models on typically developing controls—accounting for age (B-spline), sex, and imaging site—to establish normative trajectories of cortical morphology. For each ASD participant, we calculated regional Z-scores indicating deviations from these normative patterns. Deviations proved highly idiosyncratic: fewer than 12% of individuals showed extreme deviation ($|Z| > 2$) in any given region. Clustering the individual deviation profiles identified two distinct ASD subgroups. The first subgroup exhibited widespread positive deviations in association cortex, particularly frontoparietal and default-mode networks; whereas the second subgroup displayed focal negative deviations in sensory networks, with significant reductions in visual and ventral-attention systems. Notably, molecular-alignment analyses revealed that only the second subgroup's deviation map correlated with cortical gene-expression and neurotransmitter-receptor gradients. Functional validation further showed that each subgroup's deviations aligned differently with perceptual and executive-control activation maps. Our multiscale HBR normative approach demonstrates that ASD-related morphological alterations are predominantly individual-specific but coalesce into biologically and functionally interpretable variants. This precision-oriented stratification may inform targeted interventions and advance our understanding of ASD's neurodevelopmental heterogeneity.

HUMAN MODELS ON A CHIP: DISSECTING E/I BALANCE IN SYNDROMIC AUTISM SPECTRUM DISORDERS

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Autism spectrum disorders (ASDs) represent a heterogeneous group of neurodevelopmental conditions, marked by deficits in social interaction, communication challenges, and repetitive behavior, often accompanied by motor impairment and intellectual disability. Affecting roughly 1 in 100 children worldwide, there is a pressing need for improved ASD models to unravel the diverse clinical presentations and underlying pathophysiological mechanisms. The etiology of ASDs involves a multifaceted interplay between genetic, epigenetic, and environmental factors. Notably, even individuals with the same genetic variant can display strikingly variable symptoms, which poses a challenge for ASD diagnosis and treatment strategies. Syndromic forms of ASD - including Angelman, Rett, Fragile X, and Dup15q syndromes - are linked to specific genetic mutations or chromosomal anomalies. Despite their distinct genetic origins, many of these syndromes appear to share a common neurobiological mechanism: disruption of GABAergic signaling, which results in excitatory/inhibitory (E/I) imbalances in network activity.

In this project, we investigated the common and distinct aspects of E/I dysregulation in two syndromic ASD models: Rett and Dup15q syndromes. To this end, we generated patient-specific iPSC-derived neurons co-cultured with astrocytes, and performed functional characterization employing high-density microelectrode arrays (HD-MEAs), patch-clamp electrophysiology, and molecular assays.

Our data indicate early-stage hyperexcitability in both models at the single-cell and network levels. To further understand the contribution of specific neuronal subtypes to the E/I imbalance, we used a dual lineage NGN2/Ascl1 model for Dup15q syndrome. This system enables the generation of distinct excitatory and inhibitory neuronal populations in parallel cultures. Analyses of specific neuronal subtypes point to GABAergic dysfunction as a key contributor to the observed phenotypes, including disrupted burst patterns and differential sensitivity to pharmacological compounds. Furthermore, mutant neurons also exhibit reduced axonal length, suggesting a structural adaptation to buffer excessive excitability. Together, these findings point towards convergent mechanisms of circuit dysfunction that underlie distinct genetic forms of ASD. Our work highlights the utility of human neuronal models in examining early pathogenic events and identifying potential points of therapeutic intervention.

A SINGLE-NUCLEUS ATLAS OF GENETIC AND EPIGENETIC LANDSCAPES IN PARKINSON'S DISEASE MIDBRAIN

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Parkinson's disease (PD) is the second most common progressive neurodegenerative disorder, posing a substantial public health burden on the aging population. However, the underlying causes and pathogenic mechanisms of sporadic PD remain poorly understood. This is partly attributed to the challenges in obtaining human brain tissue, and the presence of diverse cell populations and their crosstalk. In this study, we aim to profile genetic and epigenetic changes in PD for the major cell types of the human midbrain. We apply single-cell combinatorial indexed assay for the assessment of DNA methylation (sciMET) to postmortem midbrain samples to construct an atlas comprising around 30,000 nuclei in total from 28 PD and 30 control donors. The application of in-house developed software ensures that the atlas is unbiased by donor genotype differences overlapping CpG loci, which we find to be an overlooked factor confounding differential DNA methylation analyses. Clustering and annotation yielded 5 major cell types in the midbrain: oligodendrocytes, oligodendrocyte precursor cells, microglia, astrocytes, and neurons. Interestingly, a cluster of neurons exhibited ~10% non-CpG methylation, which will be further characterized. Preliminary analyses identified numerous cell-type-specific differentially methylated regions associated with PD, which provide insights into functions and pathways involved in PD pathology. Additionally, we observed chromosomal and sub-chromosomal copy number aberrations (CNAs) in subsets of nuclei from both PD and control donors, with PD neurons, astrocytes and OPCs showing notably increased CNA fractions compared to control nuclei. To refine and validate these findings, further data generation and analysis is ongoing. Ultimately, our research aims to advance our understanding of PD etiopathogenesis and eventually contribute to improved therapeutic strategies.

NOTES

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Clinic	**Front Office** **Duty Manager**	86-18913579300
Engineering		
IT		
Front Office		

Dining, Bar

Dining Rooms:

CSHA Academic Center Main Cafeteria – Floor 1

Bar:

D Lounge @ Worldhotel Grand Dushulake– Floor 1
Opening Hours: 09:00 – 00:00
CSHA Academic Center Bar – Floor 4
Opening Hours: 09:00 – 23:00

Internet Access

CSHA Academic Center – Wireless
Public Area – Wireless
Room – Wireless

Gymnasium & Aerobic Room

Mezzanine Floor Opening @ Worldhotel Grand Dushulake
Hours: 06:00 – 23:00
CSHA Academic Center Gym – Floor 4
Opening Hours: 24 hours

Indoor Swimming Pool

Mezzanine Floor Opening @ Worldhotel Grand Dushulake
Hours: 06:00 – 23:00

Taxi

Local Taxi:

Tel: 86-512-67776777

Out of city - eHi Car Rental:
Tel: 4008886608
Email: booking005@1hai.cn

*You may contact **Cold Spring Harbor Asia Office** at 86-512-62729029 for any conference inquiries.*

CSHA MAP


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苏州独墅湖世尊酒店
GRAND DUSHULAKE
SUZHOU
CSHA
CSHA
Laboratory 2
实验室2
cafeteria
餐厅
Patio
庭院
Auditorium
报告厅
Patio
庭院
Main
cafeteria
主餐厅
Laboratory 1
实验室1
Office
办公
Coffee Room
茶水间
Computer
Teaching
电脑教学
Conference
Room
会议室
Lake Front
Hall
湖前厅
Moon Bay Road
CSHA OFFICE
DUSHU LAKE
独墅湖

MAP OF CSHA CONFERENCE CENTRE

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