UC Irvine
Center for Neural Circuit Mapping

Cajal Club

ALLEN INSTITUTE for BRAIN SCIENCE

2025 CONFERENCE

The Changing Brain

August 18 – 20

7:30 a.m. – 5:00 p.m. PT

Irvine Marriott, Irvine, CA

Table of Contents

Code of Conduct

The CNCM and organizers are committed to providing a professional, welcoming, and safe environment free from harassment and discrimination of any kind so that all participants can take part in advancing science through research, education, and professional development in safe fashion. All participants in attendance need to comply with this Code of Conduct Policy and in doing so also agree to conduct themselves in a professional manner. Harassment includes verbal, written, or physical conduct that denigrates or shows hostile behavior to others, which (1) has the purpose or effect of creating an intimidating, hostile, or offensive environment; (2) has the purpose or effect of interfering with an individual's performance or ability to participate in events; or (3) otherwise affects an individual's ability to participate in events due to unwelcomed verbal or physical conduct which can adversely affect the environment, or results in a decision adversely affecting the individual based upon the their rejection of such conduct.

Violations of this Policy will be taken seriously and may result in appropriate action, including removal from the event.

UC Irvine School of Medicine
Center for Neural Circuit Mapping
2025 CONFERENCE

Welcome Message

On behalf of the Organizing Committee, I would like to warmly welcome all of you to our annual 2025 Conference at the Irvine Marriott on August 18–20, 2025. Our main conference is co-sponsored by the UC Irvine Center for Neural Circuit Mapping (CNCM), the Cajal Club, and the Allen Institute for Brain Science. This marks the 5th annual in-person conference hosted by CNCM, following the success of our previous meetings. Additional workshops on spatial transcriptomics and viral-genetic tools, open to all attendees, will be held at the UC Irvine campus on August 21, 2025.

Our focus this year is “The Changing Brain.” The 2025 meeting topics reflect exciting directions in the field. We will cover topics that include cell types, neural circuits and systems, and evolution, development, and disorders of the nervous system. We have planned six sessions: 1) the Evolving Brain, 2) the Developing Brain, 3) the Learning Brain, 4) the Dynamic Brain, 5) States of the Brain, and 6) the Disordered Brain, with over 26 world-class invited speakers.

We have over 380 formal registrants from 104 diverse academic institutions and 16 industrial organizations in the U.S. and internationally. A total of 195 abstract submissions have been accepted, including 26 invited talks, 9 special/selected short talks, and 160 poster presentations. Notably, 71.3% of attendees are from outside UCI, and 7.3% are international.

One of our goals for the conference is to offer critical professional development opportunities for graduate students and postdoctoral scholars attending the meeting. This is facilitated by a R13 Conference Grant from the National Institute of Mental Health. Fourteen travel awards and 17 conference fee awards were selected from a competitive pool of over 65 submissions. Our annual conferences keep our trainees and junior investigators abreast of the most recent developments in the field and provide professional networking opportunities for them to establish relationships with leading scientists.

We acknowledge our strong institutional support, including steadfast backing from UCI School of Medicine Dean Michael Stamos. We also acknowledge support from the UCI Office of Research and Vice Chancellor for Research Pramod Khargonekar. Many people contributed to the planning and realization of this meeting. We thank the leadership of the CNCM and the Cajal Club, including Drs. Todd Holmes, Patrick Hof, Suzana Herculano and Chuck Ribak; the CNCM staff—Jane Alshami, Ginny Wu, Michele Wu, Jabez Domingo and Kaitlyn Huynh—and the staff of the Irvine Marriott for their attention to detail and logistical support.

Thank you all for coming to our conference. We look forward to stimulating interactions and meaningful discussions throughout the course of the meeting.

Sincerely,

Liqun Luo, PhD
Howard Hughes Medical Institute investigator,
Ann and Bill Swindells Professor of Biology,
Stanford University
On behalf of the Organizing Committee (Paola Arlotta, Liqun Luo, Xiangmin Xu & Hongkui Zeng)

Meet Our Organizers

Liqun Luo, PhD
Howard Hughes Medical Institute investigator,
Ann and Bill Swindells Professor of Biology,
Stanford University

Paola Arlotta, PhD
The Golub Family Professor
Department of Stem Cell and Regenerative Biology
Harvard University

Hongkui Zeng, PhD
Executive Vice President,
Director of the Allen Institute for Brain Science

Xiangmin Xu, PhD
Director, UCI Center for Neural Circuit Mapping
Chancellor's Professor, Department of Anatomy &
Neurobiology, School of Medicine
University of California, Irvine

2025 Conference Schedule

Organizers: Paola Arlotta, Liqun Luo, Xiangmin Xu, Hongkui Zeng

Day 1 - Monday, August 18	
7:20 – 8:00 a.m.	Breakfast and Registration
8:00 – 8:05 a.m.	Introduction: Christine Gall, PhD (UCI Anatomy & Neurobiology Chair)
8:05 – 8:20 a.m.	Opening Remarks: Dean Michael J. Stamos, MD (UCI SOM)
Session 1: The Evolving Brain	
8:20 – 8:25 a.m.	Introduction: Liqun Luo, PhD *Session Chair*
8:25 – 9:00 a.m.	Hongkui Zeng, PhD (Allen Institute for Brain Science) *Co-Organizer*
9:00 – 9:35 a.m.	Tom Nowakowski, PhD (University of California, San Francisco)
9:35 – 10:10 a.m.	Vanessa Ruta, PhD (Rockefeller University)
10:10 – 10:30 a.m.	Break
10:30 – 11:05 a.m.	Pierre Vanderhaeghen, PhD (VIB Leuven)
11:05 – 11:40 a.m.	Sten Grillner MD, PhD (Karolinska Institutet, Sweden)
11:40– 11:55 a.m.	Scott Owen, PhD (Stanford University)
12:05 – 1:05 p.m.	Lunch and Poster Session
1:15 – 1:25 p.m.	Speaker Group Photo (Main Lecture Hall)
1:05 – 1:50 p.m.	Poster Session Continued
Session 2: The Developing Brain	
1:50 – 1:55 p.m.	Introduction: Hongkui Zeng, PhD *Session Chair*
1:55 – 2:30 p.m.	Paola Arlotta, PhD (Harvard University) *Co-Organizer*
2:30 – 3:05 p.m.	Guillermina Lopez-Bendito, PhD (UMH-CSIC, Spain)
3:05 – 3:40 p.m.	Josh Huang, PhD (Duke University)
3:40 – 4:00 p.m.	Break
4:00 – 4:35 p.m.	Larry Zipursky, PhD (University of California, Los Angeles)
4:35 – 4:50 p.m.	Guoqiang Bi, PhD (Shenzhen Institute of Advanced Technology, China)
4:50 – 5:05 p.m.	Kelly Jin, PhD (Allen Institute for Brain Science)
5:05 – 6:20 p.m.	On-site welcome reception for all attendees
6:30 – 9:00 p.m.	Invited Speaker Dinner

Day 2 - Tuesday, August 19

7:30 – 8:30 a.m.	Breakfast

Session 3: The Disordered Brain

8:30 – 8:35 a.m.	Introduction: Paola Arlotta, PhD *Session Chair*
8:35 – 9:35 a.m.	*PJ Harman Lecture, Cajal Club:* Michelle Monje, MD, PhD (Stanford University)
9:35 – 10:10 a.m.	Li-Huei Tsai, PhD (Massachusetts Institute of Technology)
10:10 – 10:45 a.m.	Hailan Hu, PhD (Zhejiang University, China)
10:45 – 11:05 a.m.	Break
11:05 – 11:40 a.m.	Guoping Feng, PhD (Massachusetts Institute of Technology)
11:40 – 12:15 a.m.	Xiangmin Xu, PhD (University of California, Irvine) *Co-Organizer*
12:15 – 12:30 p.m.	Yves De Koninck, PhD (Laval University, Canada)
12:40 – 1:40 p.m.	Lunch and Poster Session
1:40 – 2:25 p.m.	Poster Session Continued

Session 4: The Learning Brain

2:25 – 2:30 p.m.	Introduction: Larry Swanson, PhD *Session Chair*
2:30 – 3:05 p.m.	Karel Svoboda, PhD (Allen Institute for Neural Dynamics)
3:05 – 3:40 p.m.	Elizabeth Buffalo, PhD (University of Washington)
3:40 – 4:15 p.m.	Bernardo Sabatini, MD, PhD (Harvard University)
4:15 – 4:35 p.m.	Break
4:35 – 5:10 p.m.	Nelson Spruston, PhD (HHMI Janelia Research Campus)
5:10 – 5:25 p.m.	Rózsa Balázs, MD, PhD (Brain Vision Center, Hungary)
5:25 – 5:40 p.m.	Lomax Boyd, PhD (The Rockefeller University)
6:00 – 7:00 p.m.	Special Interest Sessions / Networking Outreach Events
7:00 – 9:00 p.m.	The Art of Science (special ticketed event)

Day 3 - Wednesday, August 20

7:30 – 8:30 a.m.	Breakfast

Session 5: The Dynamic Brain

8:30 – 8:35 a.m.	Introduction: Edward Callaway, PhD *Session Chair*
8:35 – 9:10 a.m.	Liqun Luo, PhD (Stanford University) *Co-Organizer*
9:10 – 9:45 a.m.	Catherine Dulac, PhD (Harvard University)
9:45 – 10:20 a.m.	Anne Churchland, PhD (University of California, Los Angeles)
10:20 – 10:40 a.m.	Break
10:40 – 11:15 a.m.	Edward Chang, MD (University of California, San Francisco)
11:15 – 11:30 a.m.	Kuan Hong Wang, PhD (University of Rochester)
11:40– 12:40 p.m.	*Meet the Editors*: Noah Gray (Nature), Ann Goldstein (Cell), Mariela Zirlinger (Neuron), and Julio Licinio and Ma-Li Wong (Molecular Psychiatry)
12:40 – 1:40 p.m.	Lunch

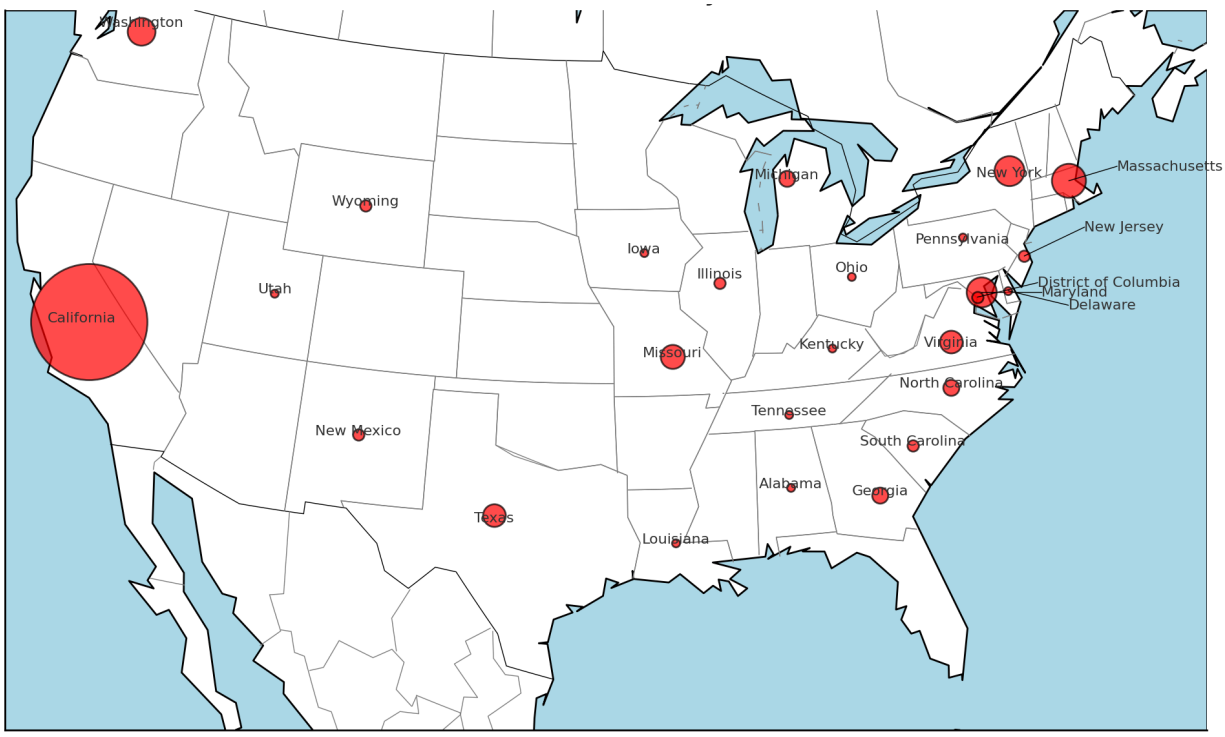
Session 6: State of the Brain

1:40 – 1:45 p.m.	Introduction: Xiangmin Xu, PhD *Session Chair*
1:45 – 2:20 p.m.	Yang Dan, PhD (University of California, Berkeley)
2:20 – 2:55 p.m.	Ishmail Abdus-Saboor, PhD (Columbia University)
2:55 – 3:30 p.m.	Zhigang He, BM, PhD (Harvard University)
3:30 – 3:50 p.m.	Break
3:50 – 4:05 p.m.	Kei Igarashi, PhD (University of California, Irvine)
4:05 – 4:20 p.m.	Cheng Lyu, PhD (Stanford University)
4:20 – 4:55 p.m.	John Ngai, PhD (NIH BRAIN Initiative)
4:55 – 5:00 p.m.	Closing Remarks

2025 Conference Attendee Graphical Locations

Attendees by Country

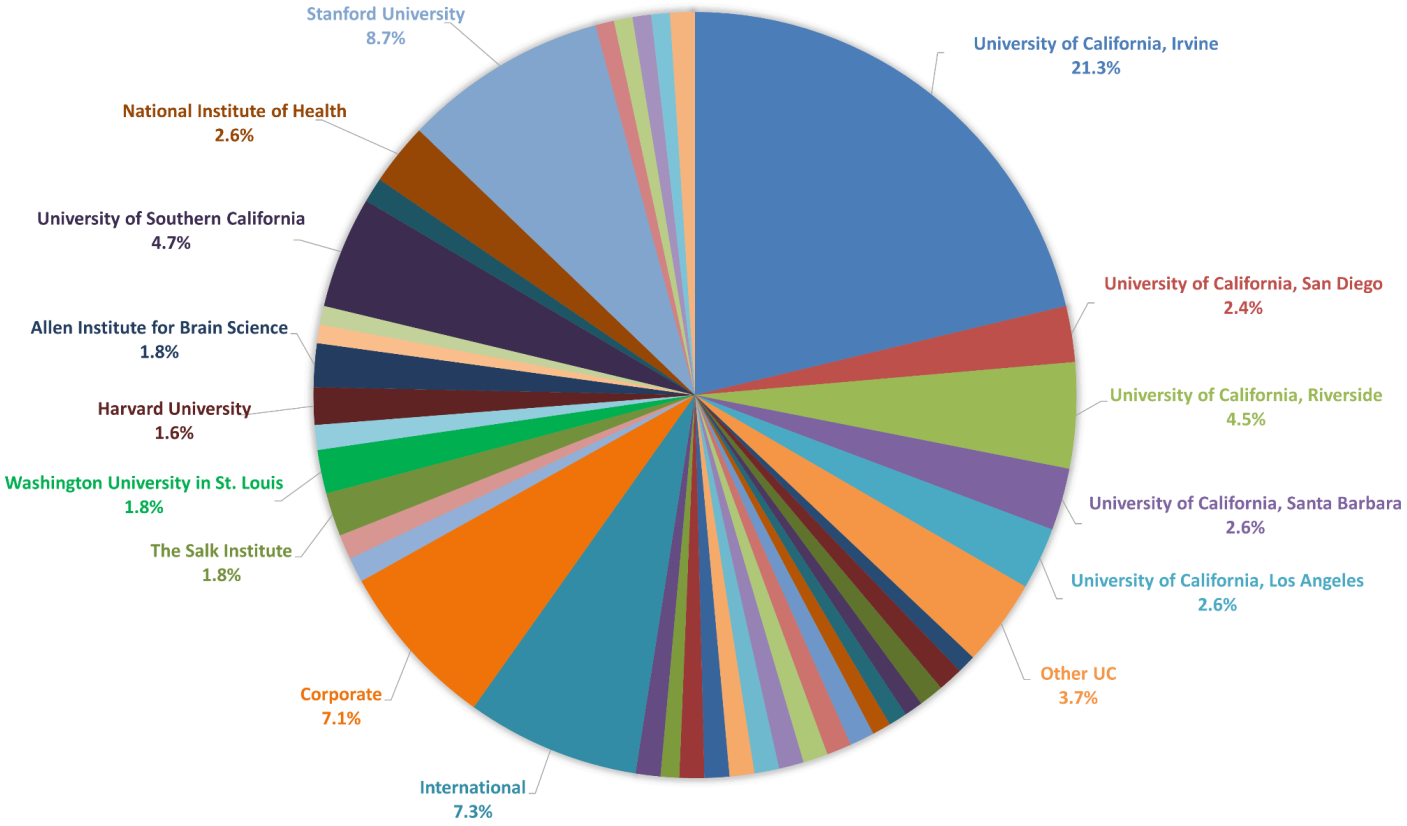
Attendees by US State



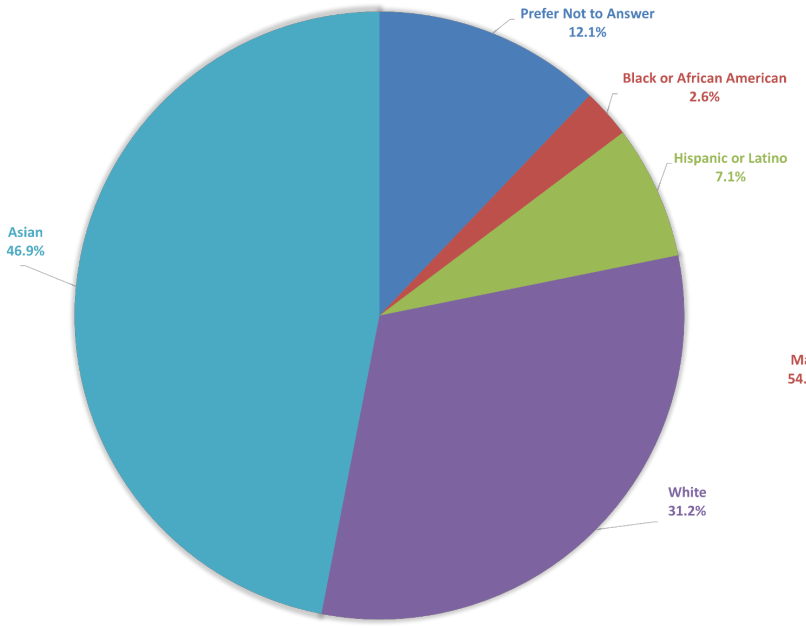
2025 Conference Attendee Demographics

Affiliation

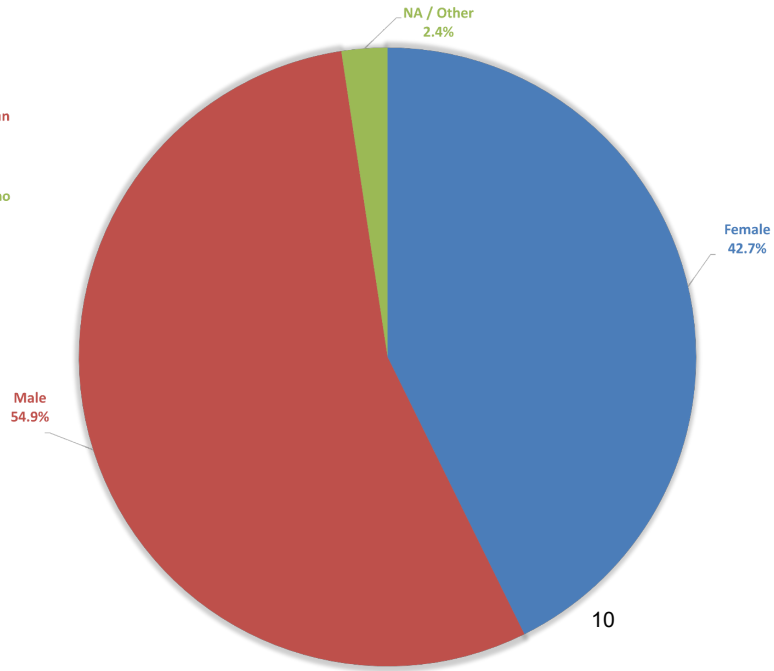
Non-UCI: 273 (71.3%); International: 27 (7.3%); UC Irvine: 81 (21.3%)



Ethnicity/Race



Gender



Conference Venue

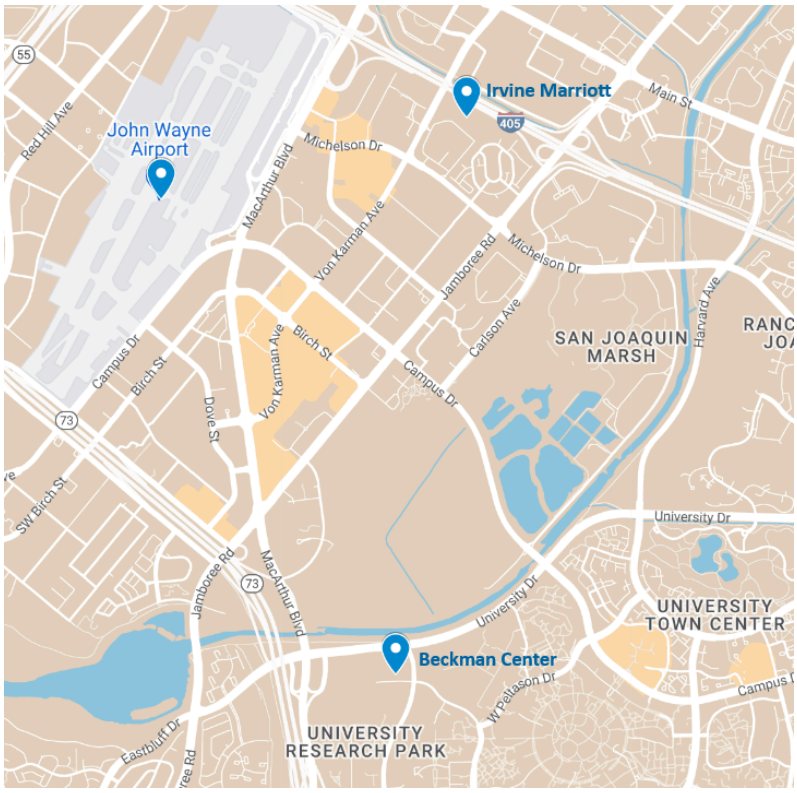
We are pleased to be hosting our conference at the Irvine Marriott for the first time. The Irvine Marriott is a 485-room, 4-star hotel known for its modern accommodation, excellent amenities, and convenient proximity to John Wayne Airport (SNA), located less than a mile away. Situated in the heart of Irvine—near South Coast Plaza and the University of California, Irvine—the venue offers over 36,000m square feet of state-of-the-art event space designed to inspire creativity and innovation.

Front Desk: +1 949-553-0100

Directions

18000 Von Karman Avenue, Irvine, California, USA, 92612

Follow San Diego Freeway (I-405 N) to Jamboree Rd. Take exit 7.



Parking

Self parking is paid at $25 per day. Electric charging stations are available on-site. Overnight parking is available for hotel guests ONLY.

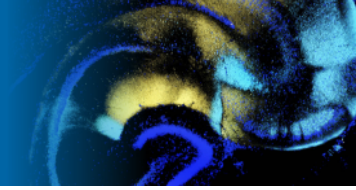
Shuttle

The hotel offers a complimentary shuttle service to and from John Wayne Airport (SNA).

Departure from the hotel: Every hour at :00 and :30, starting at 5:00 AM.

Pick-up from the airport: Every hour at :15 and :45 from the Airport Transportation Center (located across from Pillars 5 and 6) until the airport closes around 11:15 PM.

Important: Please be sure to board the "Irvine Marriott" shuttle, as there are multiple hotel shuttles serving the airport.



Session 1
The Evolving Brain

Hongkui Zeng, PhD

Department of Brain Science,
Allen Institute for Brain Science, Seattle, Washington

Dynamic changes of brain cell types in development and aging

To understand the function of the brain and how its dysfunction leads to brain diseases, it is essential to uncover the cell type composition of the brain, how the cell types are connected with each other and what their roles are in circuit function. At the Allen Institute, we have generated a comprehensive and high-resolution transcriptomic and spatial cell type atlas for the whole adult mouse brain, hierarchically organized into four nested levels of classification: 34 classes, 338 subclasses, 1,201 supertypes and 5,322 clusters. Extending from this foundational reference atlas, we have investigated the dynamic changes of transcriptomic profiles in the developing and aging brain. We generated a transcriptomic and epigenomic cell type atlas of the developing mouse visual cortex, with dense temporal sampling from E11.5 to P56. We reconstructed a transcriptomic developmental trajectory map of all excitatory, inhibitory, and non-neuronal cell types in the visual cortex, which reveals continuous cell type diversification throughout the pre- and postnatal stages of cortical development. In the aging mouse brain, through brain-wide single-cell transcriptomic profiling, we uncovered cell-type specific transcriptomic signatures of decreased neuronal structure and function and increased immune response and inflammation. We further identified a potential hotspot for aging, which is the hypothalamic region around the third ventricle, involving tanycytes, ependymal cells, and specific neuronal types with functional roles in energy homeostasis that exhibit both a decrease in neuronal function and an increase in immune response, suggesting a connection among metabolism, neuroinflammation, and aging.

Tomasz Nowakowski, PhD

Department of Neurological Surgery,
University of California San Francisco, San Francisco, California

Genetic, cellular and intercellular strategies of human brain development.

Development of the human brain takes place over many months of pre- and post-natal development. Cell types of the human brain are remarkably diverse and follow complex molecular and cellular programs to establish proper cell identity, and to develop highly non-random interactions between molecularly distinct subtypes. Here I describe our efforts to understand the logic of human neural stem cell differentiation across species using high throughput lineage tracing approaches. We uncover conserved and divergent patterns of lineage progression, including a transition from glutamatergic to gabaergic neurogenesis. By leveraging alternative viral tracing approaches, we are able to uncover patterns of cell-cell interactions in the developing human subplate, a transient zone with essential roles for brain circuit assembly. Together, my talk will highlight how molecular technologies continue to reveal remarkable complexities of human brain development, and potential vulnerabilities in disease.

Vanessa Ruta, PhD

Howard Hughes Medical Institute,
Rockefeller University, New York City, New York

Themes and variations in social circuits

Animals exhibit astonishing variation in their behavior, both within and across species. My lab has been using the elaborate courtship rituals of *Drosophila* to gain insight into the circuit mechanisms of adaptive behavior over different timescales, from how social interactions unfold on a moment-to-moment timescale to how species-specific mating displays arise over evolution. Using an interdisciplinary toolkit to probe the concise circuits of the fly, we have begun to shed light on how the interplay between internal arousal states and external sensory signals shapes ongoing behavior, allowing animals to flexibly navigate complex social landscapes. Moreover, by comparing these same circuits across closely related *Drosophila* species, our work suggests how evolution tinkers with conserved neural components to generate novel behavioral traits.

Pierre Vanderhaeghen, PhD

Stem Cell and Developmental Neurobiology,
VIB Leuven, Leuven, Belgium

Mechanisms linking human brain development, evolution, and (dys)function.

The human cerebral cortex has undergone rapid expansion and complexification during recent hominid evolution, which is thought to be at the origin of some of the higher cognitive and social skills characteristic of our species. While the mechanisms of increase in human brain size have been studied for some time, those underlying the evolution of cortical circuits only start to be unravelled. These originate from selective divergence in gene regulatory networks, the emergence of human-specific genes, as well as species-specific cellular features such as mitochondria dynamics and metabolism. Newly discovered human-specific modifiers of cortical neuron development and function shed light on human evolution, and provide unexpected links to brain diseases to which our species is particularly sensitive.

Sten Grillner, MD, PhD

Department of Neuroscience,
Karolinska Institutet, Stockholm, Sweden

The forebrain over 500 million years – a brief account

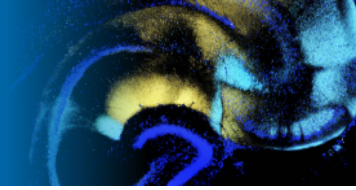
An important question is to what an extent the bauplan of the vertebrate forebrain evolved early in vertebrate evolution or rather gradually during the vertebrate evolution to mammals with cortical/pallial motor and sensory areas, the basal ganglia and the modulatory systems. The lamprey belonging to the oldest group of now living vertebrates became separate from the evolutionary line leading to mammals already some 500 million years ago. We show that the lamprey forebrain has the basal ganglia, organized in a similar way to the mammalian version with the same intrinsic nuclei (striatum, GPi, SNr, STN), same types of connectivity, types of neurons, transmitters, receptors and input from cortex/pallium and thalamus. The dopamine system has also a similar efferent and afferent connectivity. Thalamus conveys signals to a retinotopically organized visual area in the dorsal pallium/cortex, where there is also a somatopically organized area, and a motor area with separate projections to thalamus, the midbrain, brainstem and even the spinal cord. There is in addition the intratelencephalic neurons that connect both hemispheres but also project to striatum. We interpret these data to suggest that these basic features of the forebrain had evolved early in vertebrate evolution and been maintained through the amphibians, the reptile group (cynodontds) that evolves into mammals. The lamprey thus has a similar basic organization of the forebrain as mammals while the number of neurons in each part is very much reduced.

Scott Owen, PhD

Department of Neurosurgery and Department of Neurobiology,
Stanford University, Stanford, California

Dynamic regulation of neuronal excitability in the juvenile human neocortex

In early childhood and adolescence, the mammalian neocortex comprehensively rewires itself through extensive proliferation and pruning of synapses. The resulting changes in excitatory drive challenge the physiology of individual neurons, which can efficiently encode information only within a relatively narrow range of firing rates. Our limited knowledge of this maturation in the human neocortex, and the extent to which it is captured by rodent, stem-cell, or organoid models, restricts translation of new treatments for neurodevelopmental disorders including autism, obsessive-compulsive disorder, and schizophrenia. Here we combine brain slice recordings from pediatric epilepsy and vascular malformation patients with single-cell transcriptomics (Patch-Seq) to directly measure physiology and gene expression in juvenile, human Layer 2/3 neocortical neurons. We show that active physiological properties including spike rate adaptation and the hyperpolarization-activated h-current change systematically in human neurons through postnatal development (ages 4 months to 18 years). This contrasts markedly with mouse models, which exhibit changes primarily in passive properties including membrane potential and input resistance over equivalent developmental stages. Using multiple linear regression to correlate single-neuron physiology with single-cell transcriptomic data, we identify BK-type potassium channels as a primary driver of this human-specific, postnatally regulated adaptation of neuronal firing rates. Our computer simulation demonstrates how these properties can maintain firing rates within a healthy physiological range in the face of changes in excitatory synaptic drive. These results shed new insight into fundamental mechanisms of postnatal maturation in human neocortical neurons.



Session 2
The Developing Brain

Paola Arlotta, PhD

Department of Stem Cell and Regenerative Biology,
Harvard University, Cambridge, Massachusetts

Passage of time in brain organoids: the journey to understand human brain development and maturation

Much remains unknown regarding the mechanisms governing human brain maturation and aging. Human brain organoids offer a unique platform for these studies. Here, we investigated human cortical organoids cultured for periods ranging from 6 months to over 5 years in vitro. Module scores of maturations trained on the endogenous tissue show that organoids continue to develop and mature while in culture for these extended time frames. In agreement, methylation profiling revealed a strong correlation between predicted age of the organoids and time in culture. Using extracellular single-unit recordings with multielectrode arrays (MEA), we detected network bursts and action potentials with features that changed over developmental and maturation trajectories in culture. Notably, we find that human brain organoids are capable of “recording and recalling” developmental time as demonstrated by the ability of “old” progenitors to rapidly produce late progeny when exposed to inductive developmental signals. The work indicates that the human brain can develop, mature and age outside the context of the embryo.

Guillermina Lopez-Bendito, PhD

Department of Developmental Neurobiology,
Institute of Neuroscience, Alicante, Spain

Interplay Between Spontaneous Activity and Genetic Programs in Sensory-Modality Cortical Arealization

Our group has pioneered the study of the role of the early thalamic spontaneous activity in the development of sensory maps and cortical columns. Recently we showed that sensory circuits emerge as nonsegregated modules and that at birth these circuits became segregated and sensory modalities specified. We found, unexpectedly, that this segregation takes place in an evolutionary ancient subcortical structure, the superior colliculus, in a process that depends on the earliest activity from the retina. In my talk I will show these relevant data and discuss new unpublished results on the process of cortical arealization, by which the neocortex is divided into distinct functional areas. This process is traditionally attributed to embryonic morphogen gradients and postnatal experience-dependent mechanisms, however, emerging evidence challenges this linear view, suggesting that activity-dependent influences, driven by spontaneous neural activity, may play a critical role much earlier, during embryonic and perinatal development. We hypothesize that the specification of cortical sensory areas is a multilayered process involving a dynamic interplay between genetic programs and patterns of spontaneous activity. These factors converge to establish circuits uniquely tuned to process specific sensory modalities, such as the somatosensory (S1) and visual (V1) cortices. Using single-nuclei RNA sequencing and spatial transcriptomics, we have identified the genes that confer identity, "core" genetic programs, enriched in thalamo-recipient cortical layers of V1 and S1 and those genes associated with neuronal activity. Concurrently, in vivo mesoscale calcium imaging will characterize the spatiotemporal dynamics of spontaneous activity in S1 and V1. Manipulating these patterns using genetic and pharmacological tools will clarify how non-cortical inputs influence cortical identity through activity-related transcriptional changes and ultimately sensory behavior. In sum, our work will illuminate the interplay between genetic and activity-dependent mechanisms in sensory cortical development, providing insights into sensory processing and adaptation while informing strategies to address neurodevelopmental disorders involving sensory deficits.

Josh Huang, PhD

Department of Neuroscience
Duke University, Durham, North Carolina

Genetic dissection of cortical neuron type trajectories: from developmental origin to behavioral function

Neural circuits of the cerebral cortex are built from a set of basic templates shared by individuals of the species and conserved across mammalian evolution. To a great extent, cortical circuits self-assemble during development guided by genomic information and are then individually customized through learning and experience. A key challenge in understanding cortical circuitry is discovering the organization principle of the diversity of glutamatergic projection neuron (PN) “types” which, through their elaborate long axons, mediate the myriad processing streams and output channels. Meeting this challenge requires strategies to integrate multi-modal PN phenotypes and track their developmental trajectories, from lineage origin to circuit operation in behavior. In this talk, I will highlight our progress in 1) building genetic tools to systematically dissect and fate map PN types; 2) exploring the progenitor and lineage basis in the specification and laminar deployment of PN types; and 3) studying the function of genetic- and projection-defined PN types in skilled motor control. We aim to integrate the developmental genetic, systems neuroscience, and evolutionary explanations of PN diversity and organization that shape cortical circuit architecture.

Larry Zipursky, PhD

Department of Biological Chemistry,
University of California Los Angeles, Los Angeles, California

Synaptic Gradients: From continua of cell types to molecules and behavior

Continua of cell types is a common feature of excitatory neurons in the mammalian cortex. How these graded programs of gene expression contribute to neural circuit development is unclear. Recent studies in the Drosophila visual system provide insight into how continuous variation in transcriptomic architecture contributes to circuit organization. In a recent series of studies, we explored how circuits are assembled that convert specific visual stimuli, such as object location and motion, into specific directional responses. A class of cells called visual projection neurons (VPNs) function at the interface between sensory inputs and motor outputs. These neurons elaborate synaptic gradients on target neurons that drive direction-specific motor programs. Using EM-based connectomics, single cell sequencing, genetics, morphological and behavioral analyses, we discovered VPNs form a continuum of transcriptomic types, including the graded expression of cell recognition molecules, that specify these synaptic gradients. The graded expression of two Ig superfamily proteins, both members of larger families of heterophilic recognition molecules, regulate these synaptic gradients in an instructive fashion. Transcriptomic continua within a single cell type, including the expression of many cell recognition molecules, are common in the mammalian cortex. We speculate that these gradients diversify synapses in the mammalian brain.

Guoqiang Bi, PhD

Interdisciplinary Center for Brain Information,
Shenzhen Institute of Advanced Technology, Shenzhen, China

Mesoscale mapping of the nervous system: from brain to body

The nervous system is composed of a vast network of interconnected neurons that form intricate circuits spanning the brain and the entire organism. A thorough understanding of cognition, physiological regulation, and associated diseases requires mapping the brain's network architecture at cellular resolution. Toward this goal, we have developed VISoR, an ultrafast volumetric fluorescence microscopy technique capable of imaging an entire rhesus monkey brain at micron resolution within 100 hours. Tracing individual axonal fibers uncovers intricate wiring pathways and complex arborization patterns in thalamocortical projections, revealing unexpected constraints on cortical development and providing insight into the sophisticated neural architecture underlying information processing. Beyond the brain, we have further developed a blockface-VISoR system and an optimized clearing procedure to enable uniform high-resolution imaging of the entire adult mouse body. This approach allows us to visualize the complex axonal projections of somatic and parasympathetic nervons, as well as the perivascular patterns of sympathetic nerves across various organs.

Kelly Jin, PhD

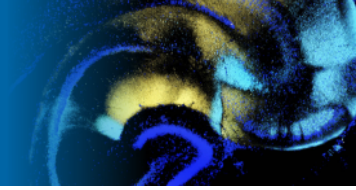
Data Science & Informatics and Molecular Genetics,
Allen Institute for Brain Science, Seattle, Washington

Brain-wide cell-type-specific transcriptomic and epigenomic signatures of healthy ageing in mice

Biological ageing can be defined as a gradual loss of homeostasis across various aspects of molecular and cellular function. Mammalian brains consist of thousands of cell types, which may be differentially susceptible or resilient to aging.

We recently presented a large single-cell RNA sequencing dataset containing roughly 1.2 million high-quality whole-cell transcriptomes of brain cells from young adult and aged mice of both sexes, from regions spanning the forebrain, midbrain and hindbrain (Jin et al, 2025 Nature). High-resolution clustering and detailed annotation of all cells using the Allen Whole Mouse Brain cell atlas revealed many age-specific clusters in both glial and neuronal cell types.

To further expand on these findings, we have now profiled the full mouse brain using 10X Multiome single-nucleus profiling, which includes both ATAC-seq and RNA-seq measured from the same cells, in young adult (2 months), aged (18 month), and geriatric (24 month) animals. Here, we present initial findings comparing the single-cell RNA-seq dataset to the newly collected single-nucleus RNA-seq and ATAC-seq dataset. We found that the distribution of overlapping regions that were collected show very similar distributions of cell types. In addition, we have found that many regions across the brain show similar transcriptomic changes with age between single-cell and single-nucleus data, but there are also changes in previously unprofiled cell types, such as in the thalamus, that show very distinctive changes with age. Furthermore, we are able to use the chromatin accessibility data to identify regulatory elements that can be used to create cell-type specific viral genetic tools for accessing age-associated cell types. Altogether, this multiomic dataset offers many promising new avenues for identifying novel insights into the molecular mechanisms that contribute to natural aging by identifying cell-type specific gene expression signatures of age, novel cell types that are enriched or depleted with age, changes in gene regulatory networks with age, as well as the potential for developing tools for the community to access and manipulate these cell types.



Session 3
The Disordered Brain

Michelle Monje, MD, PhD

Department of Neuro Oncology with Special Qualifications in Child Neurology, Stanford University, Stanford, California

Pinckney J. Harman Memorial Lecture of the Cajal Club:

Myelin plasticity in health and disease: from cognition to cancer

In the central nervous system, neuronal activity is a critical regulator of development and plasticity. Activity-dependent proliferation of healthy glial progenitors, oligodendrocyte precursor cells (OPCs), and the consequent generation of new oligodendrocytes contributes to adaptive myelination. This plasticity of myelin tunes neural circuit function and contributes to healthy cognition, while disruption of myelin plasticity contributes to cognitive impairment in a range of disease states. The robust mitogenic effect of neuronal activity on normal oligodendroglial precursor cells, a putative cellular origin for many forms of glioma, suggests that dysregulated or "hijacked" mechanisms of myelin plasticity might similarly promote malignant cell proliferation in this devastating group of brain cancers. Indeed, neuronal activity promotes the growth and progression of gliomas. Thus, neuron-glial interactions not only modulate neural circuit structure and function in the healthy brain, but neuron-glioma interactions also play important roles in the pathogenesis of glial cancers. The mechanistic parallels between normal and malignant neuron-glial interactions underscores the extent to which mechanisms of neurodevelopment and plasticity are subverted by malignant gliomas, and the importance of understanding the neuroscience of cancer.

Li-Huei Tsai, PhD

Department of Brain and Cognitive Sciences,
Massachusetts Institute of Technology, Cambridge, Massachusetts

Frequency Matters: Harnessing 40 Hz Stimulation for Alzheimer's Disease and Neuroprotection

Rhythmic neural activity in the gamma range (30–80 Hz) plays a critical role in various cognitive functions and is disrupted in several neurological conditions, including Alzheimer's disease (AD). We developed a novel approach, termed Gamma ENtrainment Using Sensory stimuli (GENUS), which employs patterned light and sound stimulation at 40 Hz in AD model mice to evaluate the effects of enhancing gamma oscillations. Our findings demonstrated that GENUS significantly increased gamma power across multiple brain regions. Moreover, daily application of GENUS led to marked reductions in amyloid and tau pathology, mitigated neuronal and synaptic degeneration, modulated neuroinflammation, restored myelination defects, and improved cognitive performance in multiple AD mouse models. Additionally, we observed that GENUS enhanced arterial vasomotion, aquaporin-4 polarization, and periarterial cerebrospinal fluid (CSF) influx, facilitating brain waste clearance. These effects were mediated by the frequency-dependent release of vasoactive intestinal peptide (VIP) from VIP interneurons, as chemogenetic silencing of these neurons abolished GENUS-induced vasomotion and amyloid clearance. Single-cell transcriptomic analysis revealed that 40 Hz stimulation induced a unique gene expression signature compared to lower or higher frequency stimulations. This signature included pathways associated with synaptic organization, membrane targeting, ATP metabolism, and oxidative phosphorylation. These insights have inspired us to explore the potential of GENUS in addressing other neurological conditions, which I will discuss in my presentation.

Hailan Hu, PhD

School of Brain Science and Brain Medicine,
Zhejiang University, Hangzhou, China

Decoding the Neural Mechanisms of Depression: Insights Through Ketamine's Pharmacological Lens

Depression, a highly polygenic and heterogeneous disorder, has long eluded mechanistic understanding due to the limitations of traditional forward genetic approaches. Here, we propose a complementary strategy: leveraging the rapid, targeted action of ketamine—a potent NMDA receptor (NMDAR) antagonist with robust antidepressant effects—to reverse-engineer the primary neural mechanisms underlying depression. By dissecting how ketamine acutely disrupts pathological circuitry, we aim to bypass indirect downstream effects and pinpoint core drivers of the disease. Our recent work demonstrates that ketamine silences NMDAR-dependent burst firing in the lateral habenula (LHb), the brain's "anti-reward" hub. In depressive-like states, LHb hyperactivity suppresses downstream aminergic reward circuits, perpetuating anhedonia and emotional dysregulation. Ketamine's rapid antidepressant action arises from its ability to suppress this hyperactivity, disinhibiting reward pathways within minutes. Furthermore, we identified that ketamine's sustained efficacy stems from a trapping blockade of LHb-NMDARs—a pharmacological mechanism that prolongs receptor inhibition even after drug clearance. Finally, ketamine's brain-region specificity is mediated by use-dependent NMDAR inhibition, selectively targeting hyperactive LHb neurons while sparing baseline activity in other regions. Collectively, by mapping ketamine's site-specific modulation of cellular and circuit dynamics, we uncover a unified framework linking NMDAR dysfunction to depression etiology and treatment. Building on this, our recent work extends this framework by unraveling a behavioral-state-dependent function of ketamine. Furthermore, I will address an emerging question in the field: Do ketamine and psilocybin—two chemically distinct rapid-acting antidepressants—converge on shared cellular and circuit-level substrates to alleviate depression?

Guoping Feng, PhD

Yang Tan Collective and McGovern Institute for Brain Research
Massachusetts Institute of Technology, Cambridge, Massachusetts

Developing therapeutic approaches for ASD

Large-scale human genetic studies have identified a large number of risk genes for autism spectrum disorder (ASD), many of which encode synaptic proteins, suggesting that synaptic dysfunction is a key pathology in ASD. Using a variety of animal models, we have identified distinct synaptic and circuitry mechanisms related to repetitive behaviors, social interaction deficits, sensory abnormalities, attention deficit and sleep disruption. Combining single cell transcriptomic analysis and cell type-specific functional manipulation, we have begun to reveal circuit-specific targets for developing potential treatment for some of the debilitating symptoms. In addition, new genome editing technologies allow us to generate non-human primate models and test gene therapy as an effective treatment for monogenic ASD.

Xiangmin Xu, PhD

Department of Anatomy and Neurobiology

University of California Irvine, Irvine, California

Single-Cell and Spatial Multi-Omics Mapping of the Human Hippocampus and Basal Ganglia

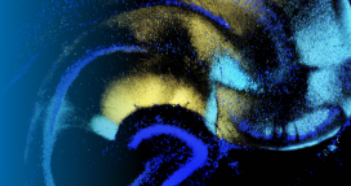
Understanding the identities and spatial organization of brain cell types is critical for linking molecular, physiological, and circuit-level data. Single-cell and spatial transcriptomics, chromatin accessibility profiling, and DNA methylation assays enable unbiased cell-type identification, while multi-modal integration reveals novel subtypes and organizational principles. In the first part of this talk, I will present our work on **epigenetic and 3D genome reprogramming during human hippocampal aging.** Using single-nucleus gene expression, chromatin accessibility, DNA methylation, and 3D genome datasets from 40 postmortem human hippocampal samples spanning the adult lifespan, we observed a marked decline in astrocytes and OPCs and identified microglial transitions to a primed inflammatory state and erosion of higher-order chromatin structure. These changes may contribute to age-related cognitive decline. In the second part, I will discuss **spatial transcriptomic mapping of the human basal ganglia** using Stereo-seq and MERFISH+. Profiling up to 1.5 million cells per experiment using five donor samples, we generated single-cell–resolution maps of over 58 transcriptionally defined clusters across basal ganglia subdivisions. D1 and D2 MSNs mapped to striosome, matrix, and ventral striatum compartments, with spatial community analysis revealing three distinct cellular modules. These large-scale tissue architectures correlated with gene expression gradients, linking molecular identity to mesoscale structural organization. Together, these studies provide a multi-omic, spatially resolved view of human brain cell-type diversity and architecture, offering new insights into lifespan changes and potential mechanisms underlying neurodegenerative and neuropsychiatric disorders.

Yves De Koninck, PhD

Department of Psychiatry & Neuroscience
Université Laval and CERVO Brain Research Centre, Québec, Canada

Chloride dysregulation in disorders of the brain; from chronic pain to neurodegenerative diseases

I will present work we have pursued to identify key mechanisms explaining aberrant pain processing by the nervous system as well as co-morbidities that develop from sustained pain hypersensitivity. This includes the discovery of impaired inhibition resulting from chloride dysregulation in neuropathic pain conditions, leading to cross-talk between sensory channels and ectopic activity possibly underlying spontaneous pain. I will illustrate how such discovery opens new perspectives to understand abnormal pain and how it affects our thinking for therapeutic design. Disrupted chloride homeostasis is now emerging as a common pathway to several brain disorders, including transition to addiction, and maladaptive responses to opioid treatment. I will conclude on recent evidence that chloride dysregulation can also be a key target for prevention, and perhaps reversal of neurodegenerative disorders, in particular ALS and Alzheimer's disease.



Session 4
The Learning Brain

Karel Svoboda, PhD

Department of Neural Dynamics,
Allen Institute for Neural Dynamics, Seattle, Washington

Illuminating synaptic learning rules

How do synapses in the middle of the brain know how to adjust their weight to advance a behavioral goal (i.e. learning)? This is referred to as the synaptic 'credit assignment problem'. A large variety of synaptic learning rules have been proposed, mainly in the context of artificial neural networks. The most powerful learning rules (e.g. back-propagation of error) are thought to be biologically implausible, whereas widely studied biological learning rules (Hebbian) are insufficient for goal-directed learning. I will report ongoing work, both experimental and theoretical, focused on understanding synaptic learning rules and credit assignment in the cortex.

Elizabeth Buffalo, PhD

Department of Neurobiology and Biophysics,
University of Washington, Seattle, Washington

Dynamic Modulation of the Hippocampal Code During Learning

Our understanding of the hippocampus has been framed by two landmark discoveries: the discovery by Scoville and Milner that hippocampal damage causes profound and persistent amnesia and the discovery by O'Keefe of hippocampal place cells in rodents. While a large body of rigorous research in rodents supports the premise that hippocampal neurons encode allocentric location, i.e., hippocampal place cells, the link between hippocampal spatial representations and memory formation is unclear. Accumulating research in rodents suggests that hippocampal place fields are not static but can be modulated by experience in that they show clustering around behaviorally salient locations and demonstrate behavioral state dependency. Work from several groups, including previous and ongoing work in our lab, demonstrates that hippocampal neurons in monkeys show complex selectivity that can be modulated by task conditions. I will discuss experiments that have examined neural activity in the hippocampus as monkeys learn new complex associations in a virtual environment. Data from these studies demonstrate that behavioral task structure has a significant influence on hippocampal activity, potentially providing a neural instantiation of a cognitive map that extends to nonspatial domains and serves as an important scaffold for memory formation.

Bernardo Sabatini, MD, PhD

Howard Hughes Medical Institute,
Harvard Medical School, Cambridge, Massachusetts

Phasic and tonic dopamine in behavioral adaptation

Animals adjust their behavior based on feedback in order to be able to adapt to and thrive in changing environments. Dopamine signaling, particularly within the basal ganglia, is essential to such outcome-dependent learning. Dopamine is typically thought to signal in two different ways consisting of slow changes in low levels of basal or "tonic" dopamine and fast "phasic" transients of high amplitude that are typically associated with important behavioral events. Here we discuss novel technology that allows us to measure and manipulate both components of dopamine signaling in real time in mice performing a variety of tasks and behaviors. We describe the relationship between changes in phasic and tonic dopamine induced by internal state and behavioral outcomes as well as how these impact signaling between and within striatal projection neurons. These results reveal previously unknown modulation of tonic dopamine levels that, we propose, dramatically alters the response to phasic dopamine transients.

Nelson Spruston, PhD

Janelia Research Campus,
Howard Hughes Medical Institute, Ashburn, Virgina

What can we learn about memory by watching it form in the hippocampus?

A major goal of neuroscience is to determine the neurobiological mechanisms responsible for learning and memory. I will describe work from the lab using cellular resolution imaging from thousands of neurons in the mouse hippocampus to study the formation of a new cognitive map during learning. The data provide insight into the dynamics of hippocampal neurons as mice learn to efficiently collect rewards in two subtly different environments. As mice learned the task, we found that hippocampal neurons developed distinct responses to identical sensory stimuli presented in the two contexts, thus producing a neural representation of latent task demands. As a population, hippocampal neurons construct a Hidden Markov Model of the environment, which has properties of a Clone-Structured Causal Graph. I will also describe strategies we intend to pursue to identify where and how synaptic plasticity contributes to this algorithmic representation of the environment in the hippocampus.

Rózsa Balázs, MD, PhD

Janelia Research Campus,

Brain Vision Center, Budapest, Hungary

Real-Time 3D Imaging and Photostimulation in Freely Moving Animals: A Novel Approach Using Robotic Acousto-Optical Microscopy

Our long-term goal is to explore the feasibility of creating a visual prosthetic using direct 3D cortical photostimulation. To achieve this aim, developing a robust and reliable behavioral protocol is just as crucial as advancing imaging technology. Current solutions either offer excellent optical quality but limit animal motion, causing significant stress that disturbs behavioral results and reliability, or they allow free movement but with limited optical quality. Beyond visual prosthetics development, there is a growing demand from researchers, pharmaceutical companies, and biotech firms to test their pharmacological and gene therapy products, as well as innovative therapeutic and diagnostic tools, in freely moving animals. While several advanced head-mounted microscopes with one-, two-, and even three-photon excitation (such as light field microscopy, Mini2p-Scope, and Inscopix's Miniscope) offer good imaging capabilities in freely moving animals, they face challenges due to their small scanners and the limited number and diameter of lenses in their objectives, compared to full-sized microscopes, which are too heavy for animals to carry. These limitations result in smaller fields of view (FOV), lower numerical aperture (NA), reduced resolution and detection efficiency, limited fast z-scanning range, and slower scanning speed.

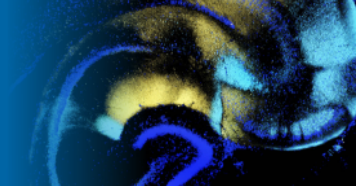
3D acousto-optical (AO) microscopy can address these technical challenges by increasing the product of detection efficiency and measurement speed by six orders of magnitude. However, conventional 3D AO microscopes weigh half a ton, making them impractical for use with freely moving animals. In this talk we will present a unique 3D AO microscope featuring a flexible objective arm with six plus one degrees of freedom, moved by a 6D robotic arm. This system can track freely moving animals with minimal force (1-10 mN) corresponding to only 0.1-1 gram. Our fast, closed-loop, real-time motion correction method, based on local intelligence, effectively eliminates optical errors caused by the deformation of the long objective arm during motion, as well as motion artifacts resulting from breathing, heartbeat, and physical movement of the mice. This cost-effective solution enables high-speed (100 kHz/ROI), real-time, motion-corrected 3D imaging with subcellular resolution, across large scanning volumes. This allows for unique 3D measurement modes such as accelerated learning and behavioral experiments, as well as simultaneous fast 3D voltage imaging and photostimulation of somata, dendrites and spines in full cortical columns in freely moving configurations. By combining the advantages of high-quality imaging with unrestricted animal movement, our system offers a promising approach for advancing visual prosthetics research and meeting the growing demands of the biomedical research community.

Lomax Boyd, PhD

Johns Hopkins Berman Institute of Bioethics,
The Rockefeller University, New York, New York

Down-regulation of PLXNA1 induces formation of cortical projections and vocal behaviors associated with the evolution of vocal learning

Several hypotheses have been proposed on the anatomical brain differences that endow some species with the rare ability of vocal learning, a critical component of spoken language. One long-standing hypothesis is the role of direct cortical projections from motor cortex layer 5 neurons to brainstem vocal motor neurons that control fine motor movement of laryngeal musculature. Cortical projections to vocal motor neurons have been reported in vocal learning songbirds and humans but appear to be absent or sparse in closely related vocal non-learning species, such as primates and rodents. The elaboration of this connection in vocal learning species is theorized to provide animals with enhanced behavioral plasticity during social interactions. While the mechanism(s) that give rise to these cortical projections remain unknown, along with their impact on vocal communication, previous comparative gene expression studies have hypothesized that specialized expression of axon guidance genes in layer 5 neurons of human speech motor cortex, and the equivalent songbird neurons of the robust nucleus of the arcopallium, are essential for cortical axons to innervate vocal motor regions in the brainstem. Here we generated mice with layer-specific knockdown of the axon-guidance receptor, PLXNA1, in motor cortex layer 5 neurons, to recapitulate the convergent human and songbird expression patterns reported in speech and song brain regions. We provide neuroanatomical and electrophysiological evidence that PLXNA1 layer 5 mutant mice possess enhanced cortical projections to brainstem vocal motor neurons, consistent with the pattern observed in vocal learners, along with decreased latencies in muscular contractions after electrical stimulation of the primary motor cortex. We also recorded mouse ultrasonic vocalizations across a variety of social contexts and found that song bouts of male mice with reduced PLXNA1 expression exhibit increased vocal plasticity, including context-specific changes in acoustic variability and increased informational encoding, compared to wildtype control mice. Our findings reveal the impact of direct cortical projections on vocal communication behavior in a vocal non-learning species. These data support the hypothesis that formation of direct cortical projections during the evolution of vocal learning expanded the plasticity of acoustic and informational features of social communications.



Session 5
The Dynamic Brain

Liqun Luo, PhD

Howard Hughes Medical Institute

Department of Biology, Stanford University, Stanford, California

Deconstructing the Serotonin System in the Mouse Brain

Serotonin powerfully modulate physiology and behavior in health and disease. In the mammalian brain, serotonin neurons are clustered in the raphe nuclei in the brainstem, but their axons innervate the entire brain. Our previous studies suggested that serotonin neurons likely comprise parallel subsystems with distinct transcriptomic features, projection patterns, input biases, physiological response properties, and behavioral functions (Ren et al., 2018; Ren et al., 2019). Building on these findings, I will describe two unpublished stories on (1) the architecture of serotonin projectome in the entire mouse brain; (2) modulation of female social behavior by projection-specific serotonin neurons

Catherine Dulac, PhD

Howard Hughes Medical Institute,
Department of Molecular and Cellular Biology,
Harvard University, Cambridge, Massachusetts

The Neurobiology of Sickness

Social interactions are essential for animals to survive, reproduce, raise their young. Over the years, my lab has attempted to decipher the unique characteristics of social recognition: what are the unique cues that trigger distinct social behaviors, what is the nature and identity of social behavior circuits, how is the function of these circuits different in males and females and how are they modulated by the animal physiological status? Social behavior is highly affected in sick animals and in this lecture I will describe our recent progress in understanding how specific brain circuits and cell types direct adaptive changes in behavior during various forms of sickness in mice, providing a new framework to understand the function and modulation of behavior circuits in health and disease.

Anne Churchland, PhD

Department of Neurobiology,
University of California Los Angeles, Los Angeles, California

Movements and engagement in decision-making

Switching between cognitive states is a natural tendency, even for trained experts. To test how cognitive state impacts the relationship between neural activity and behavior, we measured cortex-wide neural activity during decision-making in mice. Task variables and instructed movements elicited similar neural responses regardless of state, but the neural activity associated with spontaneous, uninstructed movements became highly variable during disengagement. Surprisingly, this heightened variability was not due to an increase in movements: behavioral videos showed similar motion energy in both cognitive states. But while overall movement amount remained similar, movement alignment changed: as animals slipped into disengagement, their movements became erratically timed. These idiosyncratic movements were a strong predictor of task performance and drove the increased variance that we observed in the neural activity. Taken together, our results argue that the temporal structure of movement patterns constitutes an embodied signature of cognitive state with profound impacts on neural activity

Edward Chang, MD

Department of Neurological Surgery
University of California San Francisco, San Francisco, California

Shared and language-specific phonological processing in the human temporal lobe

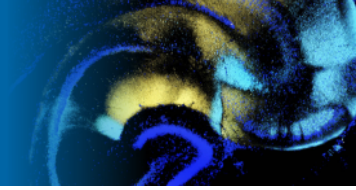
All spoken languages are produced by the human vocal tract, constraining the set of possible speech sounds. Despite this constraint, however, there exists incredible diversity in the world's 7000 spoken languages, each of which are learned through extensive experience hearing speech in language-specific contexts. It remains unknown which elements of speech processing in the brain depend on daily language experience and which do not. In this study, we recorded high-density cortical activity from adult participants with diverse language backgrounds as they listened to speech in their native language and an unfamiliar foreign language. We found that, regardless of language experience, both native and foreign languages elicited similar cortical responses in the superior temporal gyrus (STG), associated with shared acoustic-phonetic processing of foundational speech sound features, such as vowels and consonants. However, only during native language listening did we observe enhanced neural encoding of word boundaries, word frequency, and language-specific sound sequence statistics in the STG. In a separate cohort of bilingual participants, this encoding of word and sequence level information appeared for both known languages within the same individual and within the same STG regions. These results suggest that experience-dependent language processing involves dynamic integration of both shared acoustic-phonetic and language-specific sequence and word level information in local STG neural populations.

Kuan Hong Wang, PhD

Department of Neuroscience,
University of Rochester Medical Center, Rochester, New York

Dopaminergic Signaling Regulates Microglial Surveillance and Adolescent Plasticity in the Frontal Cortex

Adolescence is a sensitive period for frontal cortical development and cognitive maturation, marked by heightened structural plasticity in the dopaminergic (DA) mesofrontal circuit. However, the cellular and molecular mechanisms underlying this plasticity remain unclear. Here, we show that microglia, the brain's innate immune cells, are highly responsive to mesofrontal DA signaling during adolescence. Longitudinal in vivo two-photon imaging in mice reveals that frontal cortical microglia increase their surveillance of the parenchyma and DA axonal boutons following rewarding experiences or optogenetic stimulation of DA axons. Microglial contacts with DA axons consistently precede bouton formation, and microglia-bouton interactions are regulated by D1- and D2-type DA receptors in adolescence and adulthood. Furthermore, microglial purinergic receptor P2RY12 signaling is essential for enhanced microglial surveillance and DA bouton formation during adolescence. These results uncover bidirectional interactions between DA signaling and microglial surveillance that drive adolescent frontal plasticity and identify potential targets for restoring plasticity in adulthood.



Session 6
State of the Brain

Yang Dan, PhD

Howard Hughes Medical Institute
Departments of Molecular and Cellular Biology
University of California Berkeley, Berkeley, California

The how and why of sleep

Sleep is a fundamental biological process, and its disruption has profound impacts on human health. To identify neurons involved in sleep generation, we have performed whole-brain screening for sleep active and sleep promoting neurons, using a combination of optogenetics, electrophysiology, imaging, and gene expression profiling. We found that sleep is controlled by a highly distributed network spanning the forebrain, midbrain, and hindbrain, and the sleep neurons are part of the central somatic and autonomic motor circuits. This is summarized in a "motor theory of sleep control". To address the "why" question, 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.

Ishmail Abdus-Saboor, PhD

Howard Hughes Medical Institute
Department of Biological Sciences,
Columbia University, New York City, New York

Skin-brain axis for tactile sensations: from mice to naked mole-rats

Work in the Abdus-Saboor lab is integrating the peripheral and central nervous systems, seeking to uncover genes and neural circuits for somatosensation from the skin to the spinal cord and interconnected networks across the brain. We are elucidating the "skin-brain axis" – taking a wholistic approach that combines high-resolution behavioral mapping, brain imaging, and neural circuit manipulations. This talk will be divided into two parts, featuring discoveries and unpublished work from the Abdus-Saboor lab including 1) identification of a skin-brain neuronal pathway in mice for rewarding social touch, and 2) the role of social touch and social memory in naked mole-rat colonies.

Zhigang He, PhD, BM

Boston Children’s Hospital, Boston, Massachusetts

Organization and regeneration of brain-spinal connections

Descending pathways, composed of spinal projecting neurons (SPNs) that extend directly from various brain regions to the spinal cord, translate brain commands ("thoughts") into bodily behaviors ("actions"). The critical role of SPNs is evident in the functional deficits observed after spinal cord injury and related diseases. However, studying SPNs poses a significant challenge due to their sparse distribution across multiple brain regions, making selective labeling and manipulation difficult. To address this, we have optimized a suite of retrograde AAV-based techniques to label and manipulate SPNs effectively. First, using these methods to introduce fluorescent proteins into SPNs, we collaborated with Hongkui Zeng at the Allen Institute to create a transcriptomic atlas of brain-wide SPNs. Second, we employed channelrhodopsin-2 (ChR2) to perform functional mapping, identifying SPN populations capable of activating motor and/or sympathetic functions in the spinal cord. Ongoing studies are beginning to uncover their specific physiological roles. Third, leveraging the molecular and functional insights gained, we have started designing rational pro-regenerative strategies for spinal cord injury recovery.

Kei Igarashi, PhD

Department of Anatomy and Neurobiology
University of California Irvine, Irvine, California

Circuit plasticity of prefrontal and entorhinal neurons during olfactory learning

Memory has multiple components: “what” memory (item/object), “when” memory (time) and “where” memory (space). Research in the past decades revealed neurons involved in spatial memory, including place cells in the hippocampus and grid cells in the medial entorhinal cortex (MEC). However, circuit mechanisms of memory about item and time remain largely unclear. Our lab focuses on identifying mechanisms for item memory using olfactory cues, and how these circuits become impaired in the disease of memory – Alzheimer’s disease. We previously reported the encoding of item-outcome associative memory by layer 2a neurons of the lateral entorhinal cortex (LEC), and this encoding is controlled by dopamine signals from the ventral tegmental area (Lee et al., Nature, 2021). We recently found that neuronal populations of both the LEC (layer 5/6) and their major target, the medial prefrontal cortex, formed an internal map of pre-learned and novel items, classified into dichotomic rewarded vs. punished groups (Jun et al., Nature 2024). The formation of this internal map was mutually dependent. Our result suggests that the LEC and mPFC encodes a cognitive map of item-outcome rules. Dopamine in the LEC becomes dysfunctional in Alzheimer’s disease mouse models (Nakagawa et al., bioRxiv 2024), which suggests the critical role of dopamine in Alzheimer’s disease.

Cheng Lyu, PhD

Department of Biology
Stanford University, Stanford, California

Rewiring an olfactory circuit by altering the combinatorial code of cell-surface proteins

Proper brain function requires the precise assembly of neural circuits during development. Despite the identification of many cell-surface proteins (CSPs) that help guide axons to their targets, it remains largely unknown how multiple CSPs work together to assemble a functional circuit. Here, we used synaptic partner matching in the Drosophila olfactory circuit to address this question. By systematically altering the combination of differentially expressed CSPs in a single olfactory receptor neuron (ORN) type, which senses a male pheromone that inhibits male-male courtship, we switched its connection from its endogenous postsynaptic projection neuron (PN) type nearly completely to a new PN type that promotes courtship. To achieve this switch, we deduced a combinatorial code including CSPs that mediate both attractive and repulsive interactions between synaptic partners. The anatomical switch changed the odor response of the new PN partner and markedly increased male-male courtship. We generalized three manipulation strategies from this rewiring to successfully rewire a second ORN type to multiple distinct PN types. This work demonstrates that manipulating a small set of CSPs is sufficient to respecify synaptic connections, paving ways to explore how neural systems evolve through changes of circuit connectivity.

John Ngai, PhD

National Institute of Health, BRAIN Initiative, Bethesda, Maryland

The NIH BRAIN Initiative: A Decade+ of Innovation

The mission of the NIH Brain Research Through Advancing Innovative Neurotechnologies® (BRAIN) Initiative is to revolutionize our understanding of the human brain and to apply this knowledge toward finding cures for devastating human brain disorders. Since its launch in 2013, the BRAIN Initiative has supported the development of paradigm-shifting tools to map, monitor, and modulate neural circuits, accelerating the pace of discovery and providing new opportunities for neuroscience inquiry. In my presentation, I will highlight the progress made by BRAIN Initiative-funded investigators and investigative teams in generating comprehensive brain cell atlases, whole brain connectivity maps, tools for precision brain cell access, paradigms for linking behavior with neural circuit function, and new insights into neural circuit function in humans. I will also discuss how investments in fundamental research and technology development are opening the doors for future breakthroughs and enabling remarkable clinical advances in treating neurologic and neuropsychiatric disorders.

Poster Abstracts

Day 1 - August 18
Room A: Circuit Mapping & Neural Connectivity
Room B: Glial Function & Inflammation
Neuroscience Tools & Techniques
Room C: Single-cell and Multi-omics Applications

Day 2 - August 19
Room D: Neurodegenerative and Mental Disorders & Other Topics
Room E: Behavior, Cognition & Perception
Room F: Development of the Central Nervous System

Poster #A1: (S. Bhamidipati et al.)

Rewiring the inhibitory map: A transcriptomic blueprint of disinhibitory circuit logic

Sai Krishna Bhamidipati[1,2], Amanda Chambers[1], Edward Callaway[1,*]

[1]Systems Neurobiology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA; [2]School of Biological Sciences, University of California, San Diego, La Jolla, CA

Single-cell transcriptomic surveys have revealed dozens of inhibitory neuron subtypes in the mouse cortex—far more than the traditional PV, SST, and VIP classes—challenging long-held models of cortical inhibition. Among these, disinhibitory motifs—circuits in which inhibitory neurons suppress other inhibitory neurons—play key roles in sensory processing and cognitive flexibility. Yet these motifs remain poorly defined at cell-type resolution, and prior studies using broad Cre-lines have produced inconsistent results, likely due to unrecognized transcriptomic heterogeneity.

To address this, we apply Single Transcriptome-Assisted Rabies Tracing (START), a method developed in our lab that combines monosynaptic rabies tracing with single-nucleus RNA sequencing. START enables us to map local synaptic inputs to PV+, SST+, VIP+, and 5HT3aR+ inhibitory neurons in the mouse visual cortex (V1) while assigning each input neuron to a defined transcriptomic type using the Allen Institute's cell type taxonomy.

This project will generate the first comprehensive map of inhibitory-to-inhibitory connectivity at transcriptomic resolution. It will test, for instance, whether specific SST or VIP subtypes preferentially avoid certain excitatory targets and instead form precise disinhibitory circuits—motifs invisible to class-level genetic tools. These data promise to resolve long-standing inconsistencies in the field and offer a roadmap for building enhancer-based genetic tools targeting functionally distinct inhibitory subtypes.

By uncovering how inhibitory diversity shapes cortical circuit logic, this work redefines our understanding of cortical computation—and lays the groundwork for more precise interventions in brain disorders where disinhibition is disrupted.

Poster #A2: (K. Ishii et al.)

Brain-wide mapping of neural ensembles during morphine exposure using a novel error rate and testing strategy

Kentaro K Ishii[1,2], Daniel Kessler[4,5], Garret D. Stuber[1,2,3]

[1]Center for the Neurobiology of Exposure, Pain, and Emotion, University of Washington, Seattle, WA, [2]Department of Anesthesiology and Pain Medicine, University of Washington, Seattle, WA, [3]Department of Pharmacology, University of Washington, Seattle, WA, [4]Department of Statistics & Operations Research, University of North Carolina, Chapel Hill, NC, [5]School of Data Science and Society, University of North Carolina, Chapel Hill, NC

Opioid use disorder is a global public health crisis, with prescription opioid misuse and overdose-related deaths rising steadily over the past decade. To further develop treatment for opioid addiction, it is empirical to understand how opioids systematically impact the brain. Here, we employ an unbiased approach to map brain-wide neural ensembles activated during morphine exposure and withdrawal. Mouse brain tissue from animals that were exposed to acute or chronic morphine, or animals that were withdrawn from morphine after chronic exposure were collected and stained for immediately early gene c-Fos. Brain samples were then cleared to image under a light-sheet microscope, which allowed high-resolution, three-dimensional visualization of morphine-induced neural activity across the entire brain.

To analyze neural activation patterns, we quantified c-Fos+ cell density across >1200 brain regions and applied a novel error-rate correction and statistical testing strategy (TreeBH) to gain more power after multiple comparisons compared to alternative methods. This methodology effectively controls error rates for hierarchical tree data, such as the mouse brain dataset, allowing us to extract statistically meaningful results without diluting their significance. We first found that the acute and chronic administration of morphine induces similar neural activity patterns in comparison to withdrawal. Hierarchical clustering of brain regions by their response to each drug condition revealed two major clusters. One contained prefrontal cortical regions and ventral striatal regions that strongly responded to acute morphine conditions. Another cluster contained amygdala/extended-amygdala regions including the central amygdala and the bed nucleus of stria terminalis. These regions responded strongly to withdrawal and acute, but not in chronic morphine. Importantly, brain regions in these two clusters are known to regulate reward and fear processing respectively. Collectively, we consider that our brain-wide analysis revealed the key brain nodes that encode positive and negative valence associated with the early and late morphine exposure.

Poster #A3: (D. Zhang et al.)

Ventral hippocampal CA1 interneurons circuits revealed by monosynaptic rabies virus

Qiao Ye[1], Claire Lee[1], **Daniel Zhang[1]**, Zijing Wang[1], Xiangmin Xu[12*]

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Approximately 301 million people in the world suffer from anxiety and fear-related disorders. Different states of anxiety and fear trigger subsequent behaviors that could save a person's life or induce severe negative mental health implications. Consequently, anxiety and fear serve as important emotions regulated by important neural circuitry. The ventral hippocampal CA1 (vCA1) has been implicated in playing critical roles in long range projections responsible for anxiety and fear-inducing stimuli. Many populations of interneurons exist within vCA1, including populations expressing parvalbumin (PV), thought to exhibit anxiety behavior, and somatostatin (SOM), thought to exhibit fear behavior. Utilizing monosynaptic rabies tracing in distinct PV and SOM-expressing transgenic mouse breeds, we visualize presynaptic neural circuits of PV and SOM interneuron populations of vCA1 to understand neural circuitry differences in distinct interneuron types. Following injections, histological and data quantification processes, pre synaptic regions projections to ventral CA1 are shown to exhibit different patterns of expression which could contribute to distinct functions. As such, this project aims to identify novel characteristics of presynaptic neural circuits in distinct PV and SOM interneuron types of the vCA1 region to possibly further understand the pathology behind anxiety and fear regulation and recognition.

Poster #A4: (Y. Xiao et al.)

Multi-Modal Spatial Cellular Taxonomy of Human Hippocampus Reveals Region-Specific Activity and States

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Using proteomic, transcriptomic, and epigenomic analyses of neurotypical humans and subjects with major depressive disorder (MDD), we created the first spatially resolved multi-omic atlas of the adult human hippocampus. Through spatial proteomic and transcriptomic profiling, we identified canonical hippocampal subfields and distinct cell types within the dentate gyrus and cornu Ammonis. Comparison of healthy brains and those of subjects with MDD revealed region-specific differences in gene expression and biological pathway activation. RNA synthesis rates were more variable in MDD brains than in healthy brains, and RNA velocity analysis suggested that transcript instability may contribute to significant inter-patient heterogeneity, especially within the pyramidal neuron cluster. Spatial ATAC-seq demonstrated variations in open chromatin across hippocampal subfields, which likely influence neuronal excitability and memory formation. Our atlas provides insights into the molecular neuropathology of the hippocampus in both healthy and diseased states.

Poster #A5: (S. Srinivasan et al.)

A conserved and scalable architecture in cerebellar and cerebellar-like circuits

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The architecture of the cerebellum is conserved across myriad species, suggesting that it may function as a scalable circuit – one in which numbers of granule and Purkinje cells grow with brain size while maintaining a consistent relationship. Such scalable architectures are evolutionarily advantageous as they provide a way to preserve optimal circuit design and link performance to circuit size. The recurrence of the cerebellar motif in other brain regions, known as cerebellar-like circuits, underscores its effectiveness and suggests conserved computations. We present three lines of evidence that various cerebellar motifs are scalable and implement similar computations. First, across a wide range of mammals, the number of granule cells scales with the number of Purkinje cells as a power law. This scalability even extends to cerebellar subdivisions, mouse mutants, and other cell types such as Golgi cells. Second, examination of the optic tectum, a cerebellar-like circuit, in two species of teleost fish, shows that the optic tectum is constrained by the same scaling law. Third, using theory, we show that this scaling enables conserved computations across species while preserving information flow. Together, these findings suggest that evolution has adapted cerebellar motifs to diverse ecological niches by tuning the size of the circuit.

Poster #A6: (S. Sargolzaei et al.)

Longitudinal Analysis of Brain Functional Connectivity Network Trajectories Following Lateral Fluid Percussion Injury in Immature Rats: A Graph Theoretical Approach

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Traumatic brain injury (TBI) in pediatric populations presents unique challenges due to the rapid and complex nature of brain development. Understanding TBI's impact on pediatric brain function and structure necessitates advanced modeling techniques that can capture the intricacies of brain network alterations. Our prior study explored functional connectivity network (FCN) changes following mild to moderate lateral fluid percussion injury (lFPI) in immature rats, using functional magnetic resonance imaging (fMRI). We observed region-dependent changes in voxel-wise connectivity, with major global hypo-connectivity and localized hyper-connectivity, particularly in the hippocampus contralateral to the injury site. These findings highlighted how TBI disrupts brain connectivity development, underscoring the need for further investigation into topological variations in brain networks over time.

Building upon these results, the present study employs graph theoretical analysis to deepen our understanding of the evolving nature of brain functional connectivity following pediatric TBI. Specifically, we focus on how developmental trajectories and injury-induced disruptions in brain FCNs manifest over time in a rodent model of lFPI, with data collected at two critical time points (PID04 and PID14). Graph theory offers a powerful framework for studying brain network topology by quantifying features such as nodal strength, path length, clustering coefficient, and global efficiency. These features are essential for understanding how brain networks maintain their functional organization and adapt to both developmental processes and injury. Disruptions in these topological measures may provide key insights into how TBI alters brain network resilience and recovery.
Using a model-based approach, we construct FCNs and rigorously test correlations across time points. By capturing the dynamic changes in functional connectivity, we aim to uncover how TBI alters the normal trajectory of brain network development and how these changes may inform future therapeutic strategies. The longitudinal nature of this study allows for an in-depth exploration of how injury impacts both local and global network features over time, providing a comprehensive view of how functional connectivity evolves in response to pediatric TBI.

Ultimately, this study highlights the importance of graph theory in the analysis of brain connectivity, offering new insights into the topological disruptions caused by TBI and their potential impact on brain function. Our findings may help inform the development of targeted interventions to mitigate the long-term effects of pediatric TBI on brain networks and cognitive function.

Poster #A7: (Z. Yang et al.)

ERK Signaling Drives Evolutionary Expansion of the Cerebral Cortex

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The cerebral cortex, the seat of human intelligence, underpins our exceptional cognitive abilities. Deciphering the mechanisms governing the development of the uniquely large human cerebral cortex is crucial for understanding what distinguishes the human brain and species. Throughout primate evolution, a significant increase in the number of neurons in the cerebral cortex, particularly in the neocortex, has been observed. Remarkably, the human cerebral cortex contains approximately 16.3 billion neurons, the highest number among species. Here, we demonstrate that extracellular signal-regulated kinase (ERK) signaling promotes the self-renewal and expansion of cortical radial glial (RG) cells. Additionally, ERK signaling induces bone morphogenic protein 7 (Bmp7) expression in cortical RG cells, thereby extending the neurogenic period. We reveal a mutual inhibitory relationship between ERK signaling and Sonic Hedgehog (SHH) signaling in cortical RG cells. Furthermore, we provide evidence that ERK signaling is upregulated in cortical RG cells during both development and evolution. We propose that the expansion of the mammalian cortex, particularly in humans, is driven by the ERK signaling pathway in cortical RG cells, which engages in a positive feedback loop by antagonizing SHH signaling. Genetic evidence further indicates that ERK, PKA, YAP, and SHH signaling coordinately regulate the generation of all cell types in the cerebral cortex. In summary, we have identified a unifying principle that drives cortical expansion and evolution.

Poster #A8: (G. Vargova et al.)

Contribution of brainstem and cortical input to the computation of the auditory topographic map in the mouse superior colliculus

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Sound localization is crucial for interpreting the external environment, a skill many species rely on for survival. The superior colliculus (SC) is a midbrain region that contains a topographic map of sound space that is aligned and integrated with visual and somatosensory maps of space, forming a spatial representation of the external world and mediating sensory-driven behaviors. Given its central role in sensory processing and attention, dysfunction of SC circuitry has been linked to neurological disorders such as autism, schizophrenia, and ADHD. Unlike the visual map which is directly inherited from the retina, the auditory map must be computed from distinct brainstem nuclei that process interaural level differences (ILDs, the level of a sound arriving at each ear), interaural timing differences (ITDs, the arrival of sound at the two ears), and spectral cues (within ear frequency contrast) of the sound as it enters the ear. Our previous work showed that in the mouse SC auditory neurons use spectral cues and ILDs, but not ITDs, to create spatial receptive fields (RFs). Interestingly, anterior SC neurons with frontal RFs rely predominantly on spectral cues, while posterior neurons with lateral RFs depend on ILDs; neurons in between use a combination of both. What is not known is which brainstem areas contribute to the spatial tuning properties of SC neurons. Here, we investigate how lower brainstem nuclei, including the nucleus of the brachium of the inferior colliculus (NB) and the external cortex of the inferior colliculus (ICe), along with cortical inputs, shape auditory responses in the SC. We show that each of these areas project to the SC but have different response properties to the same stimuli. Our preliminary results reveal at least four distinct classes of auditory responses, each potentially contributing to different aspects of SC topography. Additionally, each brain region exhibits a different response time course, with the ICe responding the fastest.

Poster #A9: (Z. Li et al.)

Repulsive interactions instruct synaptic partner matching in an olfactory circuit

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Neurons exhibit extraordinary precision in selecting synaptic partners. Whereas cell-surface proteins (CSPs) mediating attractive interactions between developing axons and dendrites have been shown to instruct synaptic partner matching, it is less clear the degree to which repulsive interactions play a role. Here, using a genetic screen guided by single cell transcriptomes, we identified three CSP pairs—Toll2–Ptp10D, Fili–Kek1, and Hbs/Sns–Kirre—in mediating repulsive interactions between non-partner olfactory receptor neuron (ORN) axons and projection neuron (PN) dendrites in the developing Drosophila olfactory circuit. Each CSP pair exhibits inverse expression patterns in the select PN-ORN partners. Loss of each CSP in ORNs led to similar synaptic partner matching deficits as the loss of its partner CSP in PNs, and mistargeting phenotypes caused by overexpressing one CSP could be suppressed by loss of its partner CSP. Each CSP pair is also differentially expressed in other brain regions. Together, our data reveal that multiple repulsive CSP pairs work together to ensure precise synaptic partner matching during development by preventing neurons from forming connections with non-cognate partners.

Poster #A10: (X. Hu et al.)

A Spinoparabrachial Circuit for Visceral Pain Identification

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Interoception is the perception of internal states of the body, which can be either nonconscious, like breathing and digestion, or conscious such as feeling intestinal cramps. Despite many studies investigating circuit mechanisms in the spinal dorsal horn (SDH) and brain for sensing external stimuli from the skin, the specific molecules, cells, and circuits responsible for processing interoceptive signals from internal organs along the gut-brain axis are poorly understood. It is also unclear whether visceral interoception shares the same ascending pathways with exteroception in the spinal cord, brainstem, and higher levels of neuraxis. In this study, we revealed that Tacr3+ SDH neurons form monosynaptic contacts with visceral primary afferents and sense visceral pain stimuli. Intersectional genetic ablation or silencing of Tacr3+ SDH neurons inhibits colorectal distension (CRD)-induced VMR, while pharmacogenetic activation of Tacr3+ SDH neurons promotes CRD-induced visceral pain. Moreover, Tacr3+ SDH neurons primarily project to the parabrachial nucleus (PBN), and optogenetic activation of Tacr3+ SDH projecting to PBN terminals is sufficient to induce visceral pain without effecting somatic pain. Collectively, this study revealed a unique population of spinal dorsal horn neurons mediating visceral pain.

Poster #A11: (S. He et al.)

Intrinsic central amygdala developmental trajectories drive valence-specific circuit assembly

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The central nucleus of the amygdala (CeA) is an evolutionarily conserved forebrain structure critical for orchestrating emotional responses. Recent advances have identified transcriptomically and functionally defined cell types within the adult rodent CeA, elucidating their roles in processing opposing valences and mediating both defensive and appetitive behaviors. The CeA consists exclusively of GABAergic neurons that are organized in inhibitory microcircuits in three subdivisions: the central capsular (CeC), lateral (CeL), and medial subdivisions (CeM). Cell types with opposing functions in consummatory behavior are distributed in both CeL and CeM. Little is known about when and where during development functional and spatial identities of neurons emerge, and how the valence-specific functions are established.

Here, we provide a comprehensive transcriptomic roadmap of CeA development across embryonic and postnatal stages in mice. It revealed distinct developmental trajectories for functional, but not spatial identities: one trajectory originating from Sox5-positive precursors, giving rise to aversive neuronal populations in all three subdivisions, and a second trajectory from Htr2a-positive precursors, giving rise to appetitive populations in CeL and CeM. Contrary to expectations, we did not identify separate precursors for the spatially distinct CeA subdivisions. Clonal relationship and gene expression suggest aversive precursors originate from the caudal ganglionic eminences (CGE), while appetitive precursors derive from the lateral ganglionic eminences (LGE).

By using a computational decoder, we screened for gene families that predict valence-specific functions early on during development. Neuropeptide pathways, synaptic plasticity regulators, and axon guidance cues emerged as key determinants that predict functional identities of CeA neurons. Specifically, we identify the semaphorin family to be crucial for establishing valence-specific inputs to the CeA in a cell type-specific and stage-dependent manner. Sema3a and Sema6a are required for the establishment of inputs from multiple cortical regions to the predominantly appetitive CeA neurons marked by expression of somatostatin. Whether or not these changes alter the valence-specific functions of these neurons is currently under investigation.
In summary, our study demonstrates that distinct progenitors intrinsically give rise to valence-specific neurons, and begins to elucidate the molecular logic underpinning valence-specific circuits assembly in the CeA.

In summary, our study demonstrates that distinct progenitors intrinsically give rise to valence-specific neurons, and begins to elucidate the molecular logic underpinning valence-specific circuits assembly in the CeA.

Poster #A12: (M. Metcalfe et al.)

Bioengineered AAVs Enable Targeted Modulation and Real-Time Monitoring of Transgene Activity to Promote Functional Recovery After Spinal Cord Injury

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Spinal cord injury (SCI) results in devastating, often irreversible loss of function due to the failure of damaged axons to regenerate in the adult central nervous system (CNS). A key barrier to effective therapeutic intervention is the challenge of delivering regenerative molecules with both spatial and temporal precision, while also gaining insight into their in vivo activity during recovery. To address these challenges, we developed a multifunctional platform that integrates bioengineered adeno-associated viruses (AAVs) for cell- and region-specific gene delivery, externally regulated expression and a non-invasive bioluminescence imaging (BLI) approach to track transgene activity longitudinally in live animals.

Using a combinatorial approach of rational capsid design and transcriptional control, we used retrogradely transported AAVs (AAV-retro) that selectively transduce neurons in the somatosensory cortex projecting to the injured spinal cord. These vectors carry a short hairpin RNA targeting phosphatase and tensin homolog (PTEN), a known suppressor of the PI3K/AKT/mTOR signaling pathway. Downregulation of PTEN enhances intrinsic axonal growth capacity, making it a powerful strategy to promote regeneration following SCI. However, while permanent PTEN deletion enables significant functional recovery after cervical SCI, it has also been associated with late-onset adverse effects, highlighting the need for refined control over therapeutic gene expression. To address this, we incorporated a doxycycline (Dox)-inducible TetON promoter into the vector, enabling precise temporal regulation and allowing transgene expression to be turned on or off at defined time points during injury and recovery. This level of control is critical for minimizing late-onset adverse effects and aligning therapeutic gene expression with optimal windows for neural repair.

To monitor transgene activity in real-time, the AAV-retro construct co-expresses a luciferase reporter under the same promoter elements as the shPTEN cassette. BLI offers a non-invasive, highly sensitive method for tracking transgene activity over time in the same animal, eliminating the need for terminal endpoints and reducing variability. We utilize AkaLuc, a next-generation luciferase with emission in the near-infrared range, which offers superior tissue penetrance compared to traditional luciferases. This enables reliable detection of signals originating from deep brain structures and allows direct correlation between therapeutic gene expression, regeneration and functional recovery.

In naive mice, intraspinal injection of AAV-retro carrying shPTEN and AkaLuc, followed by Dox (2 mg/mL) administration in drinking water two days later, resulted in robust luminescence in the somatosensory cortex, confirming retrograde transport and successful Dox-dependent transgene activation. Expression was sustained over time and strictly dependent on Dox, with no detectable leakiness in its absence. Furthermore, transgene expression could be successfully turned off by withdrawing Dox and reactivated later, providing a tool to temporally modulate gene expression in response to functional needs during the recovery process.

Together, our work highlights the potential of bioengineered AAVs as both precision delivery tools and dynamic biosensors, enabling spatiotemporally controlled gene expression in the injured CNS. This controllable system supports personalized, phase-specific intervention strategies, offering a versatile platform to modulate and monitor neuroplasticity, investigate the molecular underpinnings of recovery, and optimize regenerative interventions not only for SCI but also for other disorders involving neural circuit disruption.

Poster #A13: (K. Kon et al.)

Hormone-dependent circuit maturation for male sexual behavior

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Social behaviors are innately programmed, yet remarkably flexible across the lifespan. With puberty, "adult-type" social behaviors, including aggressive, sexual, and (allo)parental behaviors, emerge and mature during adolescence concurrent with changing hormones and neural wiring. While significant progress has been made in understanding the neural circuits that control specific innate social behaviors, however, it is still unclear how the neural circuits change depending on their physiological and environmental conditions and which biological factors influence these changes. Gonadal hormones, which increase dramatically in blood levels during adolescence, directly regulate gene expression in the brain and play critical roles in brain function. Deficits in gonadal hormones or their receptors in the brain before puberty cause severe social behavior impairments, suggesting that the brain must be exposed to gonadal hormones during puberty for the proper development of social behaviors. However, the fundamental changes induced by the pubertal gonadal hormones in the functions and/or structures of the neural circuits underlying social behavior development remain unknown. Here, we investigate the neural mechanisms underlying developmental and hormone-dependent changes in male sexual behavior. Through detailed behavioral analysis of developing male mice, we identified distinct trajectories within social behaviors toward female mice. We found that the emergence of male-typical sexual behavior correlates with the physiological pubertal stage and that the absence of pubertal testosterone affects the sexual behavior in adulthood. Through circuit mapping and neural manipulation of gonadal hormone-sensitive neurons, it is supported that functional maturation of the circuit, mediated by the pubertal gonadal hormones, is involved in the emergence of male-typical sexual behavior. These findings highlight the importance of gonadal hormones in proper brain development and provide insight into how the brain controls proper behavioral timing based on physiological state.

Poster #A14: (H. Zhao et al.)

High speed neural projectome imaging of rodent brains with the TESOS2 technical pipeline

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Reconstituting neural circuits from mesoscopic to microscopic scales remains a challenge in neuroscience. Existing technologies like fMOST and MouseLight require customized equipment, limiting accessibility to specialized laboratories. Here, we introduce TESOS2 (Transparent Embedding Solvent System 2), a scalable framework that democratizes whole-brain connectivity mapping using commercially available light-sheet and confocal microscopes.

TESOS2 employs a two-step imaging workflow. First, tissue clearing enables light-sheet imaging of transparent brain samples, capturing axon projection maps across the entire brain. Second, transparent embedding of the same sample allows upright confocal imaging of neuron somas at injection sites, resolving dendritic spines and fine structural details. By integrating these complementary datasets, TESOS2 simultaneously reconstructs single-neuron dendritic spines and axon projections, bridging mesoscale connectivity with microscopic anatomy.

The workflow includes tissue clearing, polymerization, and sequential imaging, ensuring high reproducibility and data fidelity. Compatible with murine and rat brain tissues, TESOS2 eliminates the need for custom machinery, reducing technical and financial barriers. This approach enables multiscale morphological analysis within a single sample, advancing mechanistic insights into neural circuit organization and making high-resolution connectomics accessible to a broader scientific community.

Poster #A15: (H. Huang et a.)

ATLAS: a rationally designed anterograde transsynaptic tracer

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Recent advancements in virus-based neural tracing techniques have provided new opportunities to elucidate neuronal circuits. However, these techniques, which rely on the intrinsic properties of viruses, have multiple drawbacks, including toxicity, lack of specificity, and difficulties in handling. In addition, the mechanisms by which these viruses mediate transneuronal labeling remain elusive, preventing further improvement. Herein, we introduce ATLAS (Anterograde Transsynaptic Label based on Antibody-like Sensors), a rationally designed protein capable of anterograde transsynaptic labeling. ATLAS encompasses an antibody-like protein that specifically recognizes the AMPA receptor subunit GluA1 (AMPA.FingR). Following expression of ATLAS in presynaptic neurons, the AMPA.FingR and its payload, which can include Cre recombinase, is released from presynaptic sites into the synaptic cleft, after which it binds to GluA1, enters postsynaptic cells through endocytosis, and subsequently carries its payload to the nucleus. Testing in dissociate cultures verifies the presynaptic release, postsynaptic binding, and endocytosis of the ATLAS tracer. Further studies in mice show that ATLAS can mediate monosynaptic tracing in vivo from random or genetically determined cells that transsyneuronal tracing by ATLAS is strictly anterograde, synaptic, and non-toxic. Moreover, ATLAS exhibits activity dependence and can mediate transsynaptic tracing in both mice and rats. Its versatile modular design also allows for modification to map and manipulate neural circuits mediated by many different neurotransmitters and neuromodulators, including, potentially, circuits that have previously been refractory to mapping using viral methods.

Poster #A16: (C. Ran et al.)

Anatomical and functional architecture of brainstem interoceptive circuits

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The nucleus of the solitary tract (NTS) in the brainstem serves as the brain's primary interoceptive hub. It integrates and processes convergent sensory inputs from visceral organs via the vagus nerve and spinal cord, transmitting signals to higher-order brain regions to regulate behavior, physiology, and metabolism. Despite its importance, the principles by which the NTS organizes peripheral information to mediate these complex responses remain poorly understood. Here we develop a novel in vivo two-photon brainstem imaging platform, which allows us to record the activities of thousands of NTS neurons simultaneously. We discover that the NTS creates a map of internal organs that takes the shape of a "visceral homunculus". This topography requires brainstem inhibition, as blockade of inhibition broadens neuronal tuning and disrupts spatial organization. Using bulk and single-neuron analyses, we build a comprehensive map of ascending NTS neurons and identify parallel NTS interoceptive sub-systems. Combining brainstem imaging with genetic strategies, we find that each of the NTS circuits are distributed across the topographic map of internal organs and are similarly tuned to respond to various viscerosensory stimuli but differentially control behavior and physiology. In addition, we reveal discarded viscerotopic organization in certain target regions and preserved spatial order in others. The preserved viscerotopy allow downstream brain areas to differentially regulate behavior. Our work establishes the conceptual framework of the organizational logic of the brainstem interoceptive circuits.

Poster #A17: (Y. Kurmangaliyev et al.)

Decoding Synaptic Target Choice: Insights from Multimodal Connectome-Transcriptome Maps of Neural Circuits

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How do you build a neural circuit? Traditionally, the molecular mechanisms of brain wiring have been studied one neuron type and one gene at a time without considering the broader context of the circuits. The revolutions in single-cell transcriptomics and brain connectomics have transformed this approach. As of today, we have a complete synaptic connectome of the Drosophila brain covering all 135,000+ neurons and more than 8000 distinct cell types. In parallel, we can obtain a gene expression profile for any neuron in virtually any system. The combination of these technologies provides multimodal transcriptome-connectome maps of the brain, offering a detailed molecular description of both sides of every synaptic connection. This presents a unique opportunity to correlate gene expression patterns with the synaptic target choice across entire circuits. However, the key challenge remains in cross-identification and matching cell types between connectomes and transcriptomes. We have developed a high-throughput strategy for generating multimodal transcriptome-connectome maps of the developing circuits in the Drosophila brain. We employed this strategy to generate one of the most comprehensive maps of developing circuits. It covers over 100 distinct cell types in the fly visual system, with a one-to-one match between transcriptomes and connectomes, and spans all stages of circuit assembly. The analysis of this dataset revealed molecular determinants of synaptic target choice in the motion-detection circuit. Our study establishes a framework for integrating molecular and connectivity maps of neural circuits to uncover the molecular strategies underlying synaptic specificity.

Poster #A18: (H. Lacin et al.)

Lineage-based dissection of the nervous system organization

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Neuronal progenitors give rise to lineally-related groups of neurons, called neuronal lineages. Neurons within lineages tend to adopt similar morphologies and are thought to act in the same or parallel circuits to regulate specific behaviors. By focusing on the development and function of the Drosophila ventral nerve cord (VNC), our research leverages the power of the fly model system to dissect the genetic and cellular basis of neural circuit formation and behavior.

We have built a library of lines which enables us to manipulate gene and cell function in any VNC lineage during developmental and adult life. Our initial studies have focused on the 4B lineage. We found that 4B neurons control specific leg movements, those enabling flies to reach their anterior notum, likely for grooming purposes. Ablation of the 4B neurons has no apparent effect on walking behavior but eliminates the fly's ability to groom its back. Optogenetic activation of random neurons within the 4B lineage showed that different neurons control the movement of distinct leg segments, indicating a possible functional organization within the lineage. Using genetic tools, we have divided the 4B neurons into three classes of neurons based on their gene expression and birth order and we are investigating the function of each neuronal class during leg movements via loss and gain of function experiments. The results of these experiments will show whether distinct neuronal classes born at different time windows within a lineage function as modular units to control different aspects of a specific behavior.

Poster #A19: (M. Bui et al.)

Organization of parallel basal ganglia output pathways

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The basal ganglia play a central role in regulating remarkably diverse aspects of movement and behavior. Yet the circuit basis by which the basal ganglia connect to divergent downstream targets to mediate unique behavioral outcomes remains poorly understood. Here, we extend our previous work on basal ganglia outputs from the substantia nigra pars reticulata (SNr) to parcellate subdivisions of the striatum and globus pallidus based on their polysynaptic connections to the lower motor system. We apply a suite of intersectional viral tools to perform transsynaptic TRIO (Tracing the Relationship of Inputs and Outputs) experiments from distinct SNr output classes. Using high-throughput imaging and computational approaches, we have identified anatomical subdivisions of the striatum and globus pallidus that differentially connect to regions of the brainstem with distinct motor and behavioral functions. In ongoing work, we are relating these output-defined domains to their cortical inputs and their contributions to motor dysfunction in a model of Parkinson's Disease. Taken together, these findings establish an input-output architecture for separable basal ganglia subnetworks with specialized connections to broader motor and behavioral systems. This extends the traditional view of closed-loop parallel processing in the basal ganglia and defines precise anatomical boundaries in the striatum, globus pallidus, and SNr based on their pathways to the lower motor system.

Poster #A20: (I. Elkinbard et al.)

Circuitry of the Preparatory Period: A Role for Thalamocortical Circuit Dynamics in Optimizing Spontaneous Attentional Control Prior to Cognitive Engagement)

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Human cognition acts through the complex coordination of interconnected networks. Spontaneous network states prior to cognitive engagement may prime the brain for differential cognitive performance. Thalamocortical (TC) communication is known to be a crucial element of attentional processes in humans and mice. In this multi-species study, we found that spontaneous TC communication dynamics predict performance on a trial-by-trial basis. We employed a temporal expectancy task in 24 humans and 6 wild type mice, consisting of an initial cue followed by a go cue separated by a variable time interval, and a response to the go cue. Human subjects were implanted with iEEG, and mice were injected with a fluorescent Ca2+ indicator (GCAMP6m)-carrying AAV with expression targeted to CaMKIIa+ neurons of the mediodorsal thalamus (MD), and with neural activity subsequently recorded using fiber photometry in the MD and prefrontal cortex (PFC). In humans, spectral power and graph communicability (Qexp) (a measure of network-wide communication) were calculated for each of the four canonical frequency bands within the 500 ms preparatory (spontaneous, before initial cue) or anticipatory (task-activated, before go cue) periods. The feature space was reduced by performing a univariate linear regression bootstrapped 1000x with a random 80% of trials for each iteration. Features that had a statistically significant relationship with reaction time (RT) on >35% of iterations were selected for further analysis because this threshold performed best in an SVM to classify fast vs slow trials. To see if any anatomical regions had metastable effects for predicting RT across subjects, we performed a rank-sum test of selected vs non-selected features from each region. Low frequency white matter Qexp, particularly in TC circuitry, predicts upcoming RT in the preparatory period while predictive features switch to high frequency spectral activity in the anticipatory period. We further explored TC circuit dynamics in mice and observed a robust correlation between RT and activity in MD-PFC TC projections. In trials with the fastest RTs (0-20ms), TC activity surged approximately 4s prior to initial cue onset (preparatory) as well prior to the go cue (anticipatory), compared to other trials (20-500ms) ($p<0.001$). When comparing the fastest vs. the slowest third of trials with RT<100ms, activity significantly increased for the fastest trials in the preparatory period but not the anticipatory period ($p<0.001$). In concordance with our human results, these findings indicate that enhanced TC communication primes mammalian neural circuitry during the preparatory period for robust and swift performance.

Poster #A21: (S. Park et al.)

Mechanisms underlying stress-induced changes of inhibitory control in the posterior parietal cortex

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Differentiating cues that signal either reward or danger is essential for adaptive behaviors. Exposure to stress is known to substantially alter how we perceive and react to salient cues, often shifting behavioral responses from goal-oriented to habitual or impulsive. These maladaptive changes can lead to devastating consequences like pathological gambling, substance abuse, or inappropriate fear responses in non-threatening environments. Impaired impulse control has been linked to the dysregulation of the frontoparietal network, where the posterior parietal cortex (PPC) serves as a cognitive hub that links sensory perception to executive function. However, the circuit mechanisms underlying behavioral shifts in response to cues with conflicting valence remain poorly understood. To address this, we developed an opposing valence paradigm where the value of sensory cues transition from positive (sugar rewards) to negative via a secondary association with multiple foot shocks (MFS). My preliminary data show a significant decline in performance of a delayed response task after the previously rewarded cue is coupled with MFS in male C57/BL6J mice. The phenotype is driven by an increase in impulsive action. Transient inactivation of the posterior parietal cortex mimicked these effects, suggesting that the PPC plays a key role in gating inhibitory control. To determine how PPC neural dynamics may change after MFS, we used in-vivo two-photon calcium imaging in awake, head-fixed mice as they performed the task. In a longitudinal design, we tracked the same population of PPC neurons before and after MFS. We then used a logistic regression model to predict cue identity (CS+ or CS-) from the activity of individual cells and observed a decrease in stimulus selectivity across both control and MFS groups, indicating marked representational drift at the single-cell level. These findings suggest that representation of task-relevant cues in the PPC relies less on selectivity of individual neurons and more on population dynamics, highlighting the role of the PPC in integrating salient signals to guide behavior.

Poster #A22: (H. Zhang et al.)

The Analgesic Effect of Acupuncture is mediated by a Retrosplenial Cortex -Anterior Pretectal Nucleus Pathway

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Acupuncture is an effective treatment option for patients with chronic pain conditions, and it is appealing as an inexpensive, non-addictive medical alternative with a prolonged action. Earlier studies in rodent models indicate that electroacupuncture (EA) activates neurons in multiple cortical and subcortical brain regions, however, the neural circuit mechanisms underlying acupuncture effect remains elusive. In the present study, we measured the effect of EA on pain sensitivity in a mouse model of complete Freund's adjuvant (CFA)-induced chronic inflammatory pain. Applying EA (2 Hz, 5 ms, 0.1-0.2 mA for 20 min) in the ST36 (Zu San Li) acupoint in the hindlimb, which is an acupoint frequently used for pain modulation, increased mechanical pain threshold measured by the von Frey filament test, indicative of pain alleviation. The pain relief effect was confirmed by applying opto-acupuncture in ST36 in Prokr2-Cre:Ai32 mice that express channelrhodopsin-2 (ChR2) in the innervation in deep hindlimb fascia. To elucidate the neural basis of pain alleviation effects of ST36 EA, we mapped the brain activity with EA treatment in a TRAP2 mouse line (that has tamoxifen-dependent, c-fos driven recombinase CreER) crossed with the Cre-dependent Ai9 reporter mouse. Our results showed that EA in the ST36 acupoint significantly increased tdTomato labeled neuron numbers in multiple brain regions, including the secondary motor cortex (M2) and the retrosplenial cortex (RSC). In vivo calcium imaging indicated elevated activity of RSC excitatory neurons when EA was administrated in ST36 acupoint, or when opto-acupuncture was applied in Prokr2-Cre:Ai32 mice. Previous studies have reported that RSC and anterior pretectal nucleus of thalamus (APT) were involved in analgesic effect. Our preliminary virus tracing data showed that RSC neurons project to APT. To examine the role of RSC-APT in acupuncture effect, we injected retroAAV-Cre in APT and Cre dependent AAV-DIO-hM4Di in RSC, to target APT-projecting RSC neurons. The result showed that the analgesic effect of EA was abolished when APT-projecting RSC neurons were chemogenetically inhibited. Our results indicate a potential role of RSC-APT pathway in pain modulation.

Grant information: This study is supported by a UCI Susan Samueli Integrative Health Institute (SSIHI) Pilot award and a Samueli Scholar award to X.X.

Poster #A23: (M. Lam et al.)

Mechanisms of myelin membrane expansion in development and neuroplasticity

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Myelin accelerates conduction velocity along axons, and loss of myelin leads to cognitive deficits and physical disability. Cells that make myelin coordinate extreme feats of membrane trafficking to wrap axons in spiraling layers of lipids to form a compact sheath. Remarkably, in spite of its compact, multilayered structure, myelin in the central nervous system (CNS) changes in abundance and structure to adapt to new brain activity. Activity-dependent myelination can add new myelin sheaths and remodel pre-existing sheaths to fine-tune neural circuits, requiring spatiotemporal coordination of membrane trafficking in oligodendrocytes.

How does neuronal activity regulate membrane trafficking in oligodendrocytes? We previously discovered that exocytosis through the v-SNAREs VAMP2 and VAMP3 in oligodendrocytes are required for myelin membrane expansion during development (Lam*, Takeo*. 2022 Nat. Comm.). Oligodendrocyte VAMP2/3 mediate membrane fusion at myelin sheath edges and at the innermost layer, positioning VAMP2/3 to potentiate myelin addition upon neuronal activity during adulthood. We developed a tractable co-culture system to measure exocytosis in oligodendrocytes while manipulating the activity of neurons. Excitingly, activity from glutamatergic neurons doubles the rate of VAMP3 exocytosis in oligodendrocytes in a calcium-dependent manner. To test how v-SNAREs sculpt myelin in vivo, we used an optogenetic approach to induce activity-dependent myelination while inhibiting oligodendrocyte exocytosis. We found that oligodendrocyte VAMP2/3 are required for activity-dependent sheath remodeling. Thus, we uncover a cellular mechanism for spatiotemporal control of new myelin addition to facilitate dynamic sculpting of myelin in neuroplasticity.

Poster #A24: (L. Zaborszky et al.)

Cortico-cortical connections and their relations to cholinergic basalo-cortical projections

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Although schemes of cortico-cortical connections can be seen in the literature from the 1980s, data supported by connectional strengths, receptor density and computational analysis in rodents were published much later, starting around 2014 (Burwell's, Zilles, Dong's, Mao's groups and Harris at the Allen Institute). During the past several years we created a virtual basal forebrain (BF) with cellular resolution of cholinergic cell bodies from over 70 experimental rat brains using discrete cortical retrograde tracer injections. Based on that dataset, we established that the spatial location of cholinergic cell bodies projecting to 30 ontologically defined cortical targets displays systematic organizational features that deviate from the random distribution (Varsanyi et al., iSCIENCE 28, 112001, 2025), suggested by the conventional diffuse model. In fact, the combined dataset identified three hierarchically organized principal networks: somatosensory-motor, auditory, and visual, as defined by the sensory modality most predominant within them. To find out the blueprint of cortico-cortical connections in rats using some 35 cortical retrograde tracing injection cases, we registered about 200,000 cortical cells, distributed in 31 cortical areas and various layers defined by the Paxinos atlas, into our 3D database. Using hierarchical clustering, we created dendrograms that may give clues for the flow of information in the cortex. Our preliminary analysis suggests, using all layers and target clustering, that cortico-cortical connections consist of three major networks, largely corresponding to the cholinergic basalo-cortical networks. Using intersectional viral tracing strategy confirms a modular output architecture of the cholinergic system. We hypothesize that any two interconnected cortical targets (e.g., M1 and S1 whisker) receive most of their cholinergic projection from a unique BF space defined by the spatial density correlation analysis (Varsanyi et al., 2025). Furthermore, the two BF cell populations do not co-occur in other BF spaces projecting to the same two cortical targets. Thus, the system of hierarchically organized clusters of cholinergic neurons could enable the BF cholinergic system to coordinate spatially selective signaling in a hierarchical fashion, including parallel modulation of multiple interconnected yet diverse groups of cortical areas.

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Poster #A25: (S. Bolund et al.)

Mapping Molecular Architecture of the Human Cerebellum Circuitry Using High-Resolution Spatial Transcriptomics

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The cerebellum is traditionally linked to motor coordination and balance, but its contributions to cognition, emotion, and learning are becoming more evident. While recent transcriptomics studies have revealed many aspects of the cerebellum's cellular and molecular complexity, much of this work is based on model organisms including rodents and non-human primates.

Understanding cerebellar circuitry and function requires identification of connections and the molecular signatures of cell-types that directly or indirectly participate in the circuits that drive cognition and behavior. To map the molecular architecture of the human cerebellum, we integrated single-nucleus RNA sequencing (snRNA-seq) with high-resolution spatial transcriptomics to construct a spatially resolved atlas of the adult human cerebellum. By clustering genes based on gene-to-gene co-expression profiles, we defined gene modules associated with distinct cell-types or cell-populations. Using calculated spatial density of the gene modules in the Stereo-seq dataset, the location of cells and their molecular signature could be identified.

This integrative approach enabled the identification and spatial localization of all major cerebellar cell-types, including rare and layer-specific interneuron subtypes, Purkinje cells, granule cells, diverse glial populations (Bergmann glia, astrocytes, oligodendrocytes, microglia), and vascular cells such as pericytes. We also identified cell-type-specific molecular features unique to humans, as compared to macaques, marmosets, and mice, shedding light on recent evolutionary adaptations possibly underlying higher-order cerebellar functions.

Our method facilitates high-resolution cell-type mapping within human tissue and offers new insights into the structural and functional organization of the cerebellum. By integrating molecular identity with spatial context, this atlas provides a valuable resource for investigating cerebellar connectivity in both health and disease. Furthermore, it establishes a foundation for further cross-species comparisons with model organisms such as mice and non-human primates, enabling evolutionary studies of cerebellar function.

Poster #A26: (Y. Zhou et al.)

MAPSeq in young and aged mice reveals changed output projection patterns of dorsal CA1

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The output projections of dorsal CA1 are likely to be closely related to the consolidation and retrieval of cognitive memory. The deflected cognitive ability in aged human and animals might be related to the change in the projections. In order to systematically describe the changes in projections during aging, we applied MAPSeq (Multiplexed Analysis of Projections by Sequencing, Kebschull, 2016) to figure out the long-range projections with a single-cell resolution. It was the first time that this technique was applied to two groups of mice for comparison. For young mice, [1] most of the intra-hippocampal-formation(intra-HPF)-projecting cells project to dorsal subiculum, [2] a large part of extra-hippocampal-formation(extra-HPF)-projecting cells project to the retrosplenial cortex (RSP) and the lateral septal nucleus (LS) separately, [3] cells tend not to project to the RSP and LS simultaneously. For old mice, [1] the mutual exclusion between LS and RSP elapsed, [2] the projection strength to the RSC significantly decreased, [3] the projection strength to the LS significantly increased, [4] the projection pattern to extra-HPF regions showed more difference from young group than the projection pattern to intra-HPF regions. We have been verifying the differences in RSP and LS with retrograde tracing of CTB-555 in both young and old mice. Tracing showed the projections from dCA1 pyramidal layer mainly wired to agranular part of RSP(RSCa), which was possibly involved in social behavior. We injected channelrhodopsin-2(ChR2) in the dorsal CA1 and bilaterally implanted opto fibers in the RSCa. With an enhancement in projections to RSCa, we expected to see a partial rescue in the deflected social novelty preference of aged mice. We expect to expand the scope of MAPSeq and offer an idea for migrating the cognitive deflection in aging.

Poster #A27: (N. Wang et al.)

High-resolution MRI Predicted Whole Mouse Brain Cell Type Atlas using Multi-modal Fusion Network (MFNet)

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Mapping brain cell types with high spatial precision is fundamental for understanding brain structure and function. Traditional cell atlases, such as the Allen Brain Cell Atlas, rely on labor-intensive techniques like single-cell sequencing and spatial transcriptomics, and the atlas often lacks whole-brain coverage with isotropic resolution in all the dimensions (x, y, and z). Diffusion MRI (dMRI) offers a powerful alternative for studying brain cytoarchitecture and myeloarchitecture, with quantitative metrics serving as biomarkers for brain developmental and neurodegenerative disorders. While the correlation between dMRI and gene expression has been investigated, whether dMRI can directly predict the brain cell types remains unexplored. In this study, we present a deep learning framework—Multi-modal Fusion Network (MFNet)—that predicts neuronal cell types across the whole mouse brain by integrating high-resolution diffusion MRI (dMRI) and multi-modal datasets from the BICCN mouse brain atlas. Using the Allen Brain Cell Atlas as ground truth, we trained MFNet to predict dominant neuronal cell neighborhoods and classes, taking advantage of regional specificity and hierarchical cell-type structures. High spatial and angular resolution dMRI was acquired and processed with multiple biophysical models (DTI, DKI, NODDI, SANDI), then registered to the Allen Mouse Brain Common Coordinate Framework (CCFv3), along with spatial transcriptomics and epigenomics data. MFNet produces a full-scale cell type atlas at 10 μm isotropic resolution, significantly enhancing the spatial detail of the original atlas. Additionally, we examined correlations between dMRI features and gene expression of marker genes. This study offers a novel, efficient method for generating high-resolution brain cell atlases and demonstrates the potential of advanced imaging combined with deep learning to decode brain cellular architecture and its relevance to development and disease.

Poster #A28: (M. Majeed et al.)

High throughput barcoded connectomics in the primate visual cortex reveals broadcasting projections at cellular resolution

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Expansion of the cortex underlies the tremendous cognitive flexibility and rich behaviors of primates. This expansion is accompanied by diversification of transcriptomically defined cell types, but how cell-specific connectivity changes across species at the single-cell level is not completely understood. Mapping long-range projections of individual neurons in the primate brain is challenging because their large brain size makes tracing projections error-prone and time-consuming. Here, we overcome this limitation by adapting BARseq, an in situ sequencing-based barcoded connectomics technique, for the macaque brain. In BARseq, random RNA sequences, or barcodes, uniquely label thousands of neurons and fill up both somas and axons. Sequencing barcodes in situ and matching those in axons and somas reveals the projections of many neurons in parallel. Endogenous mRNAs can also be sequenced in situ and associated with projections at cellular resolution. We applied BARseq to determine the transcriptomically defined cell types of neurons in the macaque primary visual cortex (V1) and their projections to 184 voxels in 13 output regions, including higher visual areas (HVAs), the caudate, the dorsal lateral geniculate nucleus (DLG), and the colliculus. Our data recapitulated known cell type- and laminar-specificity of projections, and were consistent with the retinotopic organization of V1 projections. Surprisingly, more than half of projection neurons in V1 project to two or more HVAs. This abundance of “broadcasting” neurons is reminiscent of the mouse visual cortex and challenges the classic view that macaque V1 neurons send largely dedicated projections to individual HVAs. Our results reveal conserved organization of projections across two species and set the path forward for leveraging barcoded connectomics to understand circuit conservation and innovations across mammalian species.

Poster #A29: (R. Dickson et al.)

Brain-wide connectivity patterns of feedforward and feedback cortico-cortical neurons in the mouse secondary visual cortex

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Feedforward and feedback cortico-cortical neurons are distinct yet spatially intermingled subtypes distributed across cortical layers, playing specialized roles in sensory and cognitive processing. However, whether their presynaptic inputs differ to support these functions remains unknown. Using projection- and layer-specific monosynaptic rabies tracing, we mapped brain-wide long-range and local inputs to multiple feedforward and feedback neuron types in VISl (also known as LM), the mouse secondary visual cortex. Overall, long-range input patterns for these feedforward and feedback neurons were largely similar, as all received the majority of their inputs from VISp, the primary visual cortex, along with substantial inputs from various other cortical and visual thalamic regions. Despite their similarities, these feedforward and feedback types differed in the proportion of long-range cortical inputs originating from specific visual, retrosplenial, and auditory cortices. We also found that feedback neurons received a greater proportion of local inputs from their own layer compared to feedforward neurons. These findings reveal the input connectivity principles of cortico-cortical neurons based on feedforward and feedback projections, providing an anatomical framework for future studies on their functions and circuit integration.

Poster #A30: (M. Pratelli et al.)

Brain-wide screening for drug- and stress-induced neurotransmitter plasticity

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Neurotransmitter identity, long considered a stable phenotype, can change during both development and adulthood. Environmental stimuli that affect neuronal activity for sustained periods can cause neurons to begin expressing a new transmitter, lose one they previously expressed, or both. This transmitter plasticity, known as transmitter switching, often involves excitatory neurons acquiring inhibitory transmitters—or vice versa—and can lead to behavioral changes. We recently found that repeated exposure to drugs of abuse or a single acute traumatic experience induces transmitter switching in the prelimbic cortex or the dorsal raphe, respectively. These transmitter switches result in long-lasting cognitive deficits or in generalized fear. Do these stimuli also cause transmitter plasticity in other brain regions?

To answer this question, we developed a pipeline for brain-wide screening of transmitter plasticity involving glutamate and GABA—the most common excitatory and inhibitory neurotransmitters in the brain. We used a VGAT-Flp::VGLUT2-Cre::Con/FonTdTomato reporter mouse line, in which neurons that express, or have ever expressed, both the GABAergic marker VGAT and the glutamatergic marker VGLUT2—even if at different time points—are permanently labeled with TdTomato. Thus, when these mice are exposed to an environmental stimulus that causes glutamatergic neurons to gain GABA, or vice versa, the affected neurons become permanently labeled with TdTomato. Using two-photon array tomography, registration to the Allen Brain Atlas, and automated cell counting, we found that repeated exposure to methamphetamine (METH) as well as acute foot-shock stress produced a statistically significant increase in the number of TdTomato+ neurons in 34 and 27 brain regions, respectively.

We are now characterizing this transmitter plasticity in three brain regions that show a METH-induced increase of more than 10,000 TdTomato-labeled neurons: the retrosplenial, auditory, and visual cortices. After METH treatment, neurons that have switched transmitter co-express glutamatergic and GABAergic markers or express the glutamatergic marker alone. Preliminary data from the retrosplenial cortex indicate that these switching neurons were originally GABAergic or glutamatergic prior to METH exposure. Since transmitter switching can be stable or reverse spontaneously and only stable switches are likely to influence behavior, we are assessing whether the METH-induced changes in transmitter identity persist for one month after the end of drug exposure. Our findings demonstrate that environmental stimuli can trigger widespread transmitter switching, enabling investigation of the impact on behavior of transmitter plasticity in different regions of the brain.

Poster #A31: (B. Morales et al.)

Neuronal Networks in a Translational Model of Opioid Relapse in Addiction: Ventral Pallidum GABA Neurons as a Key Network Hub

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Brain networks involving the ventral pallidum (VP) underlie opioid drug seeking and relapse-like behaviors in rats in conventional models of self-administration-based opioid drug seeking. Here we employ a translationally relevant behavioral model of opioid relapse to understand how GABA neurons in VP (VPGABA), which are required for relapse, modulate wider reward- and stress-related brain networks they interact with, and thus participate in decisions to seek drug, or to suppress this seeking when it is maladaptive.

Here, we targeted inhibitory DREADDs (or mCherry only) to VPGABA neurons in male and female GAD1:Cre rats, allowing on-demand inhibition of these cells during behavior. All these rats were first trained in a “Safe Context” (Context A) to self-administer the rewarding opioid drug remifentanil, and a tone/light cue, by pressing a lever. They next were moved to a “Dangerous” Context B, where lever presses still yielded remifentanil and a cue, but also a foot shock punishment on 50% of trials, causing rats to voluntarily suppress their drug seeking over several days of training.

On a final test day, trained rats were placed in either context A or B, where they received cues, but not drugs or shock when they pressed the lever. All rats received clozapine-N-oxide (CNO; 5mg/kg) prior to the session, causing VPGABA neuron inhibition in rats with DREADDs (n=12), or no inhibition in rats with mCherry only (n=12). As expected, inhibiting VPGABA neurons suppressed relapse-like drug seeking. Brains from rats tested in Context A, B, or not tested (taken from home cage) were stained for c-Fos, a marker of neural activity, in numerous brain regions of interest throughout the brain.

We find that testing in an environment promoting drug seeking (Context A), and an environment suppressing seeking (Context B) altered brain-wide activity relative to no test. We also found that inhibiting VPGABA neurons altered wider network activity, and did so in a sex-dependent manner.

Results lend insight into VP’s position within addiction- and relapse-related brain networks, and may facilitate future treatment strategies in humans suffering from addiction, and struggling not to relapse to unwanted drug use.

Poster #A32: (E. Gingrich et al.)

Repeated use of Teneurin-3 and Latrophilin-2 in circuit-wide topographic target selection of the extended hippocampal network

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Precise circuit assembly is critical for nervous system function and is accomplished, in part, through cell-surface proteins (CSPs) that mediate cell-cell interaction, including contact-dependent attraction and repulsion. However, the relatively low number of CSPs compared to the vast number of connections needed presents a biological challenge to developing axons during target selection. A possible strategy to overcome this challenge is repeated use of the same receptor-ligand pair to specify multiple connections across a network. One such pair, Teneurin-3 (Ten3) and Latrophilin-2 (Lphn2), has complementary expression across multiple interconnected regions of the extended hippocampal network, following a “Ten3 to Ten3” and “Lphn2 to Lphn2” connectivity rule. For one of these projections, CA1 to subiculum (Sub), Ten3+ CA1 axons are attracted to subicular Ten3 and repelled by subicular Lphn2 while Lphn2+ CA1 axons are repelled by subicular Ten3 (Berns et al., Nature 554:328-333, 2018; Pederick et al., Science 372:1068–1073, 2021). The stereotyped, topographical connections and the circuit-wide, complementary expression make this system ideal to test whether the same mechanisms of Ten3/Lphn2-mediated repulsion and Ten3/Ten3-mediated attraction are re-used at other nodes within the circuit. Furthermore, we can use this system to study how anatomical organization of axons in relation to their targets may influence the necessity of these mechanisms. Using a conditional knockout approach in mice, we have found that Ten3 is required in entorhinal cortex (EC) axons to correctly target proximal CA1 and distal Sub (dSub). Furthermore, EC axons mistarget when Ten3 and Lphn2 are deleted from Sub, suggesting these mechanisms do generalize to other local connections. However, this mistargeting is not observed when Lphn2 alone is deleted in Sub, suggesting repulsion is less critical for targeting of EC axons to dSub than for CA1 axons. We hypothesize that this is due to the Ten3+ EC axons contacting the attractive Ten3 first, while Ten3+ pCA1 axons must traverse a large Lphn2+ region of Sub before finding its Ten3+ target. An extended connection from Sub to medial mammillary nucleus in the hypothalamus, where axons encounter the attractive and repulsive cues simultaneously, also requires Ten3 in axons and Lphn2 in the target. To our knowledge, this study is the first to examine if a single receptor-ligand pair can instruct wiring specificity across multiple nodes of a functional network using a conditional knockout approach.

Poster #A33: (Withdrawn)

Poster #A34: (D. Pederick et al.)

Inverse and topographic expression of Teneurin-3 and Latrophilin-2 across the developing brain provides insight into circuit formation and organization

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Correct segregation of neural connections is critical for processing distinct types of information and is achieved through spatially arranged topographic maps. These maps are established early in brain development, where cell surface proteins (CSP) instruct neurons to form connections with their correct partners. We demonstrated that the CSPs Teneurin-3 (Ten3) and Latrophilin-2(Lphn2) display inverse expression and mediate reciprocal repulsions that instruct the assembly of parallel hippocampal connections. Given the striking expression and function of these CSPs, we sought to define if Ten3 and Lphn2 are expressed topographically in brain regions outside of the hippocampus and whether they instruct the formation of other topographic connections. Here, we used whole brain imaging methods to identify brain regions that display inverse expression ofTen3 and Lphn2. We observed that Ten3 and Lphn2 expression matched known topographic connections in the extended hippocampus, basal ganglia and auditory brainstem. We leveraged them as markers of topographic neuron identity in the developing auditory system to generate a molecular map of developing tonotopy allowing us to identify molecules and mechanisms that may establish tonotopic connections before the onset of hearing. Intriguingly, in the cerebellumTen3 and Lphn2 expression did not follow any characterized topographic connectivity, potentially highlighting new circuit organization. We generated Ten3 conditional tag mice to provide specific labeling of axonal Ten3 and confirmed that Ten3-positive axons project to Ten3-positive target regions in the cerebellum. Furthermore, our preliminary data suggests that deletion of Lphn2leads to the mistargeting of Ten3 axons, indicating that like in the hippocampus Ten3 and Lphn2mediate precise axon targeting in the cerebellum. This whole brain characterization of Ten3 andLphn2 expression during development provides insight into their potential roles in wiring of multiple brain regions, unveils new circuit organization, and offers markers to identify other topographically expressed molecules across the brain.

Poster #A35: (C. Goodpaster et al.)

Hardwired by Hardship: A Stress-Sensitive vmPFC–BLA Circuit Fuels Adolescent Avoidance Following Early Adversity

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Early life adversity (ELA), such as neglect or maltreatment in childhood, is a major risk factor for the development of psychiatric disorders. ELA heightens responses to threats, often at the expense of goal-directed behaviors. This behavior is a hallmark of anxiety and depression, conditions that arise during adolescence, yet most studies have focused on adult outcomes of ELA. As a result, the mechanisms connecting ELA to the development of threat sensitization remain unknown. Neural circuits that process both rewards and threats, including connections between the medial prefrontal cortex (mPFC) to the basolateral amygdala (BLA), are vulnerable to early life perturbations and implicated in psychiatric disease. Additionally, corticotrophin releasing hormone (CRH), a key stress signaling neuropeptide, is affected by ELA in several brain regions. While limbic circuits and CRH are good candidates for mediating the effects of ELA on threat-induced behaviors, how CRH signaling may influence changes in limbic circuit function remains poorly understood.

To address this gap, I employ limited bedding and nesting (LBN), a model of resource scarcity, during the first week of life in mice. Once pups reach adolescence, I combined a platform-mediated avoidance (PMA) assay with in vivo neural circuit investigations. I found that adolescent mice exposed to LBN learned similarly to standard-reared (SR) controls, although the BLA showed enhanced activity during training. The following day, LBN mice exhibited higher levels of avoidance, which was strongly encoded in the BLA. Using tissue clearing and lightsheet fluorescence microscopy I found a found a significant decrease in CRH+ cells within the ventromedial PFC (vmPFC) in LBN animals. I confirmed that CRH+ cells in vmPFC project to the BLA and are overactive during PMA training in LBN mice with viral tracing and immediate early gene labeling. Using optogenetics, I found that inhibiting this pathway during PMA training reduced avoidance behavior in LBN mice to levels comparable to SR controls but had no effect on the controls themselves. Overall, this work reveals a novel, molecularly-defined neural circuit that drives maladaptive behaviors in adolescence. My work may open new avenues for therapeutic interventions targeting anxiety and depression, potentially even before symptom onset.

Poster #A36: (S. Cao et al.)

Neural control of wing and leg movements in a terrestrial social display

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California Institute of Technology

Terrestrial threat displays by flying insects communicate aggressive intent towards conspecifics using both the wings and legs, appendages whose primary functions are to control aerial vs. terrestrial locomotion, respectively. How such appendicular coordination is achieved is not well understood. Previously we identified a small (6-8 cell) cluster of AIP neurons in Drosophila that causally controls threat displays, raising the question of how this circuit node flexibly coordinates wing and leg movements. Here we have used connectomics, cell type-specific functional perturbations and calcium imaging to elucidate the circuit logic underlying male threat displays. We identified two distinct appendage-specific combinatorial modules that mediate threat actions. The wing module consists of two descending neurons (DNs), CL341 and DNp60, which act synergistically to control wing elevation and/or pumps. The leg module comprises two interneuron (IN) types, AVLP491 and 10588. AVLP491 activation promotes extended locomotion, while its co-activation with 10588 (an inhibitory IN) truncates this behavior into brief, discrete bouts of turning and charging. These findings reveal a hierarchical and combinatorial neural circuit logic that coordinates independent appendicular control systems using distinct computations, to generate a flexible social display.

Poster #A37: (M. Gongwer et al.)

Cell-type specific mechanisms driving the rapid antidepressant effects of transcranial magnetic stimulation

Michael W. Gongwer, Alex Qi, Alexander Enos, Meelan Shari, Sophia Rueda Mora, Aliza Hacking, Russel Ahmed, Cassandra Klune, Owen Williams, Jack Riley, Gary Wilke, Yihong Yang, Hanbing Lu, Andrew Leuchter, Laura A. DeNardo#, Scott A. Wilke#

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Repetitive transcranial magnetic stimulation (rTMS) is an emerging treatment for brain disorders, but the neural mechanism for its therapeutic effect is not known. We developed a novel model of rTMS to deliver highly focal clinical treatment protocols in awake, head-fixed mice. We employed a recently developed, accelerated intermittent theta burst stimulation (aiTBS) protocol that, when targeted to prefrontal cortex (PFC), can effectively treat depressed patients in days. Applying aiTBS to dorsomedial PFC (dmPFC) reversed behavioral deficits associated with chronic stress. Using fiber photometry, we showed that aiTBS drives distinct patterns of neural activity in five classes of excitatory and inhibitory neurons in mouse prefrontal cortex. Of these, intratelencaphalic (IT) excitatory neurons and somatostatin (SST) expressing inhibitory neurons showed increased activity after aiTBS, while parvalbumin (PV) and vasoactive intestinal peptide (VIP) inhibitory neurons showed decreased activity. These changes in activity were associated with unique forms of synaptic plasticity in each cell type. IT neurons, but not pyramidal tract (PT) excitatory neurons showed an increase in dendritic spine density following aiTBS. Electrophysiology recordings revealed an increase in inhibitory synaptic to PV neurons following aiTBS, which was driven by SST neurons. Using chemogenetics, we revealed that activity in IT and SST neurons, but not other cell types, is required for the antidepressant-like behavioral effects of aiTBS. Thus, we demonstrate a prefrontal mechanism linking rapid aiTBS-driven therapeutic effects to cell type-specific circuit plasticity.

Poster #A38: (I. Wickersham et al.)

Third-generation monosynaptic tracing using a nontoxic single-deletion-mutant virus

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Massachusetts Institute of Technology

Monosynaptic tracing has become a widely-used technique in neuroscience, but it has been mostly restricted to anatomical applications because of the cytotoxicity of the first-generation (ΔG) rabies viral vectors on which it is typically based. We recently introduced a second-generation monosynaptic tracing system based on nontoxic double-deletion-mutant (ΔGL) rabies virus; however, this second-generation system usually labels fewer input neurons than the first-generation system does. Separately, we also recently introduced third-generation (ΔL) rabies viral vectors, in which only one gene (L, encoding the viral polymerase) is deleted, and shown that they are as nontoxic as second-generation ones but grow more efficiently in cell culture, resulting in higher titers and therefore more labeled neurons when they are used for direct retrograde targeting in vivo. Here I describe a third-generation monosynaptic tracing system based on nontoxic single-deletion-mutant ΔL rabies viral vectors. Modification of the ΔL vectors allows pseudotyping with the avian retroviral envelope protein EnvA; this in turn allows selective infection of neurons expressing TVA by means of helper AAVs, just as for first- and second-generation vectors. Complementation of the single deletion in vivo by expression of the viral polymerase in trans using a knock-in allele allows replication of the rabies virus in the complementing cells and spread to input cells both local and distant. This ΔL-based monosynaptic tracing system comprises a new state of the art in nontoxic monosynaptic tracing and should allow diverse applications such as long-term imaging, optogenetic and chemogenetic manipulation, and transcriptomic profiling of minimally-perturbed synaptically-connected networks of neurons.

Poster #A39: (P. Gao et al.)

Differential Organization of Monosynaptic Inputs to GABAergic Neuron Subtypes in the Dorsal Subiculum

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The hippocampal formation, comprising the dentate gyrus (DG), hippocampus proper, and subiculum (SUB), is pivotal in episodic memory and spatial navigation. Although the SUB is traditionally viewed as a relay station between CA1 and downstream regions, recent data supports that SUB has distinct circuit organizational and functional characteristics. This study aims to examine the neural circuitry connections of excitatory and inhibitory cells in the distal part of the dorsal subiculum (dSUB). Utilizing our established monosynaptic rabies virus tracing system, we mapped local and long-range circuit connections to glutamatergic and GABAergic cells, including three major subtypes of GABAergic cells: parvalbumin (PV), somatostatin (SOM), and vasoactive intestinal peptide-expressing (VIP) in the dSUB. Our findings indicate that inputs to Gad2+ and Camk2a+ cells are comparable in terms of regional input patterns and strengths, primarily originating from CA1, subiculum, postsubiculum, medial septal diagonal band, thalamus, and entorhinal cortex. However, differences in input strengths were observed among the GABAergic subtypes. Compared with VIP+ cells, SOM+ and PV+ cells received significantly more inputs from the thalamus (% inputs, SOM: 0.869± 0.124, PV:1.803± 0.284, VIP: 0.205± 0.1. CSI, SOM :2.083 ± 0.265, PV: 2.036± 0.284, VIP: 0.210± 0.093.) and postsubiculum (% inputs, SOM: 0.1.379± 0.363, PV:2.694± 0.862, VIP: 0.315± 0.295. CSI, SOM :2.950± 0.911, PV: 2.525± 0.695, VIP: 0.278± 0.242). All these cell types were found to receive putative GABAergic long-range inputs from the CA1 oriens layer. These new circuit mapping results provide us with a new understanding of the neural circuit basis of specific SUB neuron types in the hippocampal formation networks.

Poster #B1: (A. Das et al.)

Circadian Control of peroxisome biogenesis and import oscillation in glial regulates sleep behavior and neural detoxification.

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Peroxisomes are responsible for metabolic activities through the regulation of antioxidant enzymes and oxidases. An important process highlighted during cellular aging is when increased oxidative stress leads to a reduced import efficiency of peroxisomal matrix proteins containing a C-terminal peroxisomal targeting signal type 1 (PTS1) - the reduced import of peroxisomal matrix proteins is a cellular hallmark of Zellweger Syndrome (Santos MJ, et al. 2000). Zellweger syndrome is one of the peroxisomal biogenesis disorders caused due to mutations in one of the 12 different PEX genes in humans accentuating the causality of impaired biogenesis of peroxisomes (Krause et al., 2009). One of the key import factors that is predicted to enable cargo proteins holding PTS1 and its delivery to the peroxisomal matrix is the PEX5 (peroxin-5) protein (Huang et al., 2020). Previous literature shows lack of functional peroxisomes led to consequential neurological abnormalities like motor and cognitive decline simultaneously with demyelination, axonal loss and gliosis (Astrid Bottelbergs et al.,2010). Thus, it made us curious to answer, “how peroxisomes mediate glial fate and metabolism?”. Drosophila is a great model to answer this question because of the functional diversity of different glial subtypes (Kremer et al. 2017; Awasaki et al. 2008; Freeman 2015). We have made a novel identification that the cortex glia has a predominance of peroxisomes in the adult Drosophila brain. We have seen the dynamic changes in transport of the peroxisomal import machinery from the cytosol to the peroxisome marked with eYFP carrying the peroxisomal targeting signal - PTS1 specifically in the glial cells. We demonstrate that eYFP.PTS1 reporter driven by Repo-GAL4, a pan- glial marker, can be used to understand the decline of peroxisomal transport in the glial cells of the adult Drosophila brain. Our data suggests that peroxisome import declines in aged glial cells thus, replicating the cellular hallmark of Zellweger syndrome where the peroxisomes lack the PTS1-positive matrix proteins also known as ghost peroxisomes (non-functional peroxisomes) (Huang et al., 2020). We have further uncovered how peroxisomal genes and proteins are oscillating at different zeitgeber (light cue induced) time points of the 24-hour daily cycle in the unique glial cell clusters. This was possible through mining of & extracting from publicly available Drosophila sleep single cell data deposited through the works of Joana Dopp et al., 2024. I have further validated this through my own experimental testing that peroxisomal cargo import & biogenesis is oscillating at different times of the day which appears to be under the control of core-circadian clock program. We are investigating how PEX5 impairment in CNS glial affects sleep as a biological process among other neuronal functions through impaired lipid metabolism and its overarching implications of how lipid oscillation during zeitgeber time course affects neural detoxification. Thus, our study finds the novel interaction of peroxisomal import and biogenesis control by the core-clock program which in turn mediates glia-neuron communication.

Poster #B2: (H. Naz et al.)

Core Activation Program and Selective Regional Responsiveness of Microglia in Aging and Parabiosis

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Aging is the primary risk factor for many neurodegenerative diseases and is associated with immune dysregulation in the brain whereas rejuvenation interventions can mediate beneficial effects. Microglia are considered as a major player in neurodegenerative disease development yet, the molecular changes underlying brain aging and rejuvenation remain poorly understood at the single cell level. Using single-cell RNA sequencing (scRNA-seq), we investigated microglia heterogeneity and their transcriptomics changes during aging and the parabiosis mediated exposure of young and old blood across four different brain regions including cerebellum, cortex, hippocampus, and striatum. We identified eight distinct microglial subpopulations shared across different ages, brain regions and treatment conditions. By comparing the expression of subpopulation-specific signature genes, (e.g. homeostatic microglia, disease associated microglia, and interferon response microglia), we benchmarked the identified microglia subpopulation, seven of which were reproducible in an independent widely used aging dataset. We nominated combinatorial signaling codes governing different reactive microglial states and defined the core microglial activation gene program. Additionally, we uncovered shared and region-specific aging signatures across the four brain regions and identified region-specific differential gene expression associated with cellular senescence, aging, amyloid metabolism and response to unfolded proteins. To investigate the effects of old blood on accelerating microglial aging, we identified genes shared by aging and old blood exposure. We found old blood-mediated aging leading to similar, albeit to a lesser extent, microglial gene expression changes as in biological aging, linked to neuroinflammation and glial activation. In contrast, exposure to young blood appeared to offer rejuvenating benefits by reverting genes dysregulated during aging. Our analysis revealed that parabiosis-mediated accelerated aging and rejuvenation had distinct effects on gene expression in specific brain regions, with the cerebellum consistently emerging as the most sensitive region and the striatum as the least affected. Pathways involved in apoptotic signaling, inflammatory responses, glial cell activation, synaptic plasticity, and phagocytic activity were differentially impacted across brain regions in response to aged and young blood exposure. Notable, we identified a core microglia activation signature including 13 genes (*Fth1, B2m, Cd9*, *Fcer1g, Selplg* etc.) that were upregulated in aging, old blood exposure, and across all reactive microglial populations within all brain regions tested. This points to a context-independent, conserved mechanism regulating microglial reactivity. This work highlights the regional variation in microglial subpopulation transcriptomic dynamics during aging and under rejuvenation conditions, providing insights into differential brain regional vulnerability and offering potential avenues for microglia-targeted modulation of brain aging.

Poster #B3: (A. Ammothumkandy et al.)

Role of Immature Neurons and Astroglia in modulating Neuronal hyperactivity and Cognition in Human Epilepsy patients

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Neuronal hyperactivity is a pathological hallmark of several brain disorders, with its most pronounced manifestation occurring in epilepsy. In addition to causing debilitating seizures, neuronal hyperactivity is associated with progressive cognitive decline. Therefore, there is an urgent need to identify new mechanisms and therapies that modulate neuronal hyperactivity and cognitive decline in the human brain. Mesial temporal lobe epilepsy (MTLE) is the most common adult epilepsy with 70% of patients being pharmacoresistant. MTLE is attributed to abnormal local firing in the hippocampus, a specific niche within the brain where newborn neurons and astroglia modify existing neural circuitry. Altered hippocampus neurogenesis can initiate epileptic seizures in rodent models through cell migration and formation of aberrant synaptic connections. In addition, chronic temporal lobe seizures result in sustained epileptic hallmarks including gliogenesis, a decline of the stem cell pool, and memory impairment. Yet, the role of aberrant neurogenesis and gliogenesis in human MTLE is unexplored due to technical limitations. A small percentage of pharmacoresistant MTLE patients undergo surgical resection of the hippocampus which provides seizure freedom in 85% of the cases. This resected tissue provides a unique window to investigate mechanisms driving the disease. Using MTLE resections, we have identified that neurogenesis declined with increasing disease duration and neuronal hyperactivity (Ammothumkandy et al 2022, Nature Neuroscience). Further, the decline in neurogenesis correlated with verbal learning impairment in epilepsy (Ammothumkandy et al 2025, Cell Stem Cell). Our findings provide the first cellular evidence of how adult neurogenesis corresponds with human cognition and signifies an opportunity to advance regenerative medicine for patients with MTLE and other cognitive disorders. In addition to neurogenesis, we observed immature astroglia persistently present during epilepsy progression. Immature astroglia activity anticorrelates with neuronal hyperactivity, suggesting a potential role for these cells in seizure modulation. Additionally, higher number of immature astroglia is associated with a decline in intelligence. Immature astroglia may represent a promising cellular target for novel epilepsy therapies, with the potential to address both seizures and cognitive decline. In conclusion, our study provides essential evidence highlighting the role of immature neurons and astroglia as key cellular contributors and therapeutic targets for alleviating neuronal hyperactivity and cognitive decline in human neurological disorders.

Poster #B4: (J. Lim et al.)

Nuclear factor one transcription factors regulate radial glia differentiation during early cortical development

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The nuclear factor one (NFI) transcription factors Nfia, Nfib, and Nfix are highly expressed in most cell types during cortical development and in the adult cortex. Haploinsufficiency of any one of these genes in humans results in neurodevelopmental syndromes that are characterized by macrocephaly, intellectual disability, corpus callosum dysgenesis and enlarged lateral ventricles. The similarities of these syndromes imply that these genes have overlapping functions during cortical development. Previous studies that examined mouse models with deletion of one or two Nfi genes have established a basis for understanding how these phenotypes may arise, but the widespread expression of these genes in most cell types as well as their potential ability to compensate for each other have made it challenging to uncover the cellular and molecular mechanisms that are regulated by NFI. To overcome these challenges, we crossed a triple conditional knockout (cKO) mouse model where all three of these genes are deleted to an Emx1-Cre mouse line. We analyzed this mouse model using a combination of histology and single-cell multi-omics and found that Nfi genes regulate the transition of neuroepithelial cells into radial glial cells during early cortical development. This defect resulted in a lateral expansion of the ventricular zone and a thinner cortical plate at developmental stages. Neurogenesis still occurred but was delayed and there was an enlargement of the marginal zone and defects in cortical layering overall. Furthermore, cortical neurons in the Emx1-Cre-driven Nfi triple cKO mouse model, which deleted Nfi genes in neuroepithelial cells, ectopically expressed preplate markers. This phenotype was absent when we used the NEX-Cre driver line to delete Nfi genes only in post-mitotic neurons. The difference in phenotypes suggested that the impaired transition of neuroepithelial cells to radial glial cells results in the formation of ectopic marginal zone cells. Moreover, we did not observe this phenotype when four or less Nfi alleles were deleted. Therefore, our Nfi triple cKO mouse model reveals new insights into the function of Nfi genes in cortical development that cannot be studied in single and double knockout mouse models.

Poster #B5: (L. Sun et al.)

A genome-wide CRISPR screen identifies novel players in central nervous system myelination

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Myelination provides critical insulation and trophic support for axons in the nervous system. Apart from demyelinating diseases such as multiple sclerosis, dysmyelination is also associated with a variety of neurological disorders, including autism spectrum disorder and Alzheimer's disease. Despite its importance in brain function, we know surprisingly little about how oligodendrocytes, long-lived myelinating glia in the central nervous system (CNS), develop and maintain myelin integrity throughout adulthood. To identify new genes regulating myelination, we developed a new platform for a genome-wide CRISPR-Cas9 knockout screen on primary murine oligodendrocytes. This viability screen covers 19,674 mouse genes and revealed several new pathways involved in oligodendrogenesis and myelination, including the mitochondria oxidative phosphorylation, glycosylphosphatidylinositol (GPI)-anchor biosynthesis, and mRNA splicing pathways. Among the hits, we identify Rad54l2 as a critical gene required for developmental myelination and myelin maintenance. Conditional deletion of Rad54l2 from oligodendrocyte lineage cells causes significantly reduced oligodendrogenesis, which is partially resolved during adulthood. Moreover, loss of Rad54l2 leads to progressive and irreversible myelin degeneration and demyelination independent of oligodendrogenesis. Further mechanistic studies suggest that Rad54l2, a DNA helicase, acts through R-loop resolution and DNA damage repair pathway to safeguard oligodendrocyte genome stability during protracted myelination. Importantly, conditional deletion of Rad54l2 in Purkinje cells causes progressive neurodegeneration and motor function deficits, suggesting that both long-lived cells, neurons and oligodendrocytes, share the same molecular machinery to maintain cell integrity. Taken together, our work discovers novel players in CNS myelination, revealing the convergent and divergent mechanisms governing neural integrity in distinct CNS cell types.

Poster #B6: (L. Otsuka et al.)

Glial Cell Coordination of Dopaminergic Development and Disease

Lauren Otsuka and Emiliana Borrelli

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Over 15 million adults in the United States alone suffer from severe mental illnesses. Among these, schizophrenia (SZ), a chronic neuropsychiatric disorder, typically manifests in adolescence and affects as many as 1 in every 300 worldwide. Despite this relatively common rate of occurrence, the etiology of SZ remains largely unknown. Due to the efficacy of neuroleptics, which primarily target dopamine D2 receptors (D2Rs), chronic alterations in dopamine (DA) signaling have long been implicated in the genesis of SZ. Similarly, drugs of abuse such as methamphetamine induce SZ-like psychotic states through disruption of dopaminergic transmission throughout the brain. This heterogeneity in the onset of psychotic disorders suggests a combination of developmental, environmental, and epigenetic factors in the etiology of SZ. Therefore, we utilize a unique transgenic mouse model (DA-D2RKO mice) in which DA neuron-specific ablation of presynaptic D2Rs leads to loss of control over DA release, widespread epigenetic reprograming of cortical neurons, and psychotic-like behavioral phenotypes. Given the well-established functions of glial cells in postnatal brain development and plasticity, we sought to characterize the brain regions and glial cell types most affected by congenital dysregulation of DA signaling. Retrograde tracing along DAergic circuits and excitatory dendritic spine analyses highlight the crucial role of DA signaling in proper neuroanatomical development. Morphological and molecular study of astrocytes and microglia along these pathways further implicate DA release as a primary driver of glial synaptic tuning during early postnatal development. Finally, we identify demyelination and oligodendrocyte dysfunctions in DA-D2RKO mice parallel to what is observed in human SZ patients. Taken together, we provide evidence of a coordinated glial cell response to DA signaling that, for better or worse, shapes dopaminergic circuitry in the developing brain.

Poster #B7: (Y. Huo et al.)

The Traditional Chinese Medicine Ming'an Decoction Regulates Glycolysis and Polarization of Brain Microglia by Activating KCNQ2/3 Channels to Alleviate Tinnitus in Rat

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Tinnitus is a refractory disease that threatens human physical and mental health, and there is currently no effective drug. Microglial polarization in the medial geniculate nucleus (MGN) of the thalamus can lead to a neuroinflammatory response in the auditory pathway and induce tinnitus. Ming-An Decoction (MAD) is composed of 5 herbal constituents, which possess neuroprotective, antioxidant, calming properties. This study aimed to investigate the central regulatory mechanism of relieving tinnitus by MAD based on the KCNQ2/3 pathway from the aspects of functional metabolism in the Medial Geniculate Nucleus (MGN), glycolysis of microglia and polarization of M1/M2. Sodium salicylate (350mg/kg/d) (SS) and MAD were intraperitoneally injected into Wistar rats, and BV2 cells were treated with 5μg/mL SS and medicated serum of MAD. We found that the inhibition rate of GPIAS in the MAD group was increased, and the SUV of MGN brain region based on small animal PET-CT images was decreased compared with the SS group. The EP/C and PL/C values in the microglia skeleton of MGN brain in the MAD group were increased. The expression level of CD206 were significantly up-regulated and CD86 were down-regulated after dealing with MAD medicated serum. The mRNA transcription and protein expression levels of KCNQ2, KCNQ3 and Glut1 increased. Also, the activities of glycolysis-related enzymes and the contents of K + , glucose and lactate decreased after dealing with MAD medicated serum. Ming An Decoction may mediate the pathogenesis of tinnitus by inhibiting glycolysis and inducing microglial M2 polarization through activating KCNQ2/3 channels, thereby inhibiting neuroinflammatory response. Further studies are necessary to elucidate the mechanism of action of MAD on neuroprotection in the SS-induced tinnitus model.

Poster #B8: (C. Kuan et al.)

Revisiting the Lineage Potential of Radial Glia Cells in the Murine Cerebral Cortex

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The mammalian cerebral cortex has a myriad of neuronal and glial cell-types that expand at different times in a fixed order. While it is generally assumed that radial glial (RG) sequentially derive these diverse neuroglial cell-types, recent studies and our findings are challenging this view. Here we present two sets of evidence. First, we performed scRNA-Seq of 49,731 sorted Nestin+ progenitors and their nascent progeny from six ages of Nestin-EGFP embryos/mice (E12, E14, E16, E18, E20/P0, and P3) for cell clustering, ontogeny projection, and the RNA velocity field analysis. This analysis showed 34 clusters including two MKi67-enriched centers that match to E12/E14 Nestin+ cells and E18/P0 Slc1a3/Glast+ RGs. RNA velocity analysis suggests that that Nestin+ clusters give rise to Neurog2+, Tbr1, and Satb+ cortical neurons, while Slc1a3/Glast+ RGs derive Aqp4+ astrocytes, Pdgfra+ oligodendrocytes, and Neurod1+ postnatal neurons. Moreover, the E12/E14 Nestin+/Sox+ progenitors can be sub-divided into Ror2+ and Ror2- cells with differentially expressed genes, e.g. Ror2+ cells express Emx1/2 and Axin2, while Ror2- cells express Slc1a3 and Wnt5a (a ligand for Ror2). Second, we used tamoxifen-induced fate-mapping to compare the lineage potential between Nestn-CreER and Glast-CreER progenitors. This experiment showed that Nestin-CreER+ progenitors generate all neuroglial cell-types, whereas Glast-CreER+ RG derive astrocytes, oligodendrocytes and postnatal neurons, but not embryonic cortical or hippocampal neurons. Together, our results suggest the existence of early-diversified neuron-restricted progenitors (NRPs) in the murine cerebral cortex, which may be marked by the co-expression of Nestin/Sox2/Ror2.

Poster #B9: (A. Cebrian-Silla et al.)

Neural Stem Cells Beyond the Ventricles: B2 Cells in Mouse and Humans

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Neurogenesis and gliogenesis continue in the ventricular-subventricular zone (V-SVZ) of the lateral ventricles in adult rodents. V-SVZ astroglial cells with apical contact with the ventricle (B1 cells) function as neural stem cells (NSCs). B1 cell numbers sharply decline during early postnatal life; in contrast, neurogenesis decreases more gradually.

Here, we characterize a second population of astroglia (B2 cells) that lack contact with the ventricle but also function as NSCs in the adult mouse brain. B2 cell numbers increase postnatally, are maintained in adulthood, and decrease with aging. We reveal the transcriptomic profile of B1 and B2 cells and show that, like B1 cells, B2 cells can exist in quiescent or activated states. Transplantation and lineage tracing experiments demonstrate that B2 cells act as primary progenitors supporting adult neurogenesis. Similar to mice, we find that the human V-SVZ contains B1- and B2-like cells in the perinatal V-SVZ. Transcriptomic analysis and spatial cell mapping of the human V-SVZ show that human B1-like cells decline sharply within the first months of life, while a B2-like population appears to persist into adolescence. Together, these results reveal a novel population of NSCs in the adult mouse brain that sustains neurogenesis into adulthood and suggest the presence of a similar population in the early postnatal human brain.

Poster #B10: (A. Kipcak et al.)

Development of Cell Type-Specific Viral Tools for Tree Shrew Midbrain Circuits

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The superior colliculus (SC) is an evolutionarily conserved midbrain structure, the function of which has adapted across species to meet specific sensory demands and evolved with the emergence of the cerebral cortex. Despite the conservation of four major SC cell types - Wide Field, Narrow Field, Stellate, and Horizontal - our understanding of their contributions to behavior remains limited, in part due to a lack of molecular tools applicable across species. Most available tools are developed in mice and rely on transgenic approaches, which are not practical in higher-order mammals. To start addressing this gap, we focused on Wide Field Vertical (WFV) neurons of the tectopulvinar pathway. These neurons receive retinal and cortical input through large dendritic arbors that span the SC and are implicated in motion detection and threat-related freezing behavior. Previous studies identified Nephronectin (NPNT) and Cerebellin 2 (CBLN2) as potential markers of WFV neurons in mice. Here, we show that CBLN2 - but not NPNT - is a conserved marker of WFV neurons in the tree shrew. Comparative analysis of the CBLN2 locus across mouse, tree shrew, and human revealed a 1.8 kb conserved region in the 5' UTR. This sequence was cloned and packaged into an AAV vector upstream of a GFP reporter (AAV-tsC2Pro-GFP). Following injection into adult tree shrew SC, the AAV labeled CBLN2+ cells with high specificity and sensitivity verified by RNA-FISH. Labeled cells displayed characteristic WFV morphological features and projected to the pulvinar. We also validated the effectiveness of this promoter to target WFV neurons in mouse SC, demonstrating its cross-species applicability. To enable intersectional strategies, we developed a bicistronic version of the virus that expresses Cre recombinase. This represents the first example of a viral tool derived from the tree shrew genome for cross-species, cell type-specific targeting. Our results provide a foundation for extending this approach to additional conserved cell types in the SC and other brain regions.

Poster #B11: (R. Madan et al.)

NeuroPathPredict: A data-driven approach to map Alzheimer's disease neuropathology distribution

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Alzheimer's disease (AD) affects over 6 million Americans, with a growing global impact and significant personal and societal costs (1). Its hallmark neuropathologies, amyloid-beta plaques, neurofibrillary tau tangles, and TDP-43 inclusions, exhibit complex and spatially heterogeneous brain distributions (2)(3). Yet, current tools for mapping these aggregates are limited: PET imaging can only detect a single pathology per scan and suffers from off-target binding and limited resolution, while histopathology, though precise, is typically confined to a few sampled regions due to cost and resource constraints. To address this, we introduce NeuroPathPredict (NPP), a novel spatial distribution prediction pipeline that leverages post-mortem data and geospatial modeling to reconstruct high-resolution brain-wide maps of neuropathological (NP) burden. NPP integrates quantitative pathology data from sampled brain regions (e.g., the middle frontal gyrus (MFG)) and aligns it with high-resolution ex vivo MRI using a custom neuroimaging transformation pipeline. It then models NP distribution patterns using elastic net regression, dimensionality reduction, and Universal Kriging (4), a spatial prediction technique adapted from environmental sciences. In a proof-of-concept analysis using data from a single brain region (MFG) from 10 participants of the ACT study (5), NPP demonstrated strong predictive performance for tau pathology (percentage area positive for tau antibody AT8 in a histological tissue section), achieving an RMSE of 0.162 and an R^2 of 0.94 under multi-fold spatial block cross-validation. The variogram model captured substantial spatial autocorrelation (range: 11 mm; sill: 0.86), supporting the feasibility of generalizing predictions beyond sampled regions. Compared to a standard linear regression model using the same covariates (RMSE: 0.87; R^2: 0.24) and a 10-fold cross-validated Elastic Net model (RMSE: 0.61; R^2: 0.62), the inclusion of spatial autocorrelation through kriging yielded a significant performance improvement, underscoring the critical value of incorporating spatial models. Our results suggest that NPP can reliably approximate NP distributions in unsampled brain regions using limited histopathological data, providing a scalable and extensible framework for brain-wide NP mapping. Beyond reconstruction, NPP also enables new lines of inquiry, such as exploring interactions between multiple pathologies, characterizing distinct distribution patterns across AD subtypes, and probing the underlying mechanisms of NP spread in the human brain.

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Poster #B12: (X. Ruan et al.)

Integrating Next-Generation Lattice Light Sheet Microscopy and Scalable Computation for Nanoscale Brain Imaging

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Unraveling rapid subcellular dynamics and intricate nanoscale organization within complex brain circuits requires pushing the boundaries of imaging and analysis capabilities. We present a new frontier in biological imaging by combining advancements in lattice light sheet microscopy (LLSM) with powerful, scalable computational tools. Our next-generation LLSM platform enables high-speed, high-resolution, large-field, and multimodal imaging while minimizing photobleaching and photodamage, thereby facilitating the capture of nanoscale structures across large brain regions. To handle the massive datasets generated by this technology, we developed PetaKit5D, a high-performance software package, optimized for real-time visualization and analysis of petabyte-scale image data. PetaKit5D incorporates novel algorithms for critical image processing tasks including image I/O, geometric transformations, stitching, and deconvolution, achieving speeds over ten times faster than existing tools. By integrating these technologies with expansion microscopy, we achieved the first complete, continuous nanoscale imaging of intact mouse olfactory bulbs from both wild type and a neurodegenerative disease model. Each sample generated about half a petabyte of data, efficiently processed using PetaKit5D. Comparative analysis revealed distinct, layer-specific myelination changes associated with neurodegeneration. This integrative approach, bridging cutting-edge optical microscopy, advanced computational methods, and large-scale expansion microscopy, opens new avenues for high-throughput, nanoscale interrogation of brain structure and pathology.

Poster #B13: (Z. Fu et al.)

A highly stable monomeric red fluorescent protein for advanced microscopy

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The stability of fluorescent proteins (FPs) is crucial for imaging techniques such as live-cell imaging, super-resolution microscopy, and correlative light and electron microscopy (CLEM). Although stable green and yellow FPs are available, stable monomeric red fluorescent proteins (RFPs) remain limited. Here, we developed an extremely stable monomeric RFP named mScarlet3-H and determined its structure at 1.5 Å resolution. mScarlet3-H exhibits remarkable resistance to high temperature, chaotropic conditions, and oxidative environments, enabling efficient CLEM imaging and rapid (less than 1 day) whole organ tissue clearing. Additionally, its high photostability allows long-term 3D structured illumination microscopy imaging of mitochondrial dynamics with minimal photobleaching. It also facilitates dual-color live-cell stimulated emission depletion imaging with a high signal-to-noise ratio and strong specificity. Systematic benchmarking against high-performing RFPs established mScarlet3-H as a highly stable RFP for multi-modality microscopy in cell cultures and model organisms, complementing green FPs for multiplexed imaging in zebrafish, mice, and Nicotiana benthamiana.

Poster #B14: (J. Zheng at al.)

Leveraging LLMs and AI Agent Networks for Community based Gene Set and Cell Type Annotation

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Single-cell RNA sequencing has transformed our ability to identify diverse cell types and their transcriptomic signatures. However, annotating these signatures—especially those involving poorly characterized genes—remains a major challenge. Traditional gene set analysis methods, such as Gene Set Enrichment Analysis (GSEA), rely heavily on well-curated annotations and often underperform in such contexts. Large Language Models (LLMs) offer a promising alternative but struggle to represent complex biological knowledge within structured ontologies. To address this, we present a novel approach that integrates free-text descriptions with ontology labels for more accurate and robust gene set annotation. Our method outperforms state-of-the-art tools, correctly annotating over 68% of gene sets within the top five predictions. By incorporating retrieval-augmented generation (RAG), we developed a robust agentic workflow that refines predictions using relevant PubMed literature to reduce hallucinations and enhance interpretability. Using this workflow, we annotated 5,322 brain cell clusters from the complete mouse brain cell atlas generated by the BRAIN Initiative Cell Census Network, creating a valuable resource to support community-driven cell type annotation efforts.

Poster #B15: (K. Baroujeni et al.)

KIASORT: Knowledge-Integrated Automated Spike Sorting for Multi-Channel Neural Recordings

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Identifying and distinguishing single units from extracellularly recorded neural signals is a key step towards understanding brain circuit dynamics. Over the past decade, with the exponential growth of large-scale neural recordings (e.g., Neuropixels probes), the demand for efficient, precise, and automated methods to parse and sort neural data has become increasingly critical. Existing approaches, however, often face challenges in meeting this demand. These challenges include variability in channel signal quality, cross-contamination of spikes, neuron-specific waveform drifts, and nonlinear temporal changes in spike shapes and polarities related to differences in neuronal morphology and electrode proximity. Here, we introduce a novel spike sorting algorithm specifically designed to address these issues. Our algorithm evaluates channel signal quality to automatically exclude noisy recordings, adaptively sets detection thresholds, robustly extracts spike waveforms, and identifies multichannel spike classes using nonlinear dimensionality reduction combined with channel-specific classifiers. It then integrates knowledge acquired from the evaluation process to achieve sorting of neural data with high precision. Tested on ground-truth simulated multichannel datasets under variable noise and neuron-specific drift conditions, our approach outperformed the current state-of-the-art Kilosort4 methods across several metrics. Specifically, our neuron-based tracking method yielded approximately 10% higher precision and a 5–15% increase in high-quality units (defined as units with an F1-score > 0.75) compared to Kilosort4, especially under conditions of heterogeneous, neuron-specific drift and pronounced nonlinear waveform changes. These performance enhancements were achieved with computational times comparable to alternative approaches (real-time sorting of simulated Neuropixels data). In addition to its automatic sorting capabilities, the algorithm provides an intuitive graphical user interface (GUI) that integrates data inspection, spike sorting, and fine-tuning within a cohesive, user-friendly platform, enabling flexible and comprehensive post-hoc manual curation.

Poster #B16: (W. Wang et al.)

ONTraC characterizes spatially continuous variations of tissue microenvironment through niche trajectory analysis across scales

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Recent technological advances have enabled simultaneous profiling of spatial coordinates and molecular features at cellular resolution across intact brain tissues. However, existing computational methods largely focus on domain-based segmentation and inter-domains differences, often overlooking the continuous variation within individual domains. To address this limitation, we introduce ONTraC, a graph neural network-based framework for niche trajectory analysis that models spatially continuous microenvironmental variations.

Unlike traditional approaches that cluster cells into discrete spatial domains, ONTraC introduces a niche-centric representation-where each “niche” is defined by a spatially localized group of cells—and infers one-dimensional trajectories that capture structural and regulatory gradients across the tissue. By leveraging a sparse graph neural network architecture to integrate spatial and molecular information, the framework enables efficient analysis of large-scale datasets comprising over one million cells from more than 100 tissue slices.

To assess its performance, we benchmarked ONTraC against five established spatial and pseudotime trajectory methods. Across multiple simulated datasets and a real mouse motor cortex dataset, ONTraC consistently demonstrated superior accuracy and robustness. It achieved higher correlation with spatial ground truths, showed improved sensitivity in capturing intra-domain heterogeneity, and was less affected by lineage-dependent biases. These results highlight ONTraC's advantage in preserving spatial continuity while resolving functional gradients.

We applied ONTraC to a whole mouse brain spatial transcriptomics dataset, where it enabled modular trajectory inference by decomposing tissue into biologically meaningful subregions. These resulting spatial trajectories revealed gradual transitions in microenvironment context, gene expression, regulon activity, and predicted cell-cell interaction patterns. These features collectively contribute to a comprehensive landscape of the cellular status dynamics, linked local environment and molecular features.

In summary, ONTraC provides a scalable and flexible framework for decoding spatial and regulatory architecture in neural tissues, offering a powerful tool for investigating how the microenvironment shapes development, function, and disease.

Poster #B17: (M. Cui et al.)

High-throughput large-scale deep-brain calcium imaging solutions

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Despite the success of genetically encoded functional indicators, most applications have been confined to the mouse neocortex. Accessing brain regions deeper than 1 millimeter remains challenging due to the random scattering of light in brain tissue. Currently, the preferred method for deep brain imaging relies on GRIN (Gradient Refractive Index) lens-based techniques. However, GRIN lenses suffer from significant inherent aberrations, which severely limit their imaging field of view (FOV). To overcome this limitation and enable large-volume two-photon calcium imaging through 0.5 mm GRIN lenses, we developed a novel objective lens specifically designed to correct these aberrations. This new design increases the imaging FOV by approximately 400%. We will present our latest volumetric calcium imaging results using this approach and discuss pathways for widespread adoption. One-photon wide-field imaging through GRIN lenses is also commonly used for deep-brain calcium recordings, especially in freely moving animals using head-mounted systems. Like two-photon systems, these recordings are also restricted by the inherent aberrations of GRIN lenses. We recently developed a simple solution that maximizes soma-resolution FOV, nearly matching the entire facet of the GRIN lens (~100%). We will describe its implementation for both head-fixed and freely moving animal studies, along with strategies for broader dissemination. To further expand the imaging volume beyond the capabilities of GRIN lenses, we introduced a fundamentally new approach: the Clear Optically Matched Panoramic Access Channel Technique (COMPACT), which enables panoramic imaging via thin-walled glass capillaries. Over the past few years, we have made significant progress, culminating in the development of COMPACT 2.0. This next-generation system offers several major improvements. First, the probe diameter has been reduced from 1 mm to 0.5 mm to better match the dimensions of the mouse brain, reducing tissue damage to one-quarter of the original volume. Second, we integrated a 12 kHz resonant galvo scanner to boost imaging throughput by a factor of 24. Third, the imaging probe can now move, spinning and translating during recording, which enables circular, vertical, and spiral scanning modes. These enhancements allow flexible, near-simultaneous recordings from large mesoscopic brain volumes. We will share the latest results from mesoscopic deep-brain calcium imaging using COMPACT 2.0 and discuss avenues for broad implementation of this technology.

Poster #B18: (X. Lu et al.)

A novel approach to map and modulate brain genomic instability across the lifespan: Implications in accelerated brain aging, and deep space exploration

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Genomic instability (increased frequency of mutations in a genome) is recognized as the foremost of the twelve hallmarks of aging. Neuronal genomic instability is featured in both aged individuals and those suffering from NDDs like AD, as shown by single-neuron sequencing studies in human brains. Neuronal DNA double-strand breaks lead to genome structural variations and 3D genome disruption in neurodegeneration. However, the mechanistic understanding and causal links between brain genomic instability, cognitive decline, and neurodegenerative disorders remain largely unclear. To map and provide genetic access to cells with genomic instability, a real-time genetic and bioluminescent sensor was developed to non-invasively trace ground-simulated brain genomic instability in vivo. Such an approach offers sufficient spatial and temporal resolution and sensitivity to disentangle the cause-and-effect relation across the lifespan in the brains of natural aging and in neurodegenerative disorders. A genetic Probe with a viRal proxy for the Instability of DNA surveillance/repair in Somatic brain Mosaicism (PRISM) sensor exploits long-standing observations that genotoxic DNA damage can significantly increase permissivity to AAV transduction due to the role of host cell DDR as an innate antiviral defense mechanism that is inhibitory to viral life cycles. We harnessed the single-strand recombinant Adeno-associated virus (rAAV) genome-processing mechanism and the instability of a hypermutable repeat sequence to detect brain genotoxic stress and visualize neuropathology and neurodegeneration at single-cell resolution. PRISM sensor mapped genomic instability in environmental and genetic models of Parkinson's disease (PD) and revealed that genomic instability is a preliminary feature of PD. We also generated the first-ever brain map of genotoxic instability in mice exposed to Moon and Mars mission-related doses of Galactic Cosmic Radiation (GCR). Our study revealed that the space exposome (stressors encountered during space exploration (e.g., altered gravity, ionizing radiation, low magnetic fields, elevated concentrations of carbon dioxide, etc.) converges on genomic instability to accelerate brain aging. Furthermore, a single-cell sensor/actuator strategy was developed for precise genetic perturbation selectively in the cells burdened with DNA damage. Casual pathogenesis was established via a genetic inhibition of overactive DNA Damage response only in cells with genomic instability, rescued α-Synuclein aggregates spreading, and cognitive and behavioral deficits in a progressive model of Lewy Body Dementia. Our approach will advance technological and conceptual innovation to improve repeated measures across longer epochs of the lifespan in the aging brain, AD/ADRD, and animal models exposed to environmental risk and resilience factors. Understanding the time course and mechanisms involved in the initiation and progression of accelerated aging and dementia may lead to potential therapeutic targets. Single-cell modulation in DNA damage-burdened cells may reveal the causal pathogenic mechanism of genomic instability, which may hold potential for "single-cell precision medicine."

Poster #B19: (L. Chen et al.)

Production, Purification and Quality Control for Enhancer Adeno-associated Virus-based Vectors

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A key challenge in the improvement of recombinant AAVs (rAAV) as tools for neural circuit mapping and studying is the expression of gene of interests in a cell-specific fashion. Early regulation systems of viral expression of the gene of interest heavily relied on the use of specific AAV serotypes, and on the usage of specific promoters. This system is very efficient but shows limit in the extent of the cell specificity it can achieve. Recent studies have extended the system to couple promoters with enhancer to enhance the specificity of gene regulatory elements to improve specificity of gene of interest expression to more restricted populations (Class/subclass/type/subtypes). Enhancer AAV vectors are engineered AAVs, designed to deliver transgenes selectively to specific cell populations by incorporating regulatory cell-type-specific enhancer elements within the viral genome. These enhancers selectively upregulate the genetic payload expression in targeted cell types using regulatory elements expressed in targeted cells of interest, allowing for precise control over gene delivery and expression. In the present study we introduce and present the production pipeline and workflow for enhancer AAVs at the UCI Center for Neural Circuit Mapping viral vector core facility, which is supported by the BRAIN Initiative Armamentarium as a production and distribution facility for brain cell-type-specific access reagents. Our collaborative group has developed a set of new enhancer AAV vectors that specifically target subclasses of brain and spinal cord cell types, including brain endothelial cells (BEC), forebrain inhibitory neuron subtypes and spinal cord prodynorphin (PDYN)-expressing inhibitory cells. Following identification of candidate cisregulatory elements identified from single-cell epigenetic datasets, such as CATlas, and pipeline PIASO to identify the enhancer sequences, a putative enhancer is genetically incorporated in the rAAV genome and recombinant viruses packaged using AAV-PHP.eB capsid for efficient transport across the BBB and high efficiency of target cell transduction. rAAV genome consists of the putative enhancer, minimal basal promoter, reporter sequences and regulatory elements. These elements are flanked by inverted terminal repeats (ITR) to recapitulate encapsidation and expression of gene of interests following viral infection. Viral production is performed using AAV293 cells, a stable cell line designed for efficient rAAV production. A co-transfection using 3 plasmids is carried out: (i) a plasmid harboring the rAAV genome, (ii) a plasmid expressing the AAV PHP.eB capsid proteins, and (iii) a helper plasmid providing Adenoviral proteins. rAAV is harvested at 5 days post-transfection; cells and media are collected and viruses are concentrated and/or purified. The resulting rAAV meeting our quality checks (viral titer + purity) are validated in primary mouse/rat neuronal cultures for infectivity and reporter expression. Validated candidates are then used for retro-orbital (RO) injection for in vivo confirmation of sensitivity and specificity of the reporter(s) expression. Brains and/or spinal cords are collected at 4 weeks post-RO injection to determine celltype-specific labeling and genetic access. In vitro and in vivo validation data for AAV products will be made available at the portal at the UC Irvine CNCM website, prior to dissemination to neuroscience researchers. We expect that enhancer AAV technologies have the potential for transformative and translational neuroscience research by bringing the precision of molecular targeting to specific cell types and neural circuits that underlie behavioral function.

Poster #B20: (J. Rink et al.)

Strategic Enhancements in Human Brain Sample Processing for the Development of a Spatially-Resolved Multi-omic Single-Cell Atlas

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While major cell type categories in the human brain are well defined, a deeper understanding of the molecular diversity of human brain cell types demands an atlas encompassing subtype-specific gene expression, epigenomic regulatory systems, spatial distribution, and developmental lineage. The Center for Multi-omic Human Brain Cell Atlas employs innovative multi-omic sequencing techniques, Paired-Tag and snm3C, alongside spatial transcriptomics with the goal of analyzing 125 distinct brain regions from 12 post-mortem neurotypical human brains across three developmental stages.

To address losses in nuclei processing, collecting 1.25 million neuronal nuclei per sample was determined necessary for sufficient coverage of rare cell types. A sampling plan was devised to maximize dissection parcellation while providing enough tissue mass for spatial transcriptomics and optimal collection of neuronal nuclei. Brain regions with insufficient tissue or high glia/neuron ratios were pooled across multiple slabs or with adjacent regions. Tissue dissociation, nuclei isolation, and FANS conditions were optimized for each sample based on myelination, cell density, and neuron-to-glia ratio to maximize neuronal nuclei recovery.For the first four brains processed of 12 in this study, donor brains from a 50-year-old male, a 47-year-old male, a 63-year-old female, and a 67-year-old female were sectioned into 4 mm thick coronal slabs and then aligned to the Allen Institute Adult Human Brain Atlas. For each brain, around 150 tissue blocks including 125 distinct brain regions were dissected and then combined into 70 unique samples. From the first 256 samples processed, a total of 5.1 billion nuclei were isolated from 103 grams of ground human brain tissue. A total of 309 million neuronal nuclei were collected by FANS using a NeuN-488 conjugated antibody as a marker of neuronal nuclei, with a significant portion processed for Paired-Tag (RNA and histone modifications) and snm3C (HiC and DNA methylation) sequencing. In conclusion, the development of strategic brain processing techniques is crucial to profiling the epigenome of rare cell types. This work underscores the importance of methodological advancements in facilitating complex neuroscientific research and advancing our understanding of brain cell diversity and function. Future studies can build upon these strategies to further refine brain processing methodologies, increase anatomical resolution of brain samples, and enhance atlas construction efforts.

Poster #B21: (M. Paquet et al.)

Engineering a genetically-encoded D-serine biosensor for studying its role in neurotransmission

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The last two decades have seen a growing interest in the role of D-amino acids within the nervous system. In particular, D-serine is now recognized as a key neuromodulator in its role as a more potent co-agonist than glycine for N-methyl-D-aspartate receptors (NMDARs), which are widely recognized as the key receptor responsible for synaptic plasticity. Accordingly, aberrations in D-serine signalling have been consistently associated with several pathological conditions, including schizophrenia, Alzheimer's disease and epilepsy. However, despite our understanding of D-serine's role in the nervous system, the molecular mechanisms that govern its dynamics remain unclear, with recent works challenging its role as a gliotransmitter. Thus, a more thorough understanding of D-serine dynamics is necessary to properly understand its role in both healthy and disease states. To address these gaps in knowledge, tools with the requisite spatiotemporal resolution, such as genetically encoded fluorescent protein-based indicators, are necessary to monitor D-serine dynamics. To date, the only genetically encoded indicator for D-serine is a FRET-based indicator, called DserFS, based on a bacterial periplasmic binding protein (PBP). PBPs are ideal scaffolds for sensor engineering because they are orthogonal to neurons, offer large changes in fluorescence in response to ligand binding and can be targeted to arbitrary cellular compartments. However, DserFS shows a limited dynamic range relative to single fluorescent protein-based indicators and requires exogenous addition for imaging in brain slices. Here we present our work on engineering a genetically encoded single fluorescent protein-based indicator for D-serine from DserFS. Indeed, preliminary results indicate that our D-serine sensor shows large fluorescence changes with micromolar affinities and good membrane localization. We anticipate that our new D-serine indicator will open new avenues for investigating D-serine dynamics within the nervous system.

Poster #B22: (J. Berry et al.)

UC Irvine's Brain Initiative Cell Atlas Network (BICAN) Brain Procurement Program for The Center for Multiomic Human Brain Cell Atlas Project

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Obtaining neurotypical postmortem brain tissues from donors with broad backgrounds that reflect the general population is challenging, but is of critical importance for large human brain mapping projects. In this paper, we introduce the UC Irvine's Brain Initiative Cell Atlas Network Brain Donation Program and share our methods and protocols for neurotypical brain collection and sample preparations towards generating high quality human brain cell multiomic atlases. We have collected, analyzed, and characterized brain tissue samples from cognitively normal adult donors that represent the United States population to support the Brain Initiative Cell Atlas Network (BICAN) consortium's construction of a human multiomic brain atlas. Here we describe our human postmortem brain procurement process and challenges encountered with brain donations of cognitively normal adults between the ages of 2-65 years in Orange County, California. We have obtained donor brains with assistance from the OC Coroner's Office, UC Irvine Willed Body program, UCI Medical Center, and Children's Hospital of Orange County. Our early experience demonstrates both feasibility and challenges in collecting cognitively normal adult human brains from communities that accurately reflect the Southern California population. In spite of our goals, our data indicate sex, age, and race biases in our procurement pipelines. The Coroner's Office donors are predominantly male, younger, and of various ethnic/racial backgrounds, whereas the Willed Body donors are generally older and ethnically/racially white. UCI Medical Center's and the Children's Hospital of Orange County's donors are of various races and ages. We plan to expand our collection pipeline and further engage in outreach with racially and ethnically diverse communities to increase the representation in our brain donation program.Obtaining neurotypical postmortem brain tissues from donors with broad backgrounds that reflect the general population is challenging, but is of critical importance for large human brain mapping projects. In this paper, we introduce the UC Irvine's Brain Initiative Cell Atlas Network Brain Donation Program and share our methods and protocols for neurotypical brain collection and sample preparations towards generating high quality human brain cell multiomic atlases. We have collected, analyzed, and characterized brain tissue samples from cognitively normal adult donors that represent the United States population to support the Brain Initiative Cell Atlas Network (BICAN) consortium's construction of a human multiomic brain atlas. Here we describe our human postmortem brain procurement process and challenges encountered with brain donations of cognitively normal adults between the ages of 2-65 years in Orange County, California. We have obtained donor brains with assistance from the OC Coroner's Office, UC Irvine Willed Body program, UCI Medical Center, and Children's Hospital of Orange County. Our early experience demonstrates both feasibility and challenges in collecting cognitively normal adult human brains from communities that accurately reflect the Southern California population. In spite of our goals, our data indicate sex, age, and race biases in our procurement pipelines. The Coroner's Office donors are predominantly male, younger, and of various ethnic/racial backgrounds, whereas the Willed Body donors are generally older and ethnically/racially white. UCI Medical Center's and the Children's Hospital of Orange County's donors are of various races and ages. We plan to expand our collection pipeline and further engage in outreach with racially and ethnically diverse communities to increase the representation in our brain donation program.

Poster #B23: (A. Ribeiro Gomes et al.)

Targeting developmentally timed neuronal cohorts in the primate brain through prenatal rAAV gene delivery

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Understanding how early developmental events unfold to shape human brain organization has been hampered by limited genetic access to neurodevelopmental processes in strategic animal models, most notably primates. To overcome this bottleneck, we recently developed a fast, flexible and scalable method for widespread neuronal transduction in marmosets using ultrasound-guided fetal intracerebroventricular injections (FIVI) of recombinant adeno-associated virus (rAAV) (Ribeiro Gomes et al., 2025). Using FIVI, we showed that fetal rAAV injections enable transgene expression in distinct neuronal populations, which are specified based on the gestational timing of injection and, unexpectedly, the rAAV serotype. Notably, certain rAAV serotypes transduced cortical neurons in a manner that was time locked to neurogenesis, with earlier injections primarily labeling deeper-layer neurons and later injections targeting more superficial layers. To test the specific connection to neuronal birth timing, we administered rAAV via FIVI at the same time as an intraperitoneal administration of the thymidine analogue 5-ethynyl-2'-deoxyuridine (EdU). As the EdU label progenitors dividing within a narrow time window, this combined approach allows for the tagging of neurons born shortly after rAAV exposure and enables assessment of how the viral transduction aligns with neuronal birthdate. In rats, pilot experiments using the same approach indicated that rAAV2 exhibited the strongest correspondence with EdU-labeled cells, prompting us to focus on this serotype in our study. In marmosets, preliminary results reveal strong spatial overlap between EdU-labeled populations and rAAV2-transduced neurons, suggesting that, as in rats, rAAV2 preferentially targets neurons generated near the time of FIVI injection and could serve as a tool for time-specific gene delivery during neurogenesis. We are currently evaluating whether the spatial distribution of EdU-labeled and rAAV2-transduced populations aligns across brain regions and FIVI gestational timepoints, as well as characterizing the specific cell types targeted. This work will help develop strategies to deliver genetic tools to map and manipulate developmentally defined cells, thereby opening new opportunities to bridge developmental trajectories with brain function across the primate's lifespan.

Poster #B24: (B. An et al.)

Sets of protein barcodes for scalable expansion microscopy-based neural morphology measurement and connectomics

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Mapping neural circuits with synaptic resolution, ideally at a density sufficient to obtain detailed maps of local neural circuits is critical to understand neural function and dysfunction. However, it remains challenging to achieve a comprehensive map of such complex and compact brain architectures with electron microscopy, the dominant method currently used. We here report a technology that allows nanoscale resolution imaging of neural circuits in a scalable way, based on expansion microscopy (ExM) which enables nanometer resolution imaging through physical magnification of biological specimens. We developed a set of protein epitopes that can be safely expressed in combinations in neurons, so that each cell gets a unique combination of epitopes, and can be distinguished during serial staining, imaging, and washing steps. The technology is also compatible with immunostaining against synaptic and other proteins, for visualization of key biomolecules within and between neurons. We have now identified 14 epitope-labeled proteins that can be combinatorially expressed in random sets, e.g. via viral delivery, in mouse hippocampus and cortex. (In theory, n epitopes would enable ~2^n different neurons to be distinguished, as an upper bound.) These epitopes uniformly label neurons throughout axons and dendrites, and are mutually orthogonally stainable with different commercially available antibodies. With 4x-fold expansion, resulting in 70 nm resolution, and antibody staining against pre and postsynaptic markers, we anticipate that this toolkit will be easily deployed in everyday neuroscience for the mapping of sparse connectomes in the short term, and in combination with recent pan-protein staining based connectomics (Tavakoli, M.R., Lyudchik, J., Januszewski, M. et al. Nature, 2025) may help with the obtaining of dense, whole mouse brain connectomes.

Poster #B25: (J. Kang et al.)

Expansion Revealing (ExR): Multiplexed ExR (multiExR) for imaging multiprotein nanostructure/ ExR of Pathology (ExRPath) for resolving nanostructures in human brain tissue

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Proteins work together in nanostructures in many physiological contexts and disease states. We recently developed expansion revealing (ExR), which expands proteins away from each other, in order to support better labeling with antibody tags and nanoscale imaging on conventional microscopes. Here, we report multiplexed expansion revealing (multiExR), which enables high-fidelity antibody visualization of >20 proteins in the same specimen, over serial rounds of staining and imaging. Across all datasets examined, multiExR exhibits a median round-to-round registration error of 39 nm, with a median registration error of 25 nm when the most stringent form of the protocol is used. We precisely map 23 proteins in the brain of 5xFAD Alzheimer's model mice, and find reductions in synaptic protein cluster volume, and co-localization of specific AMPA receptor subunits with amyloid-beta nanoclusters. We visualize 20 synaptic proteins in specimens of mouse primary somatosensory cortex. multiExR may be of broad use in analyzing how different kinds of protein are organized amidst normal and pathological processes in biology. While ExR elucidates cellular organization by separating proteins within dense nanostructures, it requires fixation procedures incompatible with human pathology specimens. Here, we report ExR of pathology (ExRPath), which attains ~20 nm resolution and decrowding of such tissues. Applying ExRPath to COVID-19-decedent brain tissue reveals periodic amyloid nanoclusters that co-localize with SARS-CoV-2, with associated neuroinflammatory phenotype, pointing towards potential brain pathology associated with COVID-19.

Poster #B26: (W. Cao et al.)

A Suite of Enhancer AAVs for Genetic Targeting of Specific Hippocampal Formation Cell Types

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Despite the central role of the hippocampal formation (HF) in learning, memory, and spatial navigation, the functional dissection of excitatory neuron subclasses within this region has been constrained by a lack of cell-type-specific genetic targeting tools. Recently, enhancer adeno-associated viruses (AAVs) have emerged as a transformative approach for achieving cell-type-specific access. These tools offer flexible and cost-effective delivery and have cross-species utility and translational potential. While enhancer AAVs have been successfully developed for neocortical, striatal, spinal cord, and various non-neuronal cell types, efforts to generate comparable tools for the HF remain limited. To address this gap, we have developed and validated around 20 enhancer AAVs that drive expression of fluorescent reporters and Cre recombinase in defined cell types within the subiculum, the principal output structure of the HF. Our toolkit enables precise genetic access to subiculum subdomains across three major organizational axes—transverse, longitudinal, and radial axes. Notably, several of our enhancer AAVs selectively target transcriptomic subclasses and supertypes of excitatory neurons. Emerging evidence indicates that superficial and deep layers of the dorsal subiculum serve distinct roles in encoding spatial and object-related memories, yet the underlying circuit mechanisms remain poorly understood. Using our enhancer AAVs, we achieve selective labeling of these layers and reveal layer-specific input and output connectivity, providing new insight into their differential contributions to learning and memory. Collectively, our new suite of viral genetic tools may provide critical resources for cell-type-specific access and manipulation of HF neuron types based on their molecular identity, connectivity, and behavioral relevance.

Poster #C1: (J. Hsieh et al.)

Comprehensive Transcriptomic and Anatomical Neuron Classification of Mouse Brainstem Trigeminal Nuclei

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The brainstem trigeminal nuclei (TGN) serve as crucial sensory relays, processing inputs from the trigeminal ganglia and transmitting them to higher brain centers, such as the thalamus and primary/secondary somatosensory regions. Due to limited data on transcriptome and morphology, neurons subtypes residing in the TGN are not well classified. To fill this gap, we aim to comprehensively map the transcriptomic profiles and reconstruct neuron morphology of the mouse TGN.

We first performed single-nucleus RNA sequencing and revealed 35 distinct cell clusters in the lateral brainstem, with 17 clusters associated with known regions of the brainstem trigeminal nuclei (PsV, SpVI, SpVO, SpVC). Significant marker genes were identified and validated by multiplex in-situ mRNA profiling, enhancing our understanding of the specific molecular profiles within these clusters. To establish a molecular connectome atlas of the mouse TGN, we developed a novel protocol to enable multi-plex in-situ mRNA-and-protein co-profiling. Neuron morphology, synaptic contacts, and mRNA markers are profiled within large, expanded sample volumes, which resulted in the classification of molecular-and-structural classification of TGN neuron subtypes.

Our finding provides critical insights into the neuron cell types and organization of the trigeminal sensory system and lays the groundwork for further exploration of its role in sensory gating and associated behaviors.

Poster #C2: (B. Parasar et al.)

Whole-genome 3D architectural screen with in-plate chromosome conformation capture (Plate-C) reveals determinants of brain DNA structure in vivo

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Genome architecture is the foundation of gene regulation. While we discovered diverse DNA structures across cell types/states that functionally drive development, their biochemical determinants remain elusive because of a lack of scalable technologies and algorithms. Here we present in-plate chromosome conformation capture (Plate-C), a multiplex, cost-effective platform that profiles hundreds of whole-genome architectures. Plate-C enabled the first chemical screen for whole-genome structural changes, with 1,000+ measurements of 150+ epigenetic compounds. In post-mitotic neurons, we discovered 5 modes of chemically induced restructuring, including rapid induction of long-range heterochromatic contacts by histone deacetylase inhibitors. We validated this finding at single-cell level in vivo, demonstrating brain-wide genome re-wiring within hours in newborn mice that highly correlated with changes in vitro, partially mirrored juvenile-to-adult transition, and drove wide-spread transcriptome changes. Plate-C brings a new era to genomics research, where massively parallel profiling of whole-genome architecture reveals new principles of DNA folding and drug mechanisms.

Poster #C3: (S. Park et al.)

Spatially Resolved Transcriptional Remodeling of the Paraventricular Thalamus During Opioid Withdrawal

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The paraventricular thalamus (PVT) is increasingly recognized as a hub for processing internal states during opioid withdrawal, in part due to its dense glutamatergic projections to the nucleus accumbens (NAc), a key node in the mesolimbic pathway also known as the dopamine tract. However, the molecular architecture underlying its role in opioid withdrawal remains largely uncharacterized. Here, we applied MERFISH-based spatial transcriptomics to quantify changes in gene expression across anterior-posterior PVT subdivisions in mice undergoing opioid withdrawal. We further identify which of the PVT glutamatergic neurons are actively participating in the withdrawal circuitry using retrograde labeling, injected into the NAc as well as quantification of immediate early genes (IEGs), a proxy for neural activity. Combining these technologies, we determined that approximately 15% of the PVT neurons actively participate in the withdrawal pathway. We identified regionally localized transcriptional programs including stress adaptation and hormonal regulation (e.g. Mt2, Deptor), neurodevelopment and synaptic plasticity (e.g. Elavl4, Kank4), and Metabolic Processes (e.g. Elovl5, Carhsp1). These results highlight the spatially heterogeneous nature of PVT responses to withdrawal, bring us one step closer to identifying the functional genetic programming of withdrawal and suggest that subregional PVT populations may differentially encode affective withdrawal states.

Poster #C4: (F. Xie et al.)

Multi-omics profiling of the interplay between cell-type and vision-dependent genetic programs in primary visual cortex

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How sensory experience during early postnatal life affects the organization of the mammalian neocortex at the resolution of neuronal cell types is poorly understood. We previously reported that the functional and molecular profiles of layer 2/3 (L2/3) cell types in the primary visual cortex (V1) are vision-dependent [S. Cheng et al., Cell 185, 311–327.e24 (2022)]. Here, we characterize the spatial organization and epigenetic signatures of L2/3 cell types with and without visual experience in several developmental time points before and after eye opening. Spatial transcriptomic profiling based on 500 genes recapitulates the zonation of L2/3 cell types along the pial–ventricular axis in V1. Single-cell multiomics profiling based on ATAC-seq reveals graded changes in chromatin signatures of L2/3 cells that are highly correlated with graded changes in transcriptomic signatures. By applying multitasking theory, we suggest that the spatial zonation of L2/3 cell types is linked to the continuous nature of their gene expression profiles, which can be represented as a 2D manifold bounded by three archetypal cell types. By comparing normally reared and dark reared L2/3 cells, we show that visual deprivation-induced transcriptomic changes comprise two independent gene programs. The first, induced specifically in the visual cortex, includes immediate-early genes and genes associated with metabolic processes. It manifests as a change in cell state that is orthogonal to cell type-specific gene expression programs. By contrast, the second program impacts L2/3 cell-type identity, regulating a subset of cell type-specific genes and shifting the distribution of cells within the L2/3 cell-type manifold. Through an integrated analysis of spatial transcriptomics with single-nucleus transcriptomics and epigenomics data, we describe how vision patterns cortical L2/3 cell types and cell states.

Poster #C5: (K. Johnston et al.)

Multimodal Transcriptomic and Genomic Analysis of Suicide and Major Depression Patients Identifies Significant Gene Pathway Dysregulation

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Major depressive disorder (MDD), or clinical depression, is a neuropsychiatric condition defined by states of depressed mood among other negative symptoms. MDD is influenced by complex genetic and environmental factors which create variability in patient symptoms and outcomes. Patients diagnosed with MDD have higher risks of suicide, yet the biological mechanisms differentiating MDD patients with and without suicidality remain unclear. This study aims to categorize and analyze the transcriptomic and epigenetic alterations amongst different cell types in the brain in the context of MDD and suicidality. In this study, we profile 28 human prefrontal cortex (PFC) gender and approximate age-matched samples using multiomic snRNA-seq and snATAC-Seq, via the 10x platform, and an additional 8 PFC samples using the MERFISH spatial transcriptomics system. From this, we construct a comprehensive spatial map of gene expression, DNA accessibility, and spatial context variations in the brains of MDD patients with and without suicidality. Focusing primarily on interneurons, we identify significant discrepancies in expression in the brains of MDD patients, particularly with IEGs (immediate early genes), and HSPs (Heat Shock Proteins). GSEA analysis compared with gene ontology identified a large number of affected biological processes associated primarily with MDD, revolving around protein folding and stabilization, and alterations in DNA transcription and RNA translation as responses to stress. Additionally, we identified linked regions to expression of the differentially expressed genes. Comparison with ENCODE data indicates that many of the identified regions were annotated either as promoters or enhancers, with less than 30% not annotated, depending on cell type. Additionally, particularly in MDD-NS vs Control comparison, many of the linked peaks were differentially accessible in interneurons and non-neuronal cells. Finally, we complemented our analysis with spatial transcriptomics, to analyze the spatial location and distributional differences of interneurons in the brains of MDD patients (with and without suicidality) compared with control. This resulted in a spatial identification of the unique impacts of MDD on brain function.

Poster #C6: (J. Harberger at al.)

Multi-Modal Spatial Omics for Single-Cell Lipid, Protein, and Transcriptomic Mapping in the Brain

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Knight Initiative for Brain Resilience

At the Brain Resilience Lab, we have developed an integrated spatial method that allows us to visualize lipid bodies, gene transcripts, and proteins within the same tissue section at single-cell resolution. By combining sequential histological staining, spatial transcriptomics, and immunohistochemistry, this approach provides a high-resolution, multi-layered view of the molecular and cellular composition of brain tissue.

We are applying this method to study lipid droplets—unique organelles that accumulate in the aging brain and are found at higher levels in neurodegenerative diseases such as Alzheimer's. Their presence reflects changes in lipid handling and storage within cells, which may play an active role in disease progression. By mapping where and how lipid droplets accumulate, we aim to uncover how specific cell types and pathological features influence metabolic pathways in Alzheimer's Disease, offering new insight into the role of lipid metabolism in neurodegeneration.

Poster #C7: (A. Liu et al.)

Regional and Functional Diversity of Human Brain Vasculature at Single-Nucleus Resolution

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Understanding the human brain vasculature is essential for learning how the brain maintains internal balance and responds to signals from the body. A key part of this system is the blood-brain barrier (BBB), which plays a critical role in protecting the brain and regulating what enters from the bloodstream. However, our knowledge of human brain vasculature is still limited, due to sparse data, technical challenges in transcriptomic profiling, and the brain's regional complexity. In this study, we applied vessel isolation and nuclei extraction from sequencing (VINE-seq) to systematically profile the human brain vasculature using 147 anatomical dissections spanning 41 brain regions and major cerebral vessels from typical middle-aged individuals. We captured 501,033 high-quality nuclei across the human brain, encompassing major vascular cell classes such as endothelial cells, mural cells, and fibroblasts, along with region-specific populations like ependymal and epithelial cells localized to distinct brain areas. Transcriptomic profiles revealed substantial brain regional variation, with endothelial cells in the cerebral cortex supporting dendrite development and dicarboxylic acid transport pathways compared to those in white matter and the brainstem. Other vascular cell types, including mural cells and fibroblasts, also displayed region-specific transcriptional signatures, underscoring the structural and functional heterogeneity of the brain vasculature. We also identified two transcriptionally distinct subtypes of capillary endothelial cells distinguished by BBB transport functions. To further resolve the spatial architecture of vascular niches, we integrated spatially resolved single-cell transcriptomic data across multiple brain regions. Building on these findings, we propose the development of a brain vasculature AI model to integrate molecular profiles and existing vascular knowledge, aiming to decode BBB heterogeneity and inform targeted drug delivery strategies across distinct brain regions. Together, our study provides a comprehensive molecular atlas of the human brain vasculature and a foundation for future investigations into cerebrovascular function, pathology, and therapeutic targeting.

Poster #C8: (M. Heffel at al.)

Single-cell Multimodal Epigenomic Atlas of the Developing Human Basal Ganglia and Cortex

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Large scale single cell atlasing projects of the developing human brain are becoming highly relevant in neuroscience research, yet largely exist only in transcriptomic space and have primarily focused on cortical regions. Here we investigated the epigenomic and three-dimensional chromatin conformational reorganization during the development of the prefrontal cortex and several basal ganglia regions including the hippocampus, striatum, globus pallidus, and more. Sequencing more than 200,000 joint single-nucleus profiles of chromatin conformation and DNA methylation generated by single-nucleus methyl-3C sequencing (snm3C-seq3) we are able to reconstruct several developmental cell lineages and explore the directionality of epigenomic changes across modalities. The inclusion of the ganglionic eminence allows for the complete trajectory dissection of inhibitory neurons before, during, and after regional migration. Using single-cell profiling and multimodal single-molecule imaging approaches, we have found that short-range chromatin interactions are enriched in neurons, whereas long-range interactions are enriched in glial cells and non-brain tissues. We reconstructed the regulatory programs of cell-type development and differentiation, finding putatively causal common variants for schizophrenia strongly overlapping with chromatin loop-connected, cell-type-specific regulatory regions. Our data provide multimodal resources for studying gene regulatory dynamics in brain development and demonstrate that single-cell three-dimensional multi-omics is a powerful approach for dissecting neuropsychiatric risk loci.

Poster #C9: (M. Cavallini et al.)

Cell-type-specific proteomic profiling in the retina

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The formation of precise synaptic connections between specific partner cells is fundamental for the development of functional neural circuits. In the retina, these highly stereotyped connections are organized into distinct synaptic layers, with the inner plexiform layer (IPL) further subdivided into five sublaminae (S1-S5), each hosting specific circuits crucial for visual information processing. Aberrant synaptic rewiring during retinal degenerative diseases represents a significant challenge for vision rescue strategies, highlighting the need to understand the molecular mechanisms underlying synaptic specificity. While transcriptomic techniques such as single-cell RNA sequencing (scRNA-seq) have provided insights into cell type heterogeneity in the central nervous system, they have limitations in detecting cell-surface recognition molecules with high resolution on a large scale. To address this gap, we implemented iPEEL (in situ cell-surface Proteome Extraction by Extracellular Labeling), a recently developed ex vivo proximity labeling technique that uses a transgenic mouse expressing a Cre-dependent, membrane-tethered horseradish peroxidase (HRP) enzyme. This system enables the labeling of cell surface and secreted proteins in specific cell types within intact tissues. We comprehensively profiled membrane proteins in the developing IPL by targeting specific amacrine cell and retinal ganglion cell populations using distinct Cre driver lines: vGlut2-Cre (all retinal ganglion cells spanning all sublaminae), JamB-Cre (J-RGCs targeting S1), ChAT-Cre (ON and OFF starburst amacrine cells in S2 and S4), Fezf1-Cre (ON starburst amacrine cells in S4), and vGlut3-Cre (VG3 amacrine cells in S3). By integrating our proteomics data with published scRNA-seq datasets from developing bipolar cells and retinal ganglion cells, we identified both novel candidates and proteins with established roles in IPL development, including sublamina-specific adhesion molecules. This comprehensive map of the IPL cell surface proteome during development offers new insights into the molecular mechanisms underlying sublamina-specific circuit assembly in the retina and provides a valuable resource for understanding the adhesion molecule code that governs synaptic specificity in neural circuits.

Poster #C10: (H. Yang et al.)

A single-cell transcriptomic atlas of the prefrontal cortex across the human lifespan

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The dorsolateral prefrontal cortex (DLPFC), which is essential for higher-order cognition, is especially susceptible to molecular and cellular changes throughout the human lifespan. We constructed a comprehensive single-nucleus transcriptomic atlas of the human DLPFC, encompassing over 1.3 million nuclei from 284 postmortem donors aged 0 to 97 years. Non-linear modeling of gene expression trends revealed ten distinct trajectories of the entire transcriptome across all cell types. Neurons and microglia, in particular, exhibited age-related expression changes linked to loci associated with neurodevelopmental and neurodegenerative disease risks. Excitatory neurons demonstrated a striking convergence toward a unified aging-related molecular signature. Pseudotime analysis revealed temporally structured gene clusters associated with development, maturation, and aging, while spatial transcriptomics confirmed a shift from gray to white matter in glial module enrichment and the laminar specificity of neuronal modules. Notably, we observed significant circadian reprogramming in late adulthood, characterized by a loss of rhythmicity in core clock genes and the emergence of novel rhythmic patterns, particularly in microglia and oligodendrocytes. This reference atlas offers a foundational resource for decoding the molecular underpinnings of human cortical development, maintenance, and aging—and their implications for psychiatric and neurodegenerative diseases.

Poster #C11: (L. Tong et al.)

Single-cell transcriptomic analysis reveals brainstem gene expression maps in response to electroacupuncture applied to treat hypertension

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Acupuncture has been used as a therapeutic intervention for a variety of diseases, including hypertension. The molecular and neural mechanisms underlying its effects are starting to emerge using modern methods. The brainstem is a critical region that regulates cardiovascular states and has been implicated as a site of action in the acupuncture-mediated treatment of hypertension. We employed single-cell RNA sequencing to characterize the multicellular gene expression programs that respond to electroacupuncture (EA) directed to blood pressure regulation to treat hypertension in distinct cell types in the mouse brainstem. We analyzed 29,732 cells and identify 9 main cell types and 4 neuronal subtypes that are EA responsive and sham control using unsupervised clustering analysis. The results reveal distinct transcriptional responses to EA in a cell-type-specific fashion. Specifically, EA responsive differentially expressed genes in excitatory neurons and inhibitory neurons are known to be associated with synaptic function. To focus on neural circuits that can be manipulated to treat hypertension, we inferred the spatial location of cholinergic neuronal clusters based on known signatures and transcription regulation networks. RNA velocity analysis reveals differential increases of RNA splicing and dynamics in oligodendrocytes and pericytes. Cell-cell communication analysis identifies astrocytes as major effectors in EA-induced changes in cell-cell communication. The results indicate that EA applied to treat hypertension evokes a coordinated response in brainstem involving different types of cells to generate changes in synaptic plasticity. Our findings unveil a thorough transcriptomic response profile in the brainstem immediately after electroacupuncture (EA) treatments targeted for hypertension.

Poster #C12: (C. J Rodriguez-Ortiz et al.)

MERFISH analysis reveals regulation of synaptic and neuronal related pathways by CSF1R inhibition in the hippocampus of App-KI mice.

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Microglia regulate normal and pathological neural circuit processes in the brain in relation to inflammatory processes. Microglia are recruited to active synapses to downregulate activity and prevent overstimulation. However, in an inflamed Alzheimer's disease-like brain, the depletion of microglia rescues dendritic spine loss and reduces cognitive impairments. But the molecular and neural mechanisms underlying how microglial depletion impacts neural function remain understudied. Pharmacological CSF1R inhibitors serve as effective tools to achieve microglial depletion. In contrast to other methods, CSF1R inhibitor-induced microglial depletion is advantageous due to its non-invasive route of administration, lack of an inflammatory response, and a path to clinical utility, that may facilitate the translation of experimental findings; the widely used CSF1R inhibitor PLX3397 has FDA approval. Here, we studied how microglia regulate hippocampal transcriptomics by using pharmacological microglia depletion in 4- and 16/17-month-old control and App knock-in (App-KI) humanized AD model mice. Recent progress in spatially resolved single-cell transcriptomics technologies allows integration of single-cell omics and spatial genomic mapping to cellular level mapping of neural circuits at high resolution. MERFISH (multiplexed error-robust fluorescence in situ hybridization) simultaneously measures the copy number and spatial distribution of hundreds of RNA species in individual cells in the preserved tissue spatial context. We performed MERFISH on brain sections containing the dorsal hippocampus to delineate cell type identities and compositions in the spatial circuit and AD pathology context. We chose 500 genes using a 23-bit coding scheme as previously published. For each experimental group, we used 4 mouse brains (1:1 F, M). A heatmap analysis of the proportions of microglia across conditions reveals almost complete elimination of microglia in PLX3397 samples (relative proportion less than 0.05). This is further confirmed by the spatial distribution loss of the microglia marker Tmem119. In comparison, astrocyte proportions do not change significantly. Intriguingly, the oligodendrocyte progenitor cell (OPC) proportions decrease with treatment. We performed pathway enrichment analysis for differentially expressed genes between conditions, with a focus on the hippocampus and glutamatergic neurons in CA1 and CA3. We also examined the gene expression intersection in different treatment conditions and found significant regulation in several neuronal and synaptic genes. We used CellChat to analyze the cellular communication pathway alterations induced by microglia depletion. The CellChat analysis enables quantitative comparisons of secreted ligand-receptor interactions among microglia and nearby neurons and identifies top differentially expressed communication pathways between conditions. The pathways we identified (APP, CSF, and CX3C) are strongly associated with microglia and Alzheimer's disease.

Poster #C13: (A. Payne and R. Park et al.)

Protein barcoding and highly multiplexed imaging in expansion microscopy enables scalable morphological reconstruction with molecular annotation

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Information processing in the brain is determined by its circuit architecture comprising neuronal morphology, synaptic connectivity, and molecular characteristics spanning nanometers to centimeters. However, capturing these features simultaneously at scale has remained challenging. Here we introduce PRISM (Protein-barcoded Reconstruction and Imaging of Synaptic Morphology), which integrates combinatorial AAV infection of cell-filling protein barcodes, highly multiplexed 6-fold expansion microscopy, and automated barcode-guided segmentation to deliver high-density morphological reconstructions with rich molecular readout. Applying PRISM to a 13 million cubic micron volume of mouse hippocampus CA3, we obtained robust readout of 18 protein barcode channels and 5 synaptic markers and observed high combinatorial diversity and reliable trafficking of protein barcodes into distal processes. We then incorporated barcode information into an affinity-graph partitioning method and showed improved automated segmentation at reduced computational costs. Furthermore, we demonstrated barcodes can enable automated proofreading by reconnection of falsely split segments. Finally, we used molecular annotations to map excitatory and inhibitory synapse distributions and putative connections between pre- and post-synaptic barcoded neurons in the CA3 volume, demonstrating extensibility of PRISM for investigating neuronal circuit architecture. Taken together, we envision PRISM provides a scalable path to integrate high resolution morphology with molecular information, opening new avenues for high-throughput circuit-targeted investigations and therapeutics.

Poster #C14: (Y. David Chen et al.)

Using single-cell RNA sequencing to generate predictive cell-type-specific genetic reagents throughout development

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Understanding how functional neuronal circuits are built requires generating cell-type-specific tools during development. Many existing genetic tools in Drosophila rely on enhancer activity to label different subsets of cells and have been extremely useful in analyzing neuronal circuits in adults. However, these tools often fail to show the same cell-type-specific expression during development. Genetic intersectional techniques such as the split-GAL4 system can refine cell-type-specificity, yet it requires significant time and resources to screen through combinations of developmental enhancer expression patterns.

Here, we use developmental single-cell RNA sequencing (scRNAseq) datasets to select gene pairs for split-GAL4 and provide a highly efficient and predictive pipeline to generate cell-type-specific split-GAL4 lines during development based on the native gene regulatory elements. These gene-specific split-GAL4 lines can be generated from a collection of coding intronic MiMIC/CRIMIC lines or by CRISPR knock-in. We use the developing Drosophila visual system as a model to demonstrate the high predictive power of scRNAseq-guided gene-specific split-GAL4 lines in targeting known cell types, annotating clusters in scRNAseq datasets, and identifying novel cell types. Recently, we further improved the marker gene selection algorithm to apply to all single-cell transcriptomic datasets. We refined the method to model expression state at the single-cell level instead of at the cluster level. This allowed the current algorithm to perform consistently regardless of the number of clusters and be robust to clusters with mixed cell types. We also develop a web application to interactively visualize and explore marker combinations and host a repository of existing gene-specific split-GAL4 reagents to streamline resource sharing and collaboration. Altogether, our approach opens new avenues for identifying cell-type-specific markers and generating cell-type-specific tools for targeted manipulations of distinct cell types throughout development that are essential for studying molecular regulators underlying various developmental processes.

Poster #C15: (T. Woo et al.)

Large Scale Single Nucleus Profiling of the Adult Mouse Spinal Cord Identifies Previously Unknown Neuronal Cell Types and New Insights into Spinal Cord Injury and Aging

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The spinal cord is the caudal extension of the central nervous system and contains complex neuronal networks mediating local sensory-motor processing of the body and communication with the brain. We generated the largest single-nucleus transcriptomic dataset of 377,462 single nuclei, including cells along the entire length of the mouse spinal cord. From 199,771 high-quality neurons, we identified 61 major neuronal clusters with multiple subtypes. The 177,691 non-neuronal cells include 4 astrocyte, 3 microglia, 4 endothelial and 3 ependymal cell clusters and a continuum of subtypes of oligodendrocyte and oligodendrocyte precursors (OPCs). Single-nucleus chromatin accessibility profiling (ATAC-seq and snRNA-Seq) and single nucleus histone modification mapping (Paired-Tag and snRNA-Seq) identified large numbers of cell type specific candidate cis regulatory elements and putative enhancers. Retrograde tracing identified multiple types of cerebellum-projecting and thalamus-projecting neurons (21,427), including three subclasses of Clarke's Column neurons (4,387), as well as a network of previously unknown neurons. This resource allowed us to identify critical changes in gene expression after spinal cord injury and different rates of aging processes in different cell types.

Poster #C16: (M. Du et al.)

Single-cell spatial transcriptomic analysis of irradiation and Riluzole treatment effect in multiple brain regions

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Irradiation is commonly used to control tumor growth but often leads to long-term cognitive impairments. Riluzole, an FDA-approved neuroprotective drug, may mitigate these effects, yet the brain-wide, cell-type-specific transcriptome responses at single-cell resolution to irradiation and Riluzole remain unclear. In this study, we apply Multiplexed Error-Robust Fluorescence In Situ Hybridization (MERFISH) to profile 20 mouse brain sections across four different experimental conditions including control (Con + Vehicle), irradiation (RT + Vehicle), Riluzole alone (Con + RZ), and irradiation with Riluzole treatment (RT + RZ). Our analysis identifies distinct irradiation-induced alterations in both non-neuronal and neuronal cell types, including microglia activation, astrocyte reactivity, oligodendrocyte demyelination, and endothelial blood-brain barrier disruption. These effects are especially pronounced in several brain regions including hypothalamus, midbrain, hippocampus, and isocortex. Riluzole treatment partially reverses these changes by reversing pro-inflammatory and reactive states in microglia and astrocytes, promoting remyelination and oligodendrocyte recovery, and enhancing blood-brain barrier stability and energy metabolism in endothelial cells. In neurons, irradiation induces gene expression changes associated with DNA damage and cell death, while Riluzole attenuates these transcriptomic alterations. Overall, this spatial transcriptomic analysis reveals glial and neuronal alterations driven by irradiation and partially rescued by Riluzole, offering insight into the cellular mechanism underlying irradiation-induced cognitive decline.

Poster #D1: (P. Wonnenberg et al.)

Modulating Parvalbumin Interneuron-Pyramidal Cell Dynamics in Depression-like Behavior: Insights into 4-Methylumbelliferone as a Therapeutic Strategy

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Major depressive disorder (MDD) is characterized by disruptions in neuronal plasticity and excitatory/inhibitory (E/I) balance within key brain regions, such as the hippocampus and amygdala. These deficits often result from chronic stress-induced neuroinflammation and extracellular matrix (ECM) alterations, specifically within perineuronal nets (PNNs) surrounding parvalbumin-positive (PV+) interneurons. While the role of PV+ interneurons in regulating pyramidal cell activity is well-documented, little is known about how chronic stress impacts their interaction or how targeting PNNs might mitigate these eTects. In this study, we employ fiber photometry to monitor calcium signaling in PV+ interneurons and pyramidal cells in real time during the induction of a depression-like state through chronic corticosterone exposure. By capturing neuronal activity within the hippocampus and amygdala, we aim to elucidate how E/I balance is disrupted under stress conditions. Additionally, we investigate the therapeutic potential of 4-methylumbelliferone (4-MU), a hyaluronic acid synthesis inhibitor, to reversibly modify PNNs and restore plasticity. Preliminary results suggest that chronic stress increases PV+ interneuron activity, leading to hypo-excitability in pyramidal cells and impairments in cognitive flexibility and emotional regulation. Treatment with 4-MU appears to normalize these interactions by reducing PNN density, enhancing plasticity, and restoring E/I balance. Behavioral assays corroborate these findings, showing improvements in memory and anxiety-related behaviors following 4-MU administration. This research provides critical insights into the mechanisms of stress-induced neuroplasticity deficits and highlights the potential of 4-MU as a novel intervention for stress-related disorders. By advancing our understanding of PV-pyramidal interactions and their modulation through ECM-targeted therapies, this study opens new avenues for addressing the pathophysiology of MDD.

Poster #D2: (L. Washiashi et al.)

Investigating the Role of Astrocytic LRP1 in Pathological Tau Clearance

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Alzheimer's disease is a common and devastating form of dementia, and currently lacks effective treatments. We seek to develop a potential therapeutic strategy via astrocytic sequestration of pathological tau. A small population in Antioquia, Columbia has been found to express an autosomal dominant mutation in PSEN1, leading to severe early-onset Alzheimer's disease. Surprisingly, a single member of this population was spared from Alzheimer's disease pathology well into her seventies, despite carrying the PSEN1 mutation. Post mortem analysis found that she exhibited high levels of amyloid deposition but reduced levels of pathological tau compared to her PSEN1-positive cohort (Arboleda-Velasquez, et al. Nature Med. 2019). Interestingly, post-mortem analysis found increased levels of lipoprotein receptor related protein1 (LRP1) (Alimeida, et al. Neuron. 2024), a protein known to be involved in tau endocytosis and the spread of pathological tau (Rauch, et al. Nature 2020). However, this overexpression was restricted to astrocytes (Alimeida, et al. Neuron. 2024), suggesting that astrocytic uptake of pathological tau might act to sequester pathological tau and to prevent uptake and spread through neurons. Therefore, we are exploring astrocyte-specific overexpression of LRP1 as a potential therapeutic for endocytosis and sequestration of extracellular tau, which will reduce the spread of pathological tau throughout the brain. We will test this by using in vivo two-photon microscopy to longitudinally image dendritic spine morphology changes on the ipsilateral side of the retrosplenial cortex. This allows us to determine how the spread of pathological tau affects downstream neuronal morphology, using the contralateral side as a within-mouse control. We have gathered preliminary data from mice that have received unilateral AAV injections to express a human variant of pathological tau (P301L) in the hippocampus. In a subset of mice, we plan to overexpress mLRP (mini-LRPs) in astrocytes. This will be accomplished using an AAV-PhP.eB-GFAP-mLRP virus injected into the ventricle for brain-wide expression. Finally, upon reaching our imaging endpoint, we will use immunohistochemistry to quantify the spread of pathological tau from the seed region. This study will elucidate whether overexpression of LRP1 in astrocytes is sufficient for sequestration of pathological tau and remediation of downstream pathology.

Poster #D3: (T. Hsu et al.)

Mesoscopic mapping of circuit deficits in autism mouse models

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Autism spectrum disorder (ASD) is a disconnectivity syndrome, making it essential to identify circuit-level deficits to understand its etiology. We developed a whole-brain mapping platform tailored for histological fluorescent images from consecutive brain cryosections to analyze circuit rewiring in ASD mouse models. Using this platform, we revealed both structural and functional alterations in multiple ASD mouse models. First, we examined circuits originating from the basolateral amygdala (BLA) in Tbr1+/− mice by expressing oChIEF-Citrine unilaterally in the BLA and analyzing Citrine+ axons and C-FOS expression. Tbr1 haploinsufficiency led to inter- and intrahemispheric connectopathies, altered neuronal activity, and impaired synchronization across brain regions. Optogenetic theta-burst stimulation of the BLA partially restored default mode network synchronization and improved nose-to-nose social interaction in Tbr1+/− mice, suggesting that amygdalar stimulation can enhance social function. Second, we used the Thy1-YFP reporter to assess circuit rewiring across three ASD models (Tbr1+/−, Nf1+/−, and Vcp+/R95G). While each model displayed distinct connectivity changes, the visual, somatosensory, and piriform areas were consistently affected. Notably, the piriform cortex showed reduced YFP signals and fewer YFP+ neurons across all three models. Correspondingly, all mutants exhibited similar olfactory discrimination deficits, underscoring the vulnerability of sensory regions—particularly the piriform area—to ASD-linked mutations. Our whole-brain mapping platform enables efficient screening of circuit abnormalities in ASD mouse models and helps pinpoint critical brain regions for further investigation. Future applications with advanced reporters may allow detailed analysis of specific or multiple circuits, facilitating deeper insights into ASD-related connectivity deficits.

Poster #D4: (J. Plank et al.)

Functional brain networks predictive of cognitive function in children with Noonan syndrome

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Cognitive difficulties are a feature of neurodevelopmental disorders with impacts on academic achievement, social relationships and emotion regulation. Efforts to map the functional brain networks that contribute to these cognitive challenges have been limited by heterogeneous samples and an absence of robust data-driven methods. To overcome these limitations, we studied a disorder with a clear genetic basis using a generalizable method for computing brain-behavior relationships. Seventy-six children, 42 with Noonan syndrome (NS) and 34 typical developing (TD), completed resting-state functional MRI scans and NIH toolbox cognitive tasks. Functional MRI data and cognitive composite scores (fluid reasoning, crystallized intelligence, and total cognition) were entered into a connectome-based predictive modelling (CPM) pipeline with internal cross-validation. Virtual lesion analysis was used to identify key brain networks that contribute to the predictive models. CPM models showed that whole-brain functional connectivity patterns strongly predicted crystallized (r_s=0.491, p_{perm}=.002) and total cognition scores (r_s=0.383, p_{perm}=.005), while fluid reasoning was only weakly predicted (r_s=0.235, p_{perm}=.056). Lesion analysis suggested frontoparietal networks were important for all models, while visual association was important for total cognition but less so for fluid reasoning. Comparison of the network strengths between-groups indicated significantly weaker connectivity in the NS group relative to TD ($p<.001$). These findings demonstrate that whole-brain functional connectivity robustly predicts crystallized and total cognition in children with NS and their TD peers. Frontoparietal networks emerged as key contributors to the models, supporting their central role in cognitive processes. Children with NS showed weaker connectivity compared to TD, highlighting wide-spread potential disruptions in network integration that may underlie cognitive difficulties in this population.

Poster #D5: (H. Yang et al.)

High-Throughput Functional Characterization of Schizophrenia Risk Variants in Neurons

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Genetic factor plays a pivotal role in the etiology of schizophrenia (SCZ), with genome-wide association studies and exome sequencing identifying thousands of common and rare risk variants. Integrated fine-mapping and multi-omics studies have implicated the neuronal cell types as key mediators of these genetic effects. However, functional interpretation of these variants remains a major bottleneck, primarily due to the lack of scalable approaches for directly assaying their effects in neural cell types. To address this gap, we employed a high-throughput PRIME editing screen to functionally characterize 1,289 candidate SCZ risk variants—comprising 976 common and 313 rare risk variants— in human induced pluripotent stem cell-derived excitatory neurons. Our screen identified 286 functional variants (214 common and 72 rare) that significantly impacted neuronal survival. Notably, SCZ risk-associated alleles were disproportionately linked to reduced neuronal fitness, a consistent pattern observed across both common and rare variant classes. Functional categorization of the identified variants revealed distinct mechanisms, including disruptions to transcriptional regulation (n=65), RNA splicing (n=167), and protein-coding sequences (n=78). These variants were further linked to 83 genes implicated in neuronal fitness, 68 of which exhibited differential expression between SCZ cases and controls. Overall, this study represents the most comprehensive functional screen of SCZ-associated variants affecting neuronal fitness to date, providing valuable and direct insights into the genetic factors contributing to neuronal fitness in schizophrenia risk.

Poster #D6: (L. Perrault et al.)

Feeding the Brain Fog: How Diet Disrupts Somatostatin Interneuron Function

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A high-fat Western diet (HFD), characterized by excessive intake of saturated fats and processed foods, has been increasingly linked to negative effects on brain health. These dietary patterns and associated metabolic disorders, such as obesity and type-2 diabetes, have been shown to be risk factors for developing neurodegenerative diseases, including Alzheimer's and Parkinson's. However, the mechanisms underlying the association between cognitive impairment and diet-associated metabolic insults are poorly understood. Previous research from our lab has shown that short-term high-fat diet (stHFD) consumption leads to impaired cognition and memory processing, which is mediated by hyperactivity of hippocampal cholecystokinin interneurons (CCK-INs). CCK-INs exhibit increased phosphorylation of the glycolytic enzyme pyruvate kinase M2 (PKM2), a crucial component in the final step of glycolysis, in response to reduced glucose uptake in the dentate gyrus (DG) of the hippocampus, along with impaired memory. However, observed projections from somatostatin-positive interneurons (SST-INs) onto CCK-INs in the DG led us to explore the relationship between these two interneuron subtypes and their role in HFD-mediated cognitive deficits. Not only did we observe a trend in increased activity of SST-INs in the DG following HFD, but we also saw a similar increase in phosphorylated PKM2 and memory impairment, which were rescued following genetic inhibition of pPKM2 in SST-INs. Furthermore, we aimed to investigate the clinical relevance of potential early interventions to prevent cognitive deficits in mouse models of diet-induced obesity (DIO). We found that chronic inhibition of DG SST-INs or long-term knockdown of pPKM2 in DG SST-INs in DIO mice improved spatial memory performance. Our study not only provides a platform to study the impact of metabolic insults on cognition, but also addresses the potential for new therapeutic targets to prevent cognitive dysfunction associated with both neurodegenerative diseases and metabolic disorders.

Poster #D7: (A. Chawla et al.)

Single-nucleus chromatin accessibility profiling identifies cell types and functional variants contributing to major depression.

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Genetic variants associated with major depressive disorder (MDD) are enriched in the regulatory genome. Here, we investigate gene-regulatory mechanisms underlying MDD compared to neurotypical controls by combining single-cell chromatin accessibility with gene-expression in over 200,000 cells from the dorsolateral prefrontal cortex of 84 individuals. MDD-associated alterations in chromatin accessibility were prominent in deep-layer excitatory neurons characterized by transcription factor (TF) motif accessibility and binding of nuclear-receptor (NR)4A2, an activity-dependent TF reactive to stress. The same neurons were enriched for MDD-associated genetic variants, disrupting TF binding sites linked to genes likely affecting synaptic communication. Furthermore, a grey matter microglia cluster exhibited decreased accessibility in MDD individuals at binding sites bound by TFs known to regulate immune homeostasis. Finally, we identified gene-regulatory effects of MDD-risk variants using sequence-based accessibility predictions, donor-specific genotypes, and cell-based assays. These findings shed light into cell-types and regulatory mechanisms whereby genetic variation may increase risk of MDD.

Poster #D8: (W. Feng et al.)

Single-cell comparison of microglia across brain regions, species, and neurodegenerative diseases

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Neurodegenerative diseases (NDs) such as Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD) are characterized by pathological protein aggregation and deposition, which trigger the activation of microglia. The activation of these brain-resident immune cells is also closely linked to chronic pain conditions, as microglia-mediated neuroinflammation can exacerbate pain signaling in both disease and injury contexts. However, understanding the roles of microglia in NDs and pain is challenging due to the heterogeneity of microglial subpopulations and variations across tissues, diseases, and analytical methods. To address this, we analyzed nine human snRNA-seq datasets from diverse brain regions and ND conditions, identifying two conserved microglial subpopulations between humans and mice. Extending the analysis to include additional brain regions and disease conditions in thirteen mouse snRNA-seq datasets, we confirmed heterogeneous microglial responses and identified shared genes and pathways across species. Differential expression analysis revealed species-specific dysregulated genes, underscoring the complexity of microglial activation. This study provides insights into conserved and divergent microglia genes and pathways, with implications for understanding their roles in both neurodegeneration and pain. By highlighting the translatability of mouse models to human conditions, this work advances efforts to develop microglia-based therapies for alleviating neuroinflammation-associated pain.

Poster #D9: (M.Garduño et al.)

Amyloid-ß neuropathology across the *Octodon degus* lifespan and it's correlation to multi-loci genomic variants

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Alzheimer's disease (AD), the most common form of dementia, currently afflicts over 55 million people worldwide and has no cure. Despite substantial efforts from the scientific community, most AD clinical trials have failed, and treatments for this cognitively debilitating condition are limited. In response to this, the field has encouraged the investigation of organisms that naturally develop neuropathological features akin to those seen in human AD, something that does not naturally occur in mice (i.e. without genetic engineering). The degu (Octodon degus), a long-lived rodent endemic to Chile, naturally exhibits many of the features seem in human AD, such as amyloid-ß plaques, phosphorylated tau, and neuroinflammation. In the present study, we quantify the amyloid-ß levels in the brains of degus across their lifespan (3 – 96 months). Animal cognitive states are gauged via burrowing behavior performance, an ethologically relevant test for degus due to the underground tunnels they inhabit in the wild. The resulting burrowing and neuropathological profiles are correlated to degu Apoe Mt4 genotypes and multi-loci genomic variant analysis using bulk high-throughput chromosome conformation capture (bHiC). Our data show degus exhibit variable drifts into AD-like profiles as they age, similar to what is seen in humans. Upon further analysis, we find that by classifying degus according to their Apoe Mt4 genotype or multi-loci variant profile, we are able to parse degus into three subpopulations exhibiting distinct temporal progression of AD-like features across their lifespan: the AD-like, intermediate, and Non-AD degus. These findings begin to untangle the degu's polygenic AD-like profile and identify naturally occurring subpopulations that could be used for AD and aging research. Our results emphasize the importance of utilizing genetically diverse (outbred) animal populations when studying polygenically-invoked diseases and highlight the promise of the degu as a translationally-relevant model for sporadic AD.

Poster #D10: (M. Snyder et al.)

Relations of Blunted Reward Positivity with Dimensional Symptoms and Traits amongst Individuals with and without Psychosis-Spectrum Disorders

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Psychosis-spectrum disorders are associated with divergences in neural processes, including atypical neural dynamics related to reward processing. Existing literature also suggests that specific symptom dimensions are associated with aberrant neural activity in response to wins and losses. For example, higher levels of negative symptoms as well as higher levels of depressive symptoms are each related to blunted reward positivity. Less evidence, however, is available for how other symptom dimensions of the psychosis spectrum are related to reward positivity following wins and losses and if these associations are consistent across schizophrenia-spectrum disorder vs. other psychotic disorder groups. Moreover, it is unclear if the hypothesized divergences in neural activity in individuals with psychotic disorders would also emerge in relation to normative (e.g., extraversion) and pathological traits (e.g., detachment). Thus, in this study, we used a monetary reward task, electroencephalography (EEG), and time-domain analysis to test how reward positivity (mean amplitude from 275-375 ms, FCz) relates to group differences and to levels of symptoms and traits in a sample of individuals with schizophrenia-spectrum disorders (*n* = 71), other psychotic disorders (*n* = 81), and no psychotic disorders (*n* = 176). The data used in this study is from the 25-year follow up time point of the Suffolk County Mental Health Project. Our findings will clarify how dimensions of symptoms and personality traits may have important links to individual differences in stimulus valuation amongst individuals with and without psychosis-spectrum disorders. Our findings may elucidate shared and distinct neural correlates of the thought disorder and internalizing spectra components and could potentially inform interventions for reward-related transdiagnostic symptoms such as anhedonia.

Poster #D11: (K. Luong et al.)

Modeling HSV-1 Latency in the Brain to Investigate Neurodegenerative Markers Associated with Alzheimer's Disease

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Herpes simplex virus 1 (HSV-1) is a highly prevalent neurotropic virus that has been implicated in the pathogenesis of neurodegenerative diseases, including Alzheimer's disease (AD). Following primary replication in mucosal epithelial cells, HSV-1 establishes lifelong latency in peripheral nervous system (PNS) neurons. Reactivation from latency enables the virus to re-infect peripheral tissues or spread to the central nervous system (CNS) via anterograde axonal transport. While peripheral neurons are well-established reservoirs, the extent to which HSV-1 establishes true latency in CNS neurons—and contributes to chronic neuropathology—remains poorly defined. To address this gap, we used primary rat cortical neurons (CNs) infected with HSV-1 strain OK14, which expresses mRFP-tagged VP26 to report productive infection. Across multiplicities of infection (MOI) from 1 to 0.001, productive replication was detectable by day 4 post-infection at the lowest MOI. To enforce a latent-like state, we applied acyclovir (ACV) to inhibit viral DNA replication. This resulted in a loss of mRFP signal and reduced infectious virus yield, consistent with latency establishment. We then assessed canonical latency hallmarks in cortical neurons: suppression of lytic gene expression, accumulation of the latency-associated transcript (LAT), and the capacity for reactivation. Using RNA-fluorescence in situ hybridization (RNA-FISH) and qPCR, we monitored LAT accumulation in infected cortical neurons, both with and without ACV, and compared this to LAT expression profiles in productively or latently infected superior cervical ganglia (SCG) neurons. We found that ACV treatment during 1 MOI HSV-1 infection was able to reduce late protein expression and sustain the infection in CNs for 5 days whereas the absence of ACV lead to neuronal death at 3 dpi. Moreover, we detected increased LAT expression when HSV-1 infected CNs were treated with ACV, following a similar pattern to HSV-1 latent infection in SCGs. These findings demonstrate that HSV-1 can enter a quiescent state in primary cortical neurons, retaining the potential for reactivation. This system will be used to monitor tau phosphorylation and amyloid-β accumulation during HSV-1 latency using specific antibodies together with FISH probes. Establishing an in vitro CNS latency model provides a critical platform to investigate how latent or recurrent HSV-1 infections might drive neurodegenerative processes, including amyloid-β accumulation and tau hyperphosphorylation, contributing to AD-like pathology.

Poster #D12: (G. Zhao et al.)

Selective vulnerability of two dopamine neuron populations with distinct spatial distribution in the human substantia nigra

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Neuropathology of Lewy bodies is reported in Parkinson disease (PD), Parkinson disease dementia (PDD) and dementia with Lewy bodies (DLBD), and the latter two disorders comprise Lewy body dementias (LBDs). LBDs and Alzheimer's disease (AD) are the most common neurodegenerative dementias with the commonality of protein deposition but distinct pathological and clinical characteristics. To investigate the cellular population and gene expression changes that may underlie disease pathogenesis mechanisms, we performed single-nucleus RNA-sequencing (snRNA-seq) on the substantia nigra (SN) tissues obtained from 45 subjects with AD, PD, PDD, or DLBD, and cognitively normal controls. We compared our data with three published snRNA-seq data of SN derived from subjects with PD or PDD and cognitively normal controls. We performed uniform data analysis for the neuronal subpopulations using the same analysis parameters across four datasets. We identified 9 neuronal subpopulations, including two Dopamine (DA) neuron populations: SOX6+ vs. SOX6- DA neurons in our data. SOX6+ DA neurons and RORB+ neurons were shared by all four datasets. However, the SOX6- DA neurons were shared only by the Martirosyan et al. data suggesting the regional differences among the four datasets. Differentially expressed gene (DEG) analysis in each disease condition revealed that ALDH1A1 expression was oppositely regulated in AD versus in PD and LBDs, which likely contributed to selective vulnerability of DA neurons to neurodegeneration in PD and LBDs. The dopamine metabolism pathway was more severely dysregulated in DLBD than in PD and PDD, whereas the activation of neuroprotective responses such as Ubiquitin-proteasome system, mitophagy, and increased superoxide dismutase (SOD) activity were observed in PD and PDD. We demonstrated that SOX6- DA neurons were more vulnerable than the SOX6+ DA neurons. Spatial transcriptomics and immunohistochemical staining of the human SN revealed spatially distinct distribution of the two DA neuron populations with SOX6- DA neurons located at ventral part of the SN. Pathway enrichment analysis revealed vast differences in gene dysregulation in the corresponding neuronal populations among the four SN datasets. Our work suggested not only the spatial heterogeneity of cell composition but also spatial heterogeneity of gene dysregulation in disease conditions, which may contribute to selective neuronal and regional vulnerability in the substantia nigra among AD, PD, PDD, and DLBD patients.

Poster #D13: (T. Nakagawa et al.)

Amyloid β-induced dopamine dysfunction in the lateral entorhinal cortex impairs associative memory

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Alzheimer's disease (AD) is the most common cause of dementia. Previous fMRI studies show that the lateral entorhinal cortex (LEC) is the primary site of dysfunction in early-stage AD patients, but it remains unclear what type of memory is affected by LEC dysfunction. We previously found that the LEC neurons in healthy animals are critically involved in associative memory encoding and dopamine facilitates associative memory encoding (Lee et al., Nature 2021).

To test whether this LEC activity is impaired in AD, we used in vivo electrophysiology to examine LEC neurons in amyloid precursor protein knock-in (APP-KI) mice performing an odor cue-reward association task. APP-KI mice showed impaired memory performance in our associative memory task, and their LEC neurons showed disrupted associative memory encoding. Photometry recordings revealed that LEC dopamine was decreased at novel rewarded odor. Optogenetic stimulation of LEC dopamine fiber rescued associative memory. These results suggest that dysfunction of LEC-projecting dopamine neurons underlies memory impairment in AD from early stages, pointing to a need for clinical investigation of LEC dopamine in AD patients.

Poster #D14: (N. Tabuchi et al.)

Intrinsic transcriptional control of serotonin axon regeneration after injury

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Most injured axons in the adult mammalian CNS are unable to regenerate and reestablish functional connections, resulting in chronic disability and neurological disease. Interestingly, however, serotonin (5-HT) axons are an exception as they spontaneously regrow extraordinarily long distances and restore function after physical or neurochemical injury. Yet currently nothing is known about the intrinsic mechanisms that enable this unusual intrinsic regrowth capacity of 5-HT axons. We reported that transcription factors, Lmx1b and Pet1, are required for long distance 5-HT axon growth during development and for maintaining the integrity of adult 5-HT axons and synapses. Given these critical roles in 5-HT axon formation and maintenance, we hypothesize that Lmx1b and Pet1 are required for 5-HT axon regeneration following injury. To investigate this, we eliminated Lmx1b and Pet1 expression in adult 5-HT neurons prior to a 5-HT neuron-specific neurochemical injury. We found that 5-HT axons failed to regrow, form new presynaptic structures, and exhibit features of morphological recovery. A major goal is to understand the intrinsic regenerative transcriptional program controlled by Lmx1b/Pet1 that endows 5-HT neuron axons to regrow after injury. In control mice subjected to neurochemical injury, 5-HT neuron transcriptomes and epigenomes undergo dramatic temporal changes that define acute injury and regrowth stages. With adult-stage targeting of Lmx1b and Pet1 prior to injury, the regrowth program is not initiated. These results strongly suggest that post-injury Lmx1b and Pet1 control drastic temporal changes in 5-HT neuronal epigenome to enhance 5-HT axonal growth and functional recovery in adult CNS after injury. Our discovery of regulatory factors underlying injury-induced 5-HT axon regrowth suggests a new model for exploration of axonal regeneration in the adult CNS and nervous system repair strategies.

Poster #D15: (A. Proddutur et al.)

Chronic stress diminishes persistent firing via increased inhibitory tone in L5 pyramidal cells of the posterior parietal cortex

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Exposure to chronic stress impairs cognitive functions, including working memory (WM). One crucial region involved in WM, attention, and decision-making is the posterior parietal cortex (PPC). A fundamental mechanism that supports WM is the sustained firing of action potentials, which is believed to be facilitated by interconnected local microcircuits and intrinsic cellular properties. Although previous research has indicated that stress negatively impacts PPC function, the specific cellular mechanisms underlying this relationship remain poorly understood. We hypothesized that chronic stress reduces persistent firing in the PPC, contributing to WM deficits. We adapted a computational model of a persistent firing network to create a framework for predicting possible synaptic parameters that could decrease persistent firing in layer 5 pyramidal cells (PCs). We then used slice electrophysiology to test these model predictions in a mouse model of repeated multimodal stress. Our results confirmed that stress exposure significantly reduced persistent firing in PPC neurons. While no significant changes were observed in intrinsic properties or excitatory synaptic responses, we found a significant increase in inhibitory tone. Specifically, stress exposure enhanced GABAB receptor-mediated inhibitory currents and increased the paired-pulse and multi-pulse ratio of GABAAR-mediated currents in L5 PCs. These findings suggest that increased inhibition, rather than changes in excitatory signaling, underlie the reduction in persistent firing following stress. Our results provide insights into how chronic stress disrupts cellular mechanisms critical for working memory and highlight the role of altered inhibition in these deficits.

Poster #D16: (A. Nguyen et al.)

Time course development of deficits in auditory brainstem responses, sensorimotor and learning behaviors in mouse models of tauopathy

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Cognitive decline is a hallmark of Alzheimer's Disease and Related Dementias (AD/ADRD), often presenting alongside deficits in sensorimotor function, auditory processing, and memory. The PS19 mouse model, which expresses the P301S humanized tau mutation, exhibits tau pathology consistent with frontotemporal dementia. When combined with the humanized ApoE ε4 knock-in (KI) allele, these mice show accelerated tau accumulation and brain atrophy as early as 3 months of age.

While auditory processing abnormalities have been documented in amyloid-based models such as APP/PS1 prior to the onset of learning and memory deficits, the effects of tau pathology on auditory function remain less understood. In this study, we investigated the progression of auditory and cognitive deficits in PS19 and ApoE4/PS19 mice at 3, 7, and 10 months of age. Our goal was to determine how age-related impairments associated with tauopathy and the ApoE ε4 allele contribute to changes in auditory and cognitive function.

To assess these outcomes, we utilized auditory brainstem response (ABR), elevated plus maze (EPM), object recognition memory (ORM), object location memory (OLM), and rotarod testing. These approaches allowed us to examine memory loss, sensorimotor deficits, and auditory processing changes over time. Our findings indicate that ApoE4/PS19 mice exhibit early-onset auditory and cognitive deficits by 3 months of age, including reduced anxiety-like behavior and ABR abnormalities characterized by increased latency and hyperexcitability at low-to-mid frequencies (4, 8, 12, and 16 kHz). Additionally, 10-month-old PS19 mice showed significant reductions in ABR Wave I and II amplitudes across both high and low frequencies, suggesting peripheral auditory dysfunction. Interestingly, ApoE4/PS19 mice demonstrated improved motor coordination and balance at 7 months compared to ApoE4 controls. These mice also showed age-dependent memory impairments, with deficits in OLM at 7 months and ORM at 10 months, while PS19 mice alone did not exhibit significant memory impairments at these time points.

Together, these results suggest that the combination of tauopathy and the ApoE ε4 allele leads to early and progressive auditory and cognitive dysfunction. These findings highlight auditory processing measures as potential early indicators of dementia progression and underscore the importance of examining sensory systems in models of AD/ADRD.

Poster #D17: (V. Ajith et al.)

Cytoarchitectural Analysis of the Aging Temporal Cortex and Ventral Hippocampus in the Canine, a Natural Animal Model of Alzheimer's Disease and Aging

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Alzheimer's disease (AD) is the most common cause of dementia in the elderly population and is characterized by hallmark neuropathological features, including amyloid-beta (Aβ) plaques and neurofibrillary tau tangles (NFTs). These abnormalities contribute to neuroinflammation, neuronal loss, and cognitive decline. In this study, we investigate the effects of neuropathology and aging on distinct neuronal subpopulations within the temporal cortex and ventral hippocampus of young (1-5 y.o.) and old (9-11 y.o.) canines—specifically, beagles and golden retrievers. Canines serve as a valuable model for AD because they naturally develop age-related cognitive decline and AD-like pathology, unlike murine models that require genetic modifications such as amyloid-beta precursor protein (APP), presenillin-1 (PSEN1), and presenillin-2 (PSEN2). By leveraging this natural model, our findings may offer enhanced translational relevance to human AD. We focused on neuronal populations expressing parvalbumin (PV), cholecystokinin (CCK), somatostatin (SST), calbindin (CB), calretinin (CR), and Purkinje cell protein 4 (PCP4), markers primarily associated with neurons that have been reported to decline in human AD. Given the hippocampus's critical role in memory and learning and the temporal cortex's close anatomical and pathological link to AD, we assessed both regions. Our study provides novel insights into how aging and neuropathology impact specific neuronal subtypes in canines and underscores their potential as a translational model for understanding human AD progression.

Poster #D18: (M. Sandoval et al.)

Assessing the localization and function of the putative schizophrenia-associated protein MDGA1 in the mouse hippocampus

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The MAM domain–containing glycosylphosphatidylinositol (GPI) anchor protein 1 (MDGA1) is a completely extracellular, GPI-anchored synaptic adhesion molecule. As such, it has been shown to be quickly diffusible within neuronal membranes. Human MDGA1 gene mutations have been associated with schizophrenia and bipolar disorder. In mice, global MDGA1 knockouts (KO) show impaired excitation/inhibition balance and defects in cognitive function. However, despite the recent advent of antibodies specific to endogenous MDGA1, understanding MDGA1 localization and function during postnatal development has been difficult. We previously generated a knock-in (KI) mouse line expressing HA-tagged endogenous MDGA1. Initially, we quantified protein expression in whole brain lysates across postnatal development and found that endogenous HA-MDGA1 expression peaks around P15 (n = 4 mice/age). Using these mice, we quantified the colocalization of immunolabeled HA-MDGA1 puncta with postsynaptic markers in area CA1 of the hippocampus at P20 (n = 5/8 mice/marker). We used Homer1b/c and NLGN2 to label excitatory and inhibitory synapses, respectively. In stratum radiatum (SR) we found that HA-MDGA1 puncta colocalized significantly more with Homer1b/c compared to its putative functional binding partner, NLGN2. However, further analysis revealed that HA-MDGA1 localization to either synapse type was proportional to the concentration of synapse type in SR. Therefore, these data indicate that in vivo, while MDGA1 transiently localizes to synapses, most MDGA1 pools are not synaptically enriched. Subcellular fractionation of mouse whole brain lysates further showed a lack of synaptosomal fraction enrichment (n = 3). Functionally, we found no changes in either excitatory or inhibitory basal synaptic transmission using dual whole cell patch clamp in acute mouse slices around P20 (n = 9-12 cells/3-4 mice), after sparse Cre-mediated MDGA1 KO from CA1 pyramidal neurons. While these functional observations are in stark contrast to constitutive MDGA1 KO mice, they are in line with non-synaptically enriched MDGA1. Importantly, we cannot rule out the possibility that acute MDGA1 KO may modulate more subtle structural aspects of synaptic development and function. Future experiments will assess possible plasticity-dependent functions of MDGA1 including long term potentiation (LTP) at CA3->CA1 excitatory synapses. Overall, our experiments systematically investigate the localization and function of endogenous MDGA1 during peak expression and synaptogenesis for the first time in vivo.

Poster #D19: (X. Ding et al.)

Family-Level Food Insecurity and Health Implications Among Latino Adults: A Focus on Cognition and Depression

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Food insecurity is a critical public health challenge, particularly in Latino communities. Chronic stress from food insecurity may trigger inflammation and gut dysbiosis, disrupting the gut-brain axis and affecting neural circuitry related to cognition and mood. This study examines the association between food insecurity, cognition, and depression in underserved Latino families through the community-based trial, Skills-based Educational strategies for Reduction of Vascular Events in Orange County (SERVE OC). We used data from our SERVE OC study, and analysis included 141 families, comprising 271 Latino adults. Family-level food security status was self-reported and categorized as high, low, or very low. Cognitive function was assessed using the validated screening tool, Montreal Cognitive Assessment (MoCA). Depressive symptoms were measured with the widely used, self-reported Center for Epidemiologic Studies Depression Scale (CES-D). Statistical analyses included t-tests to examine differences in cognitive and depression scores across food security levels for non-missing respondents. Among respondents with non-missing food security data (n=164), 66.2% were female, with a mean age of 46.1 years. Only 1 family (n=2) reported high food security. Participants with low food security (n=45) had a mean MoCA score of 21.7 (SD=4.48) and a mean CES-D score of 8.35 (SD=5.72). Those with very low food security (n=60) had a mean MoCA score of 20.7 (SD=4.64) and a mean CES-D score of 10.98 (SD=6.32). Participants with lower food security showed lower cognitive scores, though not statistically significant ($p>0.05$). However, participants with very low food security had significantly higher depressive symptoms compared to those with low food security ($p<0.05$). This Latino community study found that very low food security was significantly associated with increased depressive symptoms, and showed a similar yet non-significant trend for MoCA scores. Findings underscore the cognitive and mental health burden of severe food insecurity. Future research on the gut microbiome in Latino communities is crucial to understand the gut-brain axis pathways involved in cognition and depression related to food-insecurity.

Poster #D20: (A. Samawi et al.)

U01: Cell-type-specific neural circuit connectomes in the mouse models of aging and Alzheimer's disease

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Alzheimer’s disease (AD) is the most common cause of progressive dementia in older adults; there is no cure for this debilitating condition. We hypothesize that aging and AD-related pathologies cause maladaptive changes within hippocampal formation circuits that serve as connectome hubs for large numbers of brain regions, ultimately leading to age- and AD-related cognitive deficits.

We propose to perform large-scale, cell-type-specific mapping of hippocampal formation circuits to generate cellular resolution connectome data. To capture a more accurate composite of human AD features, we will use three complementary AD mouse models, including two next-generation AD mouse models.

These include 1) the 5xFAD mouse model with familial mutations, 2) the hAß-KI mouse that expresses human wild-type Aβ sequence from the endogenous mouse App locus to model late-onset AD features, and 3) Trem2 R47H knock-in mice that model the increased risk of the R47H coding variant for late-onset AD.

We will comprehensively map and characterize hippocampal formation brain circuits, including CA1, the subiculum (SUB), and the entorhinal cortex (EC), which all show the earliest neurodegeneration across AD mouse models and in human patients.

We will use genetically modified transsynaptic neurotropic viruses developed by our team to map brain-wide retrograde neural networks. The brain connectomes generated from viral tracing experiments will be enhanced with spatially resolved, single-cell transcriptomics-based molecular annotation using MERFISH (multiplexed error-robust fluorescence in situ hybridization). Our work will improve our understanding of brain circuits susceptible to aging and AD, moving towards developing better early diagnostic tools and new treatment strategies for AD.

Poster #D21: (A. Feshchenko et al.)

Ventral hippocampal circuitry underlying neuropsychiatric symptoms in AD

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Neuropsychiatric symptoms (NPSs) such as depression and anxiety are observed in 90% of patients with Alzheimer's disease (AD), and usually manifest long before AD onset. Although much of the field has focused on the link between depression and AD, recent clinical evidence supports that anxiety can predict the progression to AD above and beyond depression, brain atrophy, and cognitive impairment. Our lab has recently shown earlier anxiety-like behavior, cognitive decline, and alterations in brain-wide networks in AD female mice compared to controls and male mice. To determine the circuitry that drives anxiety-like behavior in AD mice, we will characterize retrograde projections from the basolateral amygdala (BLA) and lateral hypothalamus (LHA), as these regions are known to be heavily implicated in anxiety behavior and have projections from the ventral CA1 region of the hippocampus. To test our hypothesis, male and female 6-month-old APP/PS1 (AD) and control 129S6 mice were injected with retrograde virus, cholera toxin B-647 into BLA, and Cholera toxin B-488 into the LHA. After 1 week of recovery, mice were tested in a battery of behavioral paradigms to evaluate anxiety and cognitive decline and correlate these behaviors with the percentage of cells projecting from vCA. To evaluate discrete NPSs in Open Field data, we used Bout Finder, a Python-based software that we designed to assess more sophisticated elements of explorative behavior.

We found that in the Open Field test, AD females but not males cover significantly less distance in the last 10 minutes of the 30-minute trial when compared to the controls. This result may indicate that 6-month-old APP/PS1 (AD) females are less explorative and lose their motivation toward the end of the trial. Interestingly, while assessing the recordings of the Marble Burying test, we noticed the same trend where AD mice lose interest in burying the marbles and discontinue movements. For Contextual Fear Conditioning, we used 24-hour re-exposure paradigm to evaluate short-term memory retention. AD females, but not AD males, show less freezing, which indicates significant memory loss in females even after 1 day. Surprisingly, we saw no significant differences in Zero Maze. In my presentation, I will further discuss the behavioral findings, introduce a novel refined open field analysis method that enables the extraction of more sophisticated behavioral metrics, and present imaging data showing the percentage of anxiety-specific cells and changes in overlapping projections (vCA1→BLA and vCA1→LHA) in an APP/PS1 mouse model.

Poster #D22: (W. Yin Vanessa Kan et al.)

Machine Learning-Driven Identification of Distinct Behavioral Phenotypes in Cortical and White Matter Stroke

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Stroke is a leading cause of both death and disability. Ischemic strokes affect over 795,000 people every year and account for 87% of all stroke types. In cortical stroke (CS), patients typically develop acute symptoms such as weakness in one side of the body, slurred speech, and sudden trouble walking. Neurons in the affected region begin to die within minutes of a stroke, requiring immediate treatment to improve outcome. Subcortical white matter stroke (WMS), on the other hand, is a progressive condition that expands and worsens over time. It comprises 30% of all strokes and is the leading cause of vascular dementia, affecting over 90% of the population over 65 years old. Currently without effective therapies, the projected medical costs, excluding indirect costs such as productivity loss, will exceed $90 billion in the United States by 2030. To develop effective treatment options for stroke patients, we must first establish reliable methods to evaluate the effectiveness of the intervention in pre-clinical models. Traditionally, behavioral tests such as gridwalking, open field, and fear conditioning are used to evaluate motor and cognitive functions. While these tests provide reliable metrics such as foot faults, distance to center, and percentage of freezing time, they typically focus on a single behavioral metric and can be affected by observer bias. Here, we used an unbiased machine learning algorithm, Motion Sequencing (MoSeq), to study behavioral phenotypes associated with CS and WMS in young (4-month-old) and aged (10-month-old) mice at the sub-acute (7 days post injury) and chronic (30 days post injury) phases. We identified novel patterns that have not been previously reported. Specifically, MoSeq revealed that motor deficits are more pronounced in cortical stroke (CS), while cognitive deficits are more severe in white matter stroke (WMS). These changes were consistent across both age cohorts, with the severity of deficits worsening with age. Additionally, we observed improvements between the sub-acute and chronic stages in CS, whereas WMS showed a continuous decline. Our findings suggest that MoSeq can identify unique behavioral patterns associated with these two types of stroke, with spontaneous recovery observed in CS but not in WMS. This study lays the groundwork for future investigations that combine MoSeq with in vivo imaging techniques to explore the circuit mechanisms underlying these behavioral changes.

Poster #D23: (C. Crouzet et al.)

Cerebral blood flow and blood pressure changes during angiotensin II-induced hypertension and telmisartan treatment in Tg2576 mice

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Cerebrovascular changes contribute significantly to cognitive decline in Alzheimer's disease and related dementias (ADRDs). Vascular risk factors are increasingly important for understanding the development and progression of ADRDs. Specifically, midlife hypertension is a leading risk factor for vascular cognitive impairment and AD. Studies suggest impaired cerebral autoregulation reduces Aβ clearance and pathogenic brain changes. A key component in the development of hypertension is the interaction between angiotensin II (ATII) and angiotensin II type 1 receptor (AT1R). AT1R is implicated in premature aging through vascular disease, inflammation, and oxidative stress. Angiotensin receptor blockers (ARBs) are prescribed to treat hypertension by inhibiting ATII-AT1R interactions. ARBs in AD patients can preserve memory and psychomotor processing speed. Importantly, the ARB telmisartan is hypothesized to lessen cognitive impairment by controlling cerebral blood flow (CBF). To measure CBF, we used a common optical technique, laser speckle imaging (LSI). In addition to measuring CBF, cerebrovascular resistance (CVR) is a metric related to the regulation of CBF. CVR can be calculated as a ratio of peripherally-measured mean arterial pressure (MAP) and optically-derived CBF. In this study, we investigated how ATII-induced hypertension and telmisartan treatment impact MAP, CBF, and CVR in the well-established Tg2576 mouse model of AD. We compared MAP, CBF, and CVR data between baseline and final time points. Tg2576 mice receiving ATII with normal drinking water had a significant increase in MAP and CVR ($p < 0.001$) with a significant decrease in resting-state CBF ($p < 0.05$). This expected result may occur due to ATII causing hypertension and global vasoconstriction leading to reduced CBF. Tg2576 mice receiving ATII with telmisartan in the drinking water unexpectedly still had a significant increase in MAP ($p < 0.01$) and CVR ($p < 0.05$). However, CBF was normalized, suggesting that the telmisartan given was a subtherapeutic dose as the final MAP was elevated, but was sufficient to maintain CBF. Collectively these results suggest ATII-induced hypertension drastically increases MAP and CVR, and reduces CBF, while telmisartan normalizes CBF, but not MAP or CVR.

Poster #D24: (Y. Zhang et al.)

Optogenetic gamma and theta-gamma-coupling activation of Lhx6-derived inhibitory interneurons produce opposite effects on a specific subset of the transcriptome

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Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most common form of dementia worldwide. Research shows that Aβ accumulation early in AD impairs inhibitory neurotransmission, leading to dysfunction of gamma and theta brain oscillations involved in higher order cognitive processing. Optogenetic activation of parvalbumin (PV)-expressing interneurons at 40-Hz has been shown to induce gamma oscillations, improve

Aβ pathology, and alter microglia morphology. Our lab has shown that combined stimulation of PV- and somatostatin (SST)-expressing interneurons (together known as the lhx6-derived interneurons) has a larger, synergistic effect on gamma power compared to just PV-stimulation. Given this interneuron synergy discovery, we investigated how simultaneous PV- and SST- interneuron stimulation alters Aβ pathology, microgliosis, transcriptomes, and spontaneous behavior. To do this, we used the PDGF-APPSw,Ind AD model mice with cre-dependent channelrhodopsin (ChR2) expression in Lhx6-derived interneurons (Lhx6-Cre+/- Ai32+/- hAPP-J20+/-). First, mice received optogenetic stimulation of PV- and SST-interneurons in both posterior parietal cortices at theta, gamma, and theta-nested-gamma coupling frequencies respectively. Then, we analyzed functional acute behavior changes using machine learning behavioral phenotyping. We also conducted immunohistochemistry for various AD pathologies and performed single-nuclei RNA sequencing on stimulated and sham-stimulated brains to find activity-dependent differentially expressed genes. As a result of the methods above, we found a subset of differentially-expressed genes that changes expression in opposite direction as a result of gamma vs theta-gamma frequency stimulation to the interneurons, revealing a novel mechanism by which specific neuronal synchronizations by Lhx6-derived interneurons can alter the transcriptome and providing a new window into potential therapeutic gene targets.

Poster #D25: (S. Karmali et al.)

Defining a Neuromotor Behavioral Profile of Male and Female 5XFAD Mice Using Automated Continuous Behavioral Monitoring

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Alzheimer's disease (AD) is a neurodegenerative disease with an insidious onset and progressive detriments in memory, cognition, and movement. To understand behavioral deficits present in the prognosis of AD, it is paramount to measure the changes in motor behavior of mice and other animal models as a function of age. The 5xFAD mouse, one of the most widely used AD mouse models, shows increasingly severe motor and behavioral deficits over time due to its excessive amyloid buildup. Available tests for neuromotor behaviors of mouse models, which include rotarod, grip strength analysis, and wire hang, are prone to experimental or individual mouse inconsistencies. In this study, we characterize motor behavior and function in 5xFAD mice using Automated Continuous Behavioral Monitoring (ACBM), an automated and machine learning-powered animal behavior recording system developed at Brown University. Our longitudinal study analyzed the changes in behavioral and motor function in 2, 4, 6, and 8-month-old mice. ACBM is sensitive to behavioral changes in the 5xFAD mouse throughout light and dark cycles of recording and at different ages. We used a Bayesian Dirichlet model with MCMC to estimate the genotypic differences between 5xFAD and WT mice. 8-month-old mice present a novel "early activity onset" phenotype, rising about one hour earlier than WT mice of the same age. Interestingly, we also find that the first deficits in apathetic behavior occur at four months, earlier than the established onset of these behaviors at six months. We continue to employ ACBM to monitor behavioral and motor deficits of female 5xFAD mice. In short, this study provides a new robust tool for automated homecage monitoring and behavioral profiling of mice, extending our knowledge of early behavioral biomarkers and disease progression in Alzheimer's models. Next, we intend to utilize ACBM to observe the effect of anti-amyloid drugs on 5xFAD mice.

Poster #D26: (Y. Ki et al.)

Analyses of behavioral and neuronal responses during decision-making reveal deficits in Rett syndrome mice

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Rett syndrome (RTT) is a neurodevelopmental disorder characterized by a wide range of symptoms, with severe apraxia being a notable feature. Apraxia is the inability to perform motor planning and is often associated with basal ganglia dysfunction. However, our knowledge of the circuit alterations in the basal ganglia and how they relate to the behavioral symptoms in RTT is limited. Here we used a novel approach to analyze circuit malfunction underlying behavior in a mouse model of RTT that carries a methyl-CpG-binding protein 2 (Mecp2)-null allele (RTT mice). In an automated home-cage system (Hao et al, eLife, 2021), self-motivated mice engaged in tactile decision-making tasks for several months without human supervision. In the decisionmaking task, mice discriminated object location using whiskers and reported object location using directional licking. Parallel testing allowed us to assay two dozen mice at the same time. Instead of cross-sectional analysis, this approach longitudinally tracked the onset and progression of behavior deficits in the RTT mice over time relative to littermate wild-type (WT) mice. We discovered that RTT mice were able to learn the decision-making task similarly to WT mice at 12 to 16 weeks of age. Once the mice achieved proficiency in the decision-making task, we conducted additional assessments of their flexible motor planning by reversing the sensorimotor contingency. The sensorimotor contingency reversals allowed us to examine the mice's ability to adapt to new task rules. RTT mice exhibited slower reversal learning compared to WT mice at 16 to 20 weeks of age, which deteriorated with age. To examine the underlying changes in neural dynamics, we combined this behavioral paradigm with multi-Neuropixels probe recordings across a frontal cortico-basal-ganglia loop required for the tactile decision-making, including anterior lateral motor cortex (ALM), lateral striatum, and ventromedial thalamus. Preliminary analyses revealed reduced preparatory activity across these brain regions in RTT mice. Our study outlines a platform to assay motor planning deficits in the Rett mouse model and their underlying neural dynamics that could allow future interrogations of the involved brain regions.

Poster #D27: (J. Xie et al.)

Reactivation of MEC parvalbumin+ interneurons rescues grid cells in a knock-in model of Alzheimer's disease

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Alzheimer's disease (AD) patients suffer from spatial memory impairment and wandering behavior, but its underlying neural circuit mechanism remains unclear. Using the amyloid precursor protein knock-in (APP-KI) mice, we previously found that grid cells activity in the medial entorhinal cortex (MEC) was impaired earlier than the CA1 place cell remapping and spatial discrimination memory. This suggested that the earlier impairment of MEC grid cells underlies the spatial memory impairment but the mechanism how the MEC grid cell becomes impaired remains unknown. Using optogenetic assisted in vivo electrophysiology, we found that the spike activity of parvalbumin-expressing interneurons showed decreased firing in the MEC of APP-KI mice. Speed cell activity of PV cells was also impaired in APP-KI mice. By contrast, chemogenetic reactivation of PV cells in APP-KI mice improved grid cell coding of MEC neurons and rescued their spatial memory performance in a path integration task. These results suggest that the dysfunction of MEC PV cells underlies grid cell impairment and spatial memory deficit of APP-KI mice, pointing to PV cells as a potential therapeutic target of spatial memory impairments in AD patients.

Poster #D28: (C. Cazares et al.)

Leveraging the neural power spectrum to identify electrophysiological biomarkers across models of neurodevelopmental disorders

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Previous studies have identified several candidate electrophysiological biomarkers for cognitive aging in adults, with one emerging biomarker being aperiodic (1/f-like) neural activity measures. However, how these age-related changes in aperiodic neural activity differentially manifest across models of autism spectrum disorder (ASD) and genetic syndromes remains unknown. Here we used a multimodal analytical approach to investigate whether aperiodic components of neural power spectra serve as consistent biomarkers across task-free electroencephalography (EEG) and neural organoid recordings from boys with ASD, in addition to a rodent model of Rett syndrome (RTT), a genetic syndrome caused by mutations in the X-linked MECP2 gene. Our preliminary findings demonstrate that specific features of aperiodic neural activity can be quantified and tracked across both human EEG and developing neural organoid recordings from the same individual and that these features can be correlated with a patient's cognitive-behavioral assessments. Furthermore, we found that task-free and stimulus-evoked measures of aperiodic neural activity in our RTT mouse model vary across cortical layers of the primary visual cortex and can be related to performance in a visual acuity assay. By integrating field potential analyses across these complementary experimental models, our work establishes a translational framework for understanding neurophysiological disruptions in neurodevelopmental disorders. This approach offers a promising path toward the development of objective, non-invasive biomarkers that could guide therapeutic development and provide quantitative measures of treatment efficacy across the broader spectrum of neurodevelopmental conditions.

Poster #D29: (L. Gorodetski et al.)

Disruption of Dvl2-Mediated Planar Cell Polarity Signaling and Synaptic Plasticity in Mouse Models for Alzheimer's Disease

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Alzheimer's disease (AD) is characterized by neuronal loss and cognitive impairment, with early pathology attributed to the effects of Aβ oligomers and Tau aggregates on synaptic plasticity. To investigate how AD pathology contributes to synaptic dysfunction and potential synapse loss, we examined the expression and function of planar cell polarity (PCP) proteins—key regulators of glutamatergic synapse formation and maintenance—in AD mouse models. We found that Dvl2, a core PCP protein localized in the postsynaptic density (PSD), was significantly reduced in both amyloid and tau-based models of AD. Importantly, when we analyzed human postmortem brain samples, we also observed a trend of Dvl2 reduction, supporting the clinical relevance of our findings.

Poster #D30: (F. Mamdani et al.)

Cell-Specific Telomere Length in Post-mortem Human Brain of Subjects with Major Depressive Disorder

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Major depressive disorder (MDD) is a highly prevalent disorder affecting approximately 11% of the general population, with rates ranging between 3.2 - 4.6% within a given year. Several studies have shown that stressful life events and chronic stress are risk factors for MDD. It is also known that stress can lead to accelerated cellular ageing resulting in shortening of telomeres. In recent studies stress and depression have been associated with reductions in hippocampal volume and decreased neurogenesis in humans and animal models. Telomeres are special repetitive sequences of DNA located at the end of chromosomes which preserve DNA integrity, but they naturally shrink with age/cell divisions, and their loss eventually leads to chromosomal instability and cell death. We first reported a significant reduction in telomere length (TL) in the hippocampus of MDDs compared to controls a few years ago. This finding remained significant following correction for age, pH, and post-mortem interval (PMI). These results suggest a stress-mediated acceleration of cellular ageing within the hippocampus of MDDs. However, since bulk tissue homogenates were used, the cellular specificity of these MDD-Specific changes were not elucidated. This is of interest since the hippocampus and its sub-regions are composed of non-dividing neurons and dividing cells, such as glia, and both these classes of cells can undergo telomere shortening. To answer this question, we measured cell-specific telomere length in the hippocampus of 10 MDD-Suicide subjects (5 males and 5 females), 10 MDD-Non-Suicide subjects (5 males and 5 females), and 10 controls (5 males and 5 females) using Fluorescence Assisted Nuclei Sorting to isolate four cell types (N=120 samples): neurons, microglia, oligodendrocytes, and astrocytes. DNA was extracted for each cell population and TL is measured by a qPCR-based assay. In addition to diagnosis effects (MDDs versus controls) on TL, the impact of suicide status (MDD-Suicide versus MDD-Non-Suicide) and gender (females versus males) on TL is analyzed. This project provides insight into cell-specific mechanisms of telomere attrition in the hippocampus of MDD patients and novel evidence for an interaction between suicide and accelerated cellular aging.

Poster #D31: (E. Kim et al.)

Impaired risky decision-making and prefrontal-hippocampal circuit dynamics in a mouse model of Alzheimer's disease

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Alzheimer's disease (AD) research has traditionally focused on memory impairments, but evidence shows AD also disrupts decision-making under risk and ambiguity essential for daily life. However, the impact of AD pathology on risky decision-making and corticolimbic circuit function remains unclear. To address this gap, we investigated how amyloid pathology influences hippocampal-prefrontal neural dynamics and decision-making in a mouse model of AD. Five familial AD (5XFAD) mice (4-9-month-old) were tested in an ecologically relevant "approach food-avoid predator" foraging paradigm. 5XFAD and wild-type (WT) mice implanted with tetrode arrays in the medial prefrontal cortex (mPFC) and dorsal hippocampus (dHPC; CA1) underwent nest habituation, baseline foraging, and predator testing in a T-shaped maze with grain-based and chocolate-flavored pellet choices. During predator sessions, the predator (a puppet eagle on wheels) rapidly advanced each time the animal approached its preferred pellet, simulating a naturalistic threat. WT mice switched their foraging choice to less preferred but safer pellets in the presence of the predator, demonstrating adaptive foraging. However, 5XFAD mice consistently selected their preferred pellets, reflecting impaired risk assessment and behavioral flexibility. Neural recordings revealed that 5XFAD mice exhibited several circuit-level abnormalities, including reduced sharp-wave ripple (SWR) frequencies in dCA1, diminished mPFC-dHPC spike synchrony, and more rigid CA1 place-cell fields, particularly during the predator phase. Despite these deficits, both WT and 5XFAD mice exhibited comparable unconditioned and conditioned fear responses in a contextual fear conditioning paradigm, suggesting that the observed decision-making impairments are not solely due to generalized anxiety or fear deficits. These findings suggest that disrupted mPFC-dHPC neuronal communication and impaired SWR dynamics may underlie the risky decision-making deficits observed in 5XFAD mice, highlighting critical circuit-level disruptions associated with cognitive decline in AD.

Poster #D32: (Y. Huang et al.)

Early-Stage Corticostriatal Circuit Hyperactivity Impairs Cholinergic Function and Cognitive Flexibility in an Alzheimer's Model

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Cognitive flexibility deficits are a hallmark of early-stage Alzheimer's disease (AD), but the underlying circuit mechanisms remain poorly understood. Here, we show that the 5xFAD mouse model of AD pathology exhibited early deficits in instrumental reversal learning, indicating cognitive inflexibility preceding spatial memory deficits. This impairment was associated with excessive neuronal reactivation in the medial prefrontal cortex (mPFC) and dorsomedial striatum (DMS), key regions for goal-directed behavior. Electrophysiological recordings revealed that mPFC neurons in young 5xFAD mice were hyperexcitable and received elevated excitatory input. Moreover, the mPFC-to-direct pathway medium spiny neuron (dMSN) circuit and the dMSNs themselves were selectively hyperactive. These hyperactive dMSNs exerted increased inhibitory control over cholinergic interneurons (CINs) in the DMS, coinciding with reduced CIN firing and diminished striatal acetylcholine (ACh) release. Critically, sustained chemogenetic inhibition of the mPFC-to-DMS circuit in 5xFAD mice reduced cortical Aβ accumulation, normalized glutamatergic transmission in both the mPFC and DMS, restored striatal ACh levels, and rescued reversal learning deficits. Together, these findings identify a hyperactive mPFC-to-DMS circuit that disrupts corticostriatal and cholinergic signaling, contributing to cognitive inflexibility in 5xFAD mice. Targeting this circuit may offer a therapeutic strategy to preserve cognitive function in the early stages of AD.

Poster #D33: (A. Torres et al.)

Targeted SNCA Suppression via CRISPRi: Route Optimization in Non-Human Primates for Parkinson’s Gene Therapy

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Purpose Parkinson's disease (PD) is one of the most prevalent neurodegenerative disorders in aging populations and currently lacks effective disease-modifying treatments. The pathological hallmark of PD is the presence of Lewy bodies, which contain aggregated alpha-synuclein, encoded by the SNCA gene. Here, we describe the preclinical development of a gene therapy using a nuclease-deactivated S. aureus Cas9 (sadCas9) CRISPR interference system targeting the SNCA promoter to reduce alpha-synuclein expression. By comparing different routes of administration and evaluating the extent of gene downregulation, we aim to establish a solid foundation for an effective gene therapy strategy for PD. Defining the optimal route of administration Non-human primates (Macaca fascicularis) were used to determine the most effective delivery route. Animals received viral injections via intrathecal, intracisterna magna, or bilateral intraparenchymal (substantia nigra) administration. To assess transduction efficiency and target gene repression, we will perform RNAscope in situ hybridization to quantify SNCA and Cas9 transcript levels in key brain regions: substantia nigra, motor cortex, and putamen. Additionally, immunohistochemistry will be performed in these same regions to evaluate protein-level downregulation of alpha-synuclein, as well as expression of tyrosine hydroxylase (TH) and Cas9 protein. Spatial transcriptomics To evaluate cellular responses to the viral vector, we developed a custom Xenium panel that includes markers for immune responses (innate, adaptive, and macrophage-related), PD-relevant and homeostatic pathways (including autophagy, NEDDylation, mitochondrial and lysosomal function), and major brain cell types (neuronal, glial, endothelial, and ependymal cells). This approach enables detailed cell-type specific gene expression profiling across treatment groups and will guide Phase 3 studies, where the selected delivery route will be further validated alongside sham controls. Conclusion We identify the optimal delivery route for our neuromodulatory gene therapy in Parkinson’s disease by assessing Cas9 and SNCA expression levels in three critical brain regions. The optimal route will be defined as the one achieving the greatest downregulation of alpha-synuclein protein and the highest transduction efficiency (as determined by Cas9 expression) in the substantia nigra, motor cortex, and putamen. Preliminary spatial transcriptomic data in non-human primates will provide additional context on cellular responses across treatment groups.

Poster #D34: (G. Acharya et al.)

Leveraging Temporal Dynamics to Enhance Seizure Forecasting in a Virtual RNS Framework

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Seizure management systems such as NeuroPace's Responsive Neurostimulation (RNS) device are currently far more capable of detecting seizures than predicting them. In contrast, state-of-the-art forecasting algorithms trained on labeled interictal and preictal intracranial EEG (iEEG) data can reach decent performance (e.g., ≥ 80% accuracy, AUC ≥ 0.8), but often fall short in pseudo-prospective evaluations, particularly in their ability to minimize 'time in warning'. These algorithms generally rely on static features and often underutilize the strong temporal dynamics embedded in iEEG.

In this study, we explore the potential of modeling temporal dynamics–of both iEEG features and seizure risk–to improve forecasting in a virtual RNS framework. Using iEEG.org data from n = 30 patients with mesial temporal lobe (MTL) epilepsy at the Hospital of the University of Pennsylvania, we simulated an RNS-like seizure predictor that uses the main iEEG features used by the RNS device (namely, line length, variance, area, half-wave feature, and 5 band powers) extracted from four MTL channels and tested its performance in pseudo-prospective prediction of acute seizures. We find that forecasting iEEG features is not only feasible up to 10 minutes ahead, but incorporating them into the classifier improves pseudo-prospective performance by 36% over baseline. Additionally, temporal smoothing of predicted seizure risks using historical risk trends results in an additional 25% gain, yielding a 46% improvement when combined with feature forecasting. Interestingly, we also find that excluding iEEG data immediately preceding seizure onset during training can enhance baseline performance, suggesting that the preictal period comprises distinct early and late phases–with the early phase potentially offering more generalizable information for long-horizon prediction. This complementary observation further underscores the value of tailoring training strategies to better capturing seizure-related dynamics.

Furthermore, we quantified the difficulty of predicting each seizure, measured as the minimum time-in-warning required to successfully block out the corresponding preictal and ictal period, and found that seizure difficulty strongly correlates with the duration of the interictal period preceding each seizure ($\rho = -0.15$, $p < 0.05$), a correlation that did not exist in the RNS-like baseline predictor ($\rho = -0.01$, $p = 0.91$). In other words, inclusion of dynamics resulted in improved predictability especially for isolated and lead seizures, which are also the ones with greatest impact on patients' quality of life.

Poster #D35: (M. Shanmugam et al.)

Mapping the Second-Order Projections of Ventral Hippocampus CA1 Neurons Using a Novel Transsynaptic Tracing Approach

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The ventral subregion of the rodent hippocampus (anterior hippocampus in humans) is implicated in body weight regulation, anxiety, and various emotionally relevant learning and memory processes. Understanding the neuroanatomical connectivity of this brain region with striatal and hypothalamic regions associated with energy balance and reinforcement learning may help identify novel central targets for weight loss and psychiatric disorders. Here we identified transsynaptic anterograde neural pathways originating from the CA1 neurons in the ventral hippocampus (CA1v) of male rats. Certain first-order targets of the CA1v field have already been established by our group and others; namely, the nucleus accumbens shell (ACBsh) and the lateral hypothalamic area (LHA). To identify the second-order targets of these pathways, a novel rationally designed transsynaptic viral tracing approach, “ATLAS”, was employed to identify downstream targets of CA1v-ACBsh and CA1v-LHA pathway projections. ATLAS viral targeting of CA1v projection neurons drives Cre expression in downstream neurons in a strictly anterograde, glutamatergic, activity-dependent, and monosynaptic manner (ref: Rivera et al.). A cre-dependent anterograde tracer (AAV1-FLEX-tdTomato) was then injected in the ACBsh or LHA to reveal second-order targets of these pathways. Projection density was characterized using immunohistochemistry-based amplification of transsynaptic labeling, then quantified systematically using the recently-developed Axiome C platform for a whole-brain characterization of neural pathway density (ref: Hahn et al.). Results reveal that the highest total axon projection density from the CA1v-ACBsh pathway was observed in the lateral septum (LS), LHA, and the bed nucleus of the stria terminalis (BNST). While the quantification of 2nd order tracing from the CA1v-LHA pathway is still in progress, preliminary results suggest that the septofimbrial nucleus (SF), medial septal nucleus (MS), and LS are primary downstream targets of this pathway. Ongoing complementary experiments will utilize transsynaptic retrograde tracing methods to better identify brain systems that input to these CA1v pathways. Overall, this study gives us insight into transsynaptic neural circuits connecting CA1v neurons to brain regions that are critical for reinforcement learning, anxiety, and energy balance.

Poster #E1: (X. Wang et al.)

Altered Spontaneous Brain Activity in Obese Children: A Resting-State Functional MRI study

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To investigate brain function changes in obese children using resting-state functional magnetic resonance imaging (rs-fMRI). Method: Fifty-five obese children (35 simple obesity [Simple OB], 20 obesity with metabolic syndrome [OB+MS]) and 24 healthy controls (HC) underwent rs-fMRI, anthropometric measurements, and laboratory tests. Group differences in amplitude of low-frequency fluctuations (ALFF) and fractional ALFF (fALFF) were analyzed via analysis of covariance (ANCOVA) and post-hoc Tukey tests. Correlations between brain regions with significant differences and clinical metrics were assessed. Results: (1) ALFF Analysis: Compared with the HC group, ALFF values significantly increased in the right fusiform gyrus, right superior occipital gyrus, and left inferior temporal gyrus in the Simple OB group ($p < 0.001$). The OB+MS group showed similar increases in these regions, along with the right precuneus ($p < 0.001$). ALFF values in the right fusiform gyrus and right precuneus were higher in the OB+MS group than in the Simple OB group ($p < 0.01$). ALFF in the right fusiform gyrus correlated positively with body mass index (BMI), total body fat percentage (TBF%), and liver enzymes (ALT/AST; $r = 0.27–0.69$). Right superior occipital gyrus ALFF correlated with BMI and diastolic blood pressure (DBP; $r = 0.30–0.64$), while left inferior temporal gyrus ALFF linked to BMI and liver markers. Right precuneus ALFF associated with BMI, triglycerides (TG), DBP ($r = 0.27–0.37$), and inversely with high-density lipoprotein cholesterol (HDL-C; $r = -0.32$). (2) fALFF Analysis: Both obese groups exhibited elevated fALFF in the left, inferior temporal gyrus, right orbital middle frontal gyrus, and right median cingulate/paracingulate gyri compared to HC ($p < 0.01$). The OB+MS group showed higher fALFF in the right orbital middle frontal gyrus than the Simple OB group ($p = 0.0002$). Left inferior temporal gyrus fALFF correlated with waist-hip ratio (WHR; $r = 0.27$), and right orbital middle frontal gyrus fALFF with BMI and fasting blood glucose (FBG; $r = 0.31–0.42$). Conclusion: Increased ALFF/fALFF in obese children were localized to visual and emotional processing pathways. The right fusiform gyrus, precuneus, and orbital middle frontal gyrus demonstrated specificity in the OB+MS group, suggesting their role as central pathological markers of metabolic syndrome-related obesity.

Poster #E2: (Z. Wang et al.)

Wirefree Miniscope Calcium Imaging in 3D Staircase

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The hippocampal CA1 region plays a critical role in memory encoding, temporal processing, and spatial navigation, yet its function in three-dimensional (3D) environments remains underexplored. In this study, we employ a wire-free calcium imaging miniscope to record neural activity in the dorsal CA1 of freely behaving mice navigating a custom-designed 3D staircase. By pairing neural recordings with behavioral tracking from dual orthogonal cameras, we reconstruct the animals' trajectories and correlate spatial behavior with neuronal dynamics. Using DeepLabCut for behavioral pose estimation and SCOUT (Single-Cell spatiOtemporal longitUdinal Tracking) for neuron identity registration across sessions, we build a comprehensive dataset linking position and directionality to CA1 activity. Further analysis includes CEBRA (Consistent EmBeddings of high-dimensional Recordings using Auxiliary variables) to uncover the latent topological and temporal structure of population-level dynamics. This project offers new insights into the neural coding of 3D navigation and advances the use of untethered imaging and AI-driven analysis in neuroscience.

Poster #E3: (Q. Sun et al.)

Two interneuron subtypes differentially involved in information valuation during learning

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Wyoming Sensory Biology Center

Learning involves evaluating multiple dimensions of information and generating appropriate actions, yet how the brain assigns value to this information remains unclear. In this study, we show that two types of interneurons (INs) in the primary somatosensory cortex—somatostatin-expressing (SST-INs) and parvalbumin-expressing (PV-INs) neurons—differentially contribute to information evaluation during trace eyeblink conditioning (TEC). An air puff (unconditioned stimulus, US) delivered after a whisker stimulus (conditioned stimulus, CS) elicited both reflexive eye closure and stress-related locomotion. However, only self-initiated, anticipatory eye closure during the CS window, measured via electromyography (EMG), was directly relevant to learning performance. We found that SST-IN activity was primarily associated with the generation of anticipatory eye blinks during the CS period, showing a negative correlation with EMG responses. In contrast, PV-IN activity was positively correlated with stress-related locomotion following the US, suggesting a role in processing the emotional or aversive component of the task. Furthermore, cholinergic signaling via nicotinic receptors modulated both SST- and PV-IN activities, linking these interneurons to the regulation of learning-related actions and emotional responses. These findings demonstrate that distinct interneuron populations evaluate different dimensions of information—SST-INs for predictive, adaptive actions and PV-INs for stress-related emotional responses—to guide learning and behavior.

Poster #E4: (Withdrawn)

Poster #E5: (D. Martins et al.)

Retinocentric to egocentric transformation in visuoparietal cortex of freely moving mice

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To perceive and interact with a complex and changing environment, animals use visual inputs to build an internal model of their local space. Early visual regions represent object locations in a retinotopic reference frame, preserving the relative topography of retinal photoreceptors. Significant cortical processing is required to transform these retinocentric representations to the egocentric reference frame found in association cortex, in which object locations are mapped relative to the animal's head or body. Computational models have proposed that egocentric object location can be calculated by integrating retinocentric location and pupil orientation. However, the neural circuitry underlying this critical transformation is not well understood, especially under conditions of active visual sampling. Here, we used a head-mounted miniaturized two-photon microscope to perform calcium imaging of mouse visuoparietal cortical neurons during free movement through a chamber containing a salient visual object. By combining body pose tracking with a head-mounted camera to measure pupil movements, we determined the location of the object in both egocentric and retinocentric coordinates. We mapped the distribution of reference frame coding between primary visual cortex (V1) and posterior parietal cortex (PPC) to determine which neural populations compute this transformation. Next, we measured idiothetic pupil orientation signals from lateral posterior thalamus (LP), the rodent homolog of primate pulvinar, by recording LP axonal projections into V1 and PPC during free movement. Next, we will chemogenetically silence LP to eliminate idiothetic inputs from the retinocentric to egocentric transformation circuit while recording activity in V1 and PPC. These experiments aim to reveal the biological implementations underlying a critical reference frame transformation in the mammalian neocortex.

Poster #E6: (C. Wang et al.)

Imaging Acetylcholine Dynamics across Cortical Layers during Behaviors

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Traditionally, acetylcholine is thought to be globally released in the cortex, acting as a broadcast signal to modulate the brain state and cortical neuronal activity. Recent anatomical studies revealed specific input-output relationships for distinct motifs within Basal Forebrain cholinergic neurons (BFCNs; Gielow & Zaborsky, 2017) and layer-specific innervation of BFCNs in the primary Somatosensory Cortex (S1; Allaway et al., 2020). These suggest the possibility that BFCNs projecting to different layers of S1 receive inputs from distinct sets of brain regions. However, direct evidence for distinct, layer-specific spatiotemporal dynamics of cortical acetylcholine is missing. Hence, we imaged the acetylcholine dynamics across S1 layers using an aluminum-coated microprism and a novel acetylcholine sensor GRAB-ACh4m during spontaneous behavior. To assist layer segmentation for prism imaging, we retrogradely traced from L2/3 and L5A of vibrissal Motor Cortex (vM1) to label L2/3 and L5 of S1. We tracked facial movements and pupil fluctuations during imaging and regressed the contributions of different movement predictors to acetylcholine dynamics across layers. To further test potential functional divergence of cortical acetylcholine, we delivered odors, air puffs, and social interactions in separate imaging sessions. Together, these results delineate the contributions of stimulus and stimulus-evoked movements to acetylcholine dynamics across cortical layers during a diverse set of behaviors.

Poster #E7: (K. Christopher et al.)

Cholinergic control of vocal-emotional behavior in the developing marmoset (Callithrix jacchus)

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Cholinergic neurons play an essential role in refining cognitive-emotional components of executive function by interacting with the activity of prefrontal-hippocampal networks. A major population of these neurons originate from the basal forebrain nuclei that lie as a continuum extending from the medial septum, vertical and horizontal diagonal bands of broca, through the nucleus basalis of Meynert. During development, acetylcholine modulates neuronal proliferation, differentiation, and synaptic plasticity but its contribution to the development and maturation of cognitive, social and emotional processing remains unknown. In this study we show how early life degeneration of medial septal cholinergic projections in infant marmosets alters the normal development of vocal behavior and fear expression. We targeted the medial septal-basal forebrain nuclei by injecting ME20.4-IgG-saporin (saporin) in 14-day old neonatal marmosets (n=6). Saporin is an immunotoxin that selectively destroys the p75-positive cholinergic cells projecting to the hippocampus. Another group of infant marmosets received sham control surgery (n=6). We first examined changes in isolation induced vocal calls before and after the saporin immunolesion. Vocal calls such as phee, twitter, trill and tsik were preserved before and after the saporin immunolesion. The infant specific cry-call that triggers parental feedback was also intact but there was a notable discontinuation of cry-calls by week 5. In addition, their cries were short in duration, low in frequency and they made fewer long distance social contact calls (muti-syllabic phees). We also assessed their emotional responses to a fear inducing object (i.e., a fake owl) or neutral objects (e.g., toy car, color blocks) that were not fear inducing. We noted that at 4 weeks of age, the control infants seemed oblivious to the fear object; they neither approached nor exhibited defensive reactions to it. This apparent fearlessness was also evident at 12 weeks of age, with a complete absence of tisk alarm calls. In contrast, animals with medial septal cholinergic depletions displayed defensive reactions and their vocal repertoire was dominated by alarm tsik calls as early as 4 weeks of age and present at 12 weeks of age as well. At 9 weeks of age, we examined their social behavior towards a parent and a stranger. Control infants spent more time in close proximity to the parent, and were equally social towards a stranger. In contrast, those with medial septal cholinergic depletions appeared ambivalent in their behavior showing a mixture of approach, avoidance, and disinterest towards the parent, even when the parent tried to touch their infant. Together, these data suggest that the normal development of medial septal-basal forebrain cholinergic projections are integral to the postnatal development of vocal-emotional and social behavior.

Poster #E8: (X. Zheng et al.)

Parametric modulation of a shared midbrain circuit drives distinct vocal modes in a singing mouse

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Neural circuits capable of generating multiple outputs are essential for behavioral flexibility, yet their organizational principles remain poorly understood. In this study, we investigated whether distinct vocal behaviors of singing mice (Scotinomys teguina) are controlled by separate pathways or by shared circuits operating in different parametric regimes. Using a novel behavioral assay, we found that singing mice alternate between two major vocal modes: loud, temporally patterned songs lasting several seconds that are used in long-distance communication and soft, unstructured ultrasonic vocalizations (USVs) employed during close-range interactions. Notably, the song rhythm can be accurately described by a simple linear model with just three parameters. Despite their dramatic acoustic and contextual differences, both songs and USVs share peripheral sound production mechanism, vocal-respiratory coupling, and central neural control by the caudolateral periaqueductal gray (clPAG). To probe the role of the clPAG neurons in song motor control, we silenced them with Tetanus toxin light-chain (TeLC), which progressively lowered song amplitude and duration before eliminating all vocalizations. In particular, our model revealed that the patterning parameter most affected by the clPAG silencing — which controls song termination — also accounts for natural sexual dimorphism in song rhythm. This suggests that the clPAG may drive the natural variability of song production. Our findings reveal how differential amplitude and frequency modulation of shared neural circuits produces categorically distinct behavioral outputs and provide a mechanistic basis for how behavioral innovations can emerge through evolutionary tinkering of ancestral neural pathways.

Poster #E9: (Z. Liang et al.)

Activation of The Sour Receptor OTOP1 by Ammonium Chloride, a Key Component of Salty Licorice

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Humans and most other vertebrates detect and discriminate among five basic tastes, for which receptors are now known. In addition, high concentrations of salts, such as ammonium or potassium chloride, evoke an aversive taste distinct from the ENaC–mediated attractive sodium taste. Ammonium and other non-sodium salts are detected by both Type II taste receptor cells (TRCs), which mediate bitter, sweet, and umami tastes, and Type III TRCs, which mediate sour taste. Type III TRCs express the proton channel OTOP1 which functions as a sour receptor. Because NH4Cl alkalinizes the cell cytosol, we hypothesized that OTOP1 might function as a sensor for the NH4Cl taste. Indeed, extracellular NH4Cl evoked large inward currents in OTOP1-transfected HEK-293 cells. The current magnitude correlated with the degree of intracellular alkalization measured with a fluorescent pH sensor, pHlourin. Similar responses were observed for human OTOP1, whereas relative NH4+ sensitivity was diminished in zebrafish OTOP1 and enhanced in chicken OTOP1. The large current magnitude and species variation led us to hypothesize that OTOP1 channels were gated by the intracellular pH change, rather than passively responding to the pH gradient. Indeed, a charge-neutralizing mutation (R292A) of a conserved arginine selectively reduced NH4+ sensitivity without affecting acid responses. Finally, using Otop1-/- mouse strain, we showed that OTOP1 is required for sensory responses of both the gustatory nerve and isolated Type III TRCs to NH4+. The behavioral aversion showed a significant reduction to NH4Cl in Otop1-/- mice, and this aversion was entirely abolished in a double knockout mouse strain with Skn-1a−/−. These data together reveal an unexpected role for the proton channel OTOP1 in mediating a major component of the NH4Cl taste and a novel channel regulation mechanism conserved across species. These findings also suggest a potential role for OTOP channels in mediating responses to additional taste-related stimuli, indicating broader functional significance in gustatory signaling and regulation of cellular pH homeostasis.

Poster #E10: (G. Agarwal et al.)

News without the buzz: reading weak theta rhythms in the hippocampus

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Local field potentials (LFPs) reflect coordination among neural populations, yet their exact relationship to neural computation remains unknown. One exception is the theta rhythm of the rodent hippocampus, which organizes sequential firing among place cells, enabling spike timing to track the animal's path through its environment. But when the animal stops, the theta rhythm becomes irregular, which is assumed to disrupt its ability to carry information. We challenge this assumption by developing an artificial neural network that discovers position-tuned theta rhythms (pThetas) from LFPs even in the absence of strong theta oscillations. We provide evidence that pTheta is distinct from the dominant theta rhythm, while reflecting rhythmic coordination among place cell populations. Our work suggests that weak and intermittent oscillations, as seen in many brain regions and species, can convey information commensurate with population spike codes.

Poster #E11: (L. Korobkova et al.)

The Zona Incerta Encodes Sensory Stimuli Valence

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Background: Navigating a complex world to survive and balance approach and avoidance behaviors requires integrating multisensory cues associated with food availability and danger. While the zona incerta (ZI), a mostly GABAergic subthalamic brain region, is implicated in processing and modulating various sensory inputs, its role in encoding the valence of sensory stimuli remains unclear.

Methods:We used in vivo fiber photometry recordings to measure neuronal activity in GABAergic cells of the ZI of mice exposed to appetitive and aversive stimuli during operant and fear conditioning tasks. Additionally, to identify overlap or independence in valence-specific processing of stimuli by ZI neurons, we combined Targeted Recombination of Active Populations (TRAP) to label neuronal ensembles active at the time of encoding appetitive or aversive conditioning with immunohistochemistry (IHC) for the early immediate gene C-FOS at later time points.

Results: Fiber photometry revealed that GABAergic neurons in the ZI respond to both appetitive and aversive sensory stimuli learned through conditioning. Moreover, TRAP and C-FOS visualization identified that aversive and appetitive stimuli were processed by both, overlapping and distinct, neuronal ensembles in the ZI.

Conclusions:These results highlight the role of the ZI in encoding sensory valence and suggest that it integrates learned associations with sensory stimuli to guide behavioral responses. Together with our previous findings on the involvement of the ZI in motivation, these data support the idea that the ZI serves as a crucial hub that flexibly links sensory processing with appropriate behavior.

Poster #E12: (S. Landi et al.)

Value schemas dictate hippocampus dynamics during rapid learning

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A concept bridging spatial representation and memory that may be a powerful principle for the primate hippocampus is that of the cognitive map, a form of internal model which allows one to organize knowledge gained from experience, assimilate new information and plan. However, it is unclear how such representations develop during learning. To study this, we recorded the activity of hippocampal neurons in nonhuman primates learning a set of naturalistic foraging tasks in a virtual reality environment. Using a joystick, monkeys learn to navigate in an open-field arena and sequentially harvest rewards by colliding with visual targets. Harvested targets provide rewards based on an underlying rule which constitutes the schema, defined either by the color or the spatial location of the targets in the arena. Critically, on each trial, monkeys are given a limited time to harvest targets, such that they can only harvest about half of the targets before they disappear. Therefore, learning the underlying schema leads to more efficient foraging and greater reward. Monkeys learn new schemas each recording session. Unlike other behavioral tasks that can take many months for monkeys to learn, our paradigm enables efficient and robust learning within a single recording session. We recorded hippocampal activity using 32- and 64- channel Plexon S-probes and 1.0 NHP Neuropixels along the anterior-posterior axis of the hippocampus while monkeys performed interleaved blocks of spatial and color schema foraging. We examined representations of sensory inputs, their associated values, and the abstract task structure at both the level of single units and the simultaneously recorded neural ensemble. We used principal component analysis to identify the leading covariance patterns in the population. Although individual neurons showed significant mixed selectivity, we identified a low dimensional subspace in the hippocampus that captures information about reward value and task schema. Value maps emerged in a schema-dependent manner, i.e., spatial value maps were elicited only during learning under a spatial schema. We observed a similar trend at the single unit level, with hippocampal neurons evolving flexible, schema-selective firing rate maps. The hippocampus thus emerges as a key node in linking crucial aspects of ongoing experiences with their outcomes, weighting reward value maps in a schema-dependent manner.

Poster #E13: (S. Acosta-Mendoza et al.)

Distance Representation in Mouse V1 Arising from Integration of Multiple Depth Cues

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Animals must estimate the distance of external objects to effectively interact with their environment. While the retina encodes a two-dimensional projection of the world, the visual system reconstructs three-dimensional structure using multiple depth cues. Among these, binocular disparity—the difference in input between the two eyes—has been extensively studied and is known to drive disparity-selective responses in the mouse primary visual cortex (V1). However, behavioral studies have shown that mice can judge distance using monocular vision alone. Further, V1 is required for successful performance, suggesting that it encodes a representation of distance beyond binocular cues. Despite decades of study, it is not well understood how binocular and monocular cues are integrated in V1 to support distance perception. To address this, we are using two-photon calcium imaging in head-fixed mice to measure V1 activity in response to stimuli presented at varying distances, while systematically manipulating the available depth cues. Preliminary findings show that a subset of V1 neurons exhibits reliably distance-tuned responses. We are currently extending these findings to freely moving animals using a head-mounted two-photon microscope in order to assess whether distance tuning is preserved during natural behavior. This work aims to provide new insights into how distance cues are encoded and integrated in V1, and how these representations support visually guided behavior in ethological contexts."

Poster #E14: (S. Haga-Yamanaka et al.)

Hypothalamic encoding of chemosensory predator threat in mice

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Animals innately select context-appropriate defensive behaviors of varying intensities. In mice, the accessory olfactory system (AOS) is a key sensory pathway for detecting predator-derived chemosignals, relaying the information to medial hypothalamic nuclei, particularly the dorsomedial/central subdivision of the ventromedial hypothalamus (VMHdm/c), a critical center for defensive behavioral responses. Although it is well established that certain predator-derived chemical cues activate accessory olfactory neurons and trigger innate defensive behaviors, the downstream neural pathways and circuit mechanisms through which AOS-derived sensory information is transformed into specific behavioral outputs remain largely unresolved. We recently identified fresh cat saliva as a potent source of predator cues that convey imminent threat and elicit freezing behavior via the AOS. Notably, the number of VMHdm/c neurons activated by cat saliva exposure positively correlates with the duration of freezing, suggesting that VMHdm/c neurons play a critical role in mediating defensive responses to predator threats detected by the AOS. To test this, we examined whether VMHdm/c neurons are activated during cat saliva-induced freezing and whether their activity is required for the expression of this behavior. We performed real-time calcium imaging of VMHdm/c neurons using fiber photometry in SF1-Cre mice expressing GCaMP8m. Exposure to cat saliva markedly increased freezing behavior, which was accompanied by the VMHdm/c activity significantly higher than those during freezing episodes prior to exposure or during control swab exposure. Furthermore, to test the necessity of VMHdm/c neurons, we silenced SF1+ neurons using hM4Di DREADDs and Compound 21 during cat saliva exposure. Inhibition of these neurons significantly reduced freezing behavior compared to controls. Collectively, our findings demonstrate that VMHdm/c neurons are both strongly activated by predator cues and required for the expression of freezing behavior, establishing their critical role in encoding chemosensory threat. These results provide direct functional evidence that the VMHdm/c serves as a key hypothalamic node for integrating accessory olfactory inputs and translating them into appropriate defensive responses.

Poster #E15: (A. Lam et al.)

Lateral spread in sensory cortex as a novel gain mechanism in selective detection

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Neocortex is a context-dependent connectivity machine. And yet, the mechanisms underlying dynamic functional connectivity within the neocortex are poorly understood. One likely contributing factor is gain modulation of the upstream region. Here, we investigate the contribution of lateral spread as a potential gain mechanism in the context of a selective detection task in mice. In this task, mice learn to selectively respond to a single paddle deflection in the whisker field of one side of the face (target) and ignore identical deflections of the opposite side (distractor). Target and distractor stimuli evoke similar responses within their primary somatosensory cortex (S1) center response fields. And yet, target stimuli effectively propagate beyond S1 to multiple cortical and subcortical regions while distractor stimuli do not. Using widefield Ca2+ imaging, we determined the extent of target vs. distractor lateral spread within sensory cortex. For expert performing mice, we found that target stimuli evoke significantly larger volumes of activation compared to distractor stimuli. Moreover, we found a stronger relationship between peak (center response field) activation and lateral spread for target compared to distractor stimuli, which emerges with learning. Using a simplified neural circuit model, we demonstrate that modulations of lateral inhibition can account for the differences in target vs. distractor evoked spatial activations. From these findings, we propose that modulation of lateral spread in sensory cortex could be a potent mechanism of stimulus gain underlying dynamic functional connectivity.

Poster #E16: (A. Hwang et al.)

Cortical Coordination: Cholinergic and Noradrenergic Dynamics in Sensorimotor Task Learning and Execution

Anya Hwang, Kevin Hodo, Samuel Andrew Hires

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Cholinergic and noradrenergic systems are among the most vulnerable neural circuits affected in age-related neurodegenerative disorders like Alzheimer's Disease (AD) where their dysregulation contributes directly to many symptoms of cognitive decline. The effect of this degeneration on coordinated neuromodulator dynamics and the degree to which the resulting dysregulation impairs cognitive performance remains unresolved. Understanding the systemic role of these widely projecting neuromodulatory circuits in the manifestation of cognitive decline will provide insight towards how disruption of signaling contributes to the development of neuropathological diseases. Given that noradrenergic and cholinergic signaling drives network-wide brain state coordination to promote learning, arousal, and memory, we aim to characterize cholinergic and noradrenergic release dynamics across learning and execution of a sensorimotor task in wildtype and AD models of neurodegeneration. To simultaneously record cholinergic and noradrenergic release activity, we developed a two-photon in vivo imaging technique using dual-colored GPCR Activation Based sensors (GRABs) broadly expressed in cortical neurons. Here we demonstrate across learning, consistent codeveloped patterns of neuromodulatory release emerge in cortex of wildtype mice, where distinct periods of synchronization and desynchronization of acetylcholine (ACh) and NE dynamics are observed across behavioral events. These results suggest a level of coordination between task related noradrenergic and cholinergic release. Understanding the role of coordinated neuromodulatory dynamics in this context will provide a foundation for clarifying the impact of cholinergic and noradrenergic dysregulation in the progression of neurodegenerative diseases.

Poster #E17: (B. Isaac Cohen et al.)

Global and Modular Acetylcholine Dynamics to Movement, Sound and Reinforcement

B Isaac Cohen, Garrett Goodrum, Robert Harutyunyan, Samuel Andrew Hires

University of Southern California

The neuromodulator and neurotransmitter acetylcholine (ACh) is necessary for normal cognition. However, the functional organization of the cortical cholinergic system remains elusive. Cortical ACh largely originates from subcortical projections in the basal forebrain which innervate the entire cortex with a modular architecture. Whether this intricate anatomy translates into modular ACh release during task-related behavior remains unknown. We used whole-brain expression of a next-generation G-protein-coupled receptor-activation-based ACh sensor (GRAB-ACh4m) and mesoscopic imaging to record ACh dynamics across the entire dorsal cortex during conditioning to reward and punishment. Our preliminary findings suggest that the cortical cholinergic system responds in a remarkably global fashion to activity and stimuli, while simultaneously organizing into distinct modules with unique response properties. This work advances our understanding of the degree of spatial and temporal flexibility of the cholinergic system and its potential role in modifying cognitive processes.

Poster #E18: (J. Ong et al.)

GABAergic Over-inhibition or Excitation: Investigating Conflicting Hypotheses about Down Syndrome Sleep Deficits

Jessie Ong, Stella Lopez, Sadie Bella Peroulas, Tula Kurashige, Angelica Alvarado, Rebecca Pizzitola, Elsa Pittaras, Craig Heller

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Down syndrome (DS) affects 6 million people worldwide and results in intellectual disability and an altered sleep phenotype. Since 2007, The Heller Lab at Stanford has proposed that neuronal over-inhibition is the cause of DS intellectual disability based on their finding that GABA antagonists restore cognitive abilities of Ts65Dn mice. In parallel, the Cancedda Lab has proposed the exact opposite hypothesis that neuronal over-excitation is the cause of DS intellectual disability based on their discovery that a chloride cotransporter antagonist also restores cognitive abilities of Ts65Dn mice.

To understand the reasons for our different results, we administered a KCC2 agonist (CLP290) that decreases intracellular [Cl-] so that GABA is hyperpolarizing and inhibitory to achieve the following:
Test the excitatory and inhibitory GABAergic hypotheses with respect to their effects on sleep in Ts65Dn mice
Determine the effect of decreasing excitatory GABAergic signaling on learning/memory

Ts65Dn mice and control mice were divided into baseline (no drug), vehicle (saline), and KCC2 agonist groups in a crossover experimental design (n=25). Saline and KCC2 agonist were administered intraperitoneally. Seep was evaluated with Electroencephalogram/Electromyography electrodes and learning/memory was evaluated with Novel Object Recognition and T-Maze.

Learning/Memory
Ts65Dn mice display worse short-term and long-term recognition memory compared to control mice.
Short-term and long-term recognition memory is significantly improved in KCC2 compared to vehicle treatment in Ts65Dn mice.
Sleep
Ts65Dn mice display significantly decreased NREM and increased waking compared to control mice.
NREM is significantly increased and waking is significantly decreased in KCC2 compared to vehicle treatment of Ts65Dn mice.

Ts65Dn mice demonstrate decreased NREM, increased waking, and worse short- and long-term memory than control mice. The KCC2 agonist treatment, CLP290, increased NREM, decreased waking, and improved memory in Ts65Dn mice. We demonstrate that increasing GABAergic inhibition with a KCC2 agonist alleviated sleep and memory deficits in Ts65Dn mice. This study supports the hypothesis that intellectual disability observed in DS is caused by overexcitation of GABAergic neurons.

Poster #E19: (C. Dupre et al.)

Locomotion in radially symmetric animals

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Hydra is a useful animal model to study basic behavior, representing the first instances in evolution to have a nervous system. A freshwater polyp mostly found in ponds and lakes, it is radially symmetric with a body essentially made of a cylinder with a head and tentacles at one extremity and a foot at the other extremity. It can move its body column in various directions in order to explore its surroundings and contract it to hide and escape threats.

Using these simple motion primitives Hydra spends most of its time foraging, defined as an exploration of the local environment for food items. The number of components that make foraging behavior is not known, and the temporal structure of this behavior still needs to be elucidated. Hydra is most of the time sessile, i.e. it stays attached with its foot to a substrate. However, it can sometimes move to a different location by somersaulting, which is done by attaching its head to the substrate and detaching its foot in order to reattach it somewhere else.

To decipher the structure of movement in Hydra, we created a paradigm where we can track it in 3D over multiple days and reconstruct it with a model. We found that Hydra somersault in bursts separated by intervals of a few hours, which creates sequences of nomadic states interspersed with sequences of sessile states.

We suggest that somersaulting and sessile states alternate as a strategy to explore the environment. Further, within somersaulting and sessile states there are other substates that exist and can help the animal tune its exploration. Together, this provides a description of an algorithm that can be used to implement foraging behavior in a radially symmetric animal.

Poster #E20: (E. Azevedo et al.)

Neurotensinergic circuits controlling threat avoidance in mice

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Novelty is an essential aspect of life that drives exploratory behavior in animals. Exploration in response to novelty is a highly conserved process and fundamental to survival. However, novelty also presents the potential for danger. Avoidance behaviors (i.e. latency to explore new foods or open environments) is an adaptive response to threats that delay exploration and ensure survival. Avoidance behaviors are enhanced by psychological stress and are a hallmark of neuropsychiatric disorders in humans. Neural circuits that control threat avoidance via integration of stressful information and that modulate exploration remain unknown. Elucidating the behavioral function of this well-conserved phenomenon and its underlying neural mechanisms are important open-ended questions. Here we identify and characterize neurotensinergic circuits in the lateral septum (LSNT) in stress-induced threat avoidance in mice. To simulate predator stress, mice were exposed to undiluted 2,4,5-trimethylthiazole in a filter paper for 1h acutely or 1h/day for 7, 15 or 30 days. Control mice were exposed in the same context to water. We assessed anxiety-like behaviors using novelty-suppressed feeding (NSF) and elevated plus maze (EPM). Using predator odor to increase neophobic behaviors in mice, we identified a neural population in the lateral septum (LS) that integrates predator information and modulates the latency to explore food in the NSF test or open environments in the EPM test. We observed that avoidance behaviors only appeared in animals chronically exposed to predator odor at days 15 and 30, but not earlier. Calcium recordings in freely exploring mice, activity-based and single nuclei RNA-seq analysis revealed that predator-responsive LS neuronal clusters are GABAergic and express neurotensin (LSNT). Furthermore, chronic chemogenetic activation of LSNT neurons induced avoidance behaviors in the absence of a predator odor while synaptic silencing can completely abrogate stress-induced avoidance behaviors. Using genetic mouse models to indelibly tag predator odor-responsive neurons, we defined the downstream circuit that connects the encoding of predator information to the lateral hypothalamus and supramammillary nucleus. Projection-specific activation of LSNTLH neurons increase stress-induced avoidance behaviors while LSNTSUM projections alleviate these effects in mice. Our findings offer two opposing neurotensinergic circuits that are genetically- and projection-defined and link psychological stress and anxiety-like behaviors in chronically stressed mice. These molecularly defined opposing circuits may serve as framework to understand the basis of foraging in predator-rich environments, where novelty may mean danger.

Poster #E21: (L. Carrette et al.)

Analgesic brain states through mu and kappa opioid receptor agonism: Whole-brain reactivity and functional connectivity induced by heroin and salvinorin A

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A better understanding of the impact of opioids on the brain is essential for the development of improved analgesics with reduced abuse liability. Activation of both the mu or kappa opioid receptors (MOR and KOR, respectively) induces analgesic effects, but MOR agonists are generally rewarding and widely abused, while KOR agonists are dysphoric, which may protect against misuse. To visualize similarities and differences between MOR and KOR agonism, we use single-cell whole-brain imaging of Fos reactivity. Mice (N=8/group, 4M+4F) were treated with the MOR agonist heroin (20 mg/kg), the KOR agonist salvinorin A (2 mg/kg), or vehicle, administered 30 minutes before an open field test to assess locomotion and general condition and a tail immersion test to assess analgesia. The brains were perfusion-extracted, 90 min post-injection, immunolabeled, and cleared using the iDisco+ protocol, and imaged using light-sheet microscopy. Images were processed using the ClearMap pipeline for cell detection and counting. As expected and previously reported, agonism at both receptors induced analgesia, but resulted in opposite effects on mobility, increased by heroin and reduced by salvinorin A. This behavior aligned with overall increased Fos activation of the brain by heroin, driven by heightened activity in the isocortex, hippocampus, cortical subplate, midbrain, and hindbrain, compared to salvinorin A. Only a subset of thalamic regions, involved in pain and arousal like the habenula, showed increased activity under salvinorin A, as observed under heroin. The centromedian-parafascicular complex of the thalamus, on the other hand, showed decreased activation under both conditions. Heroin produced a disruption in forebrain organization and a functional disconnection of the midbrain and hindbrain from the isocortex, hippocampus, and amygdala, relative to saline. Salvinorin A led to a mild disconnection across the thalamus, hypothalamus, basal ganglia, and midbrain and a pronounced decoupling of the isocortex from the rest of the brain, which may contribute to the dissociative properties of KOR agonism. The differences and similarities in regional reactivity and the derived connectomic differences associated with these treatments will help improve our understanding of their brain-wide impact and develop predictive network models, which in turn can inspire the development of non-addictive and non-dysphoric analgesics.

Poster #E22: (E. Schmidt et al.)

Human-specific SRGAP2C as a modifier of cortical circuit function and behavior

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The human brain is characterized by distinct traits of neuronal circuit organization, which are thought to underlie our cognitive abilities. However, we currently have little knowledge about the underlying mechanisms that shape these human traits of neuronal connectivity, nor how these traits impact brain functions such as learning and cognition. Our previous work has shown that the human-specific genetic modifier (HSGM) SRGAP2C induces human-specific traits in neuronal circuit organization and improves learning performance in mice. However, how HSGMs like SRGAP2C shape the functional properties of cortical circuits underlying learning and behavior remains unknown.

SRGAP2C modifies synaptic development, increases the number of cortico-cortical inputs that cortical pyramidal neurons (PNs) receive, and alters neuronal response properties. Together, these changes may enable SRGAP2C to alter how cortical circuits process information. To study this, we perform in vivo 2-photon imaging while mice perform in a whisker-based texture discrimination task. Our results show that in this learning task, a larger fraction of stimulus-coding neurons emerges in mice that express SRGAP2C in cortical PNs. Strikingly, however, the discriminability of this larger fraction of stimulus-coding neurons is reduced. Moreover, cortical PN responses in SRGAP2C mice display higher variability and a subset of cortical PNs exhibit improved encoding of multiple task variables.

Together, our findings point towards a shift in coding strategy from one where a few highly selective neurons encode task-relevant information, to a more distributed code where a larger population of neurons contributes to the overall encoding of behaviorally relevant information. Interestingly, our preliminary findings suggest that this altered coding scheme allows for a more robust and complex population code in SRGAP2C mice.

Additional ongoing experiments include in vivo widefield calcium imaging of the whole dorsal cortex to investigate how changes in cortical PN response properties alter mesoscale dynamics of cortical circuits. Together with computational modeling approaches, this work suggests that expression of SRGAP2C leads to more effective distribution of relevant task information across the cortex.
By using SRGAP2C to investigate how modifying specific circuit motifs impacts neural dynamics and behavior, we provide insights into the unique role human-specific genes play in shaping the structure and function of cortical circuits. Considering that SRGAP2C acts within known neurodevelopmental disease pathways, our work may provide insight into the role that human-specific genes play in shaping the phenotypic expression of neurodevelopmental diseases.

Poster #E23: (A. Grig et al.)

Artificial Consciousness and the Evolving Brain: Can Theories of Consciousness Guide the Design of Brain-Inspired Systems?

Aliya Grig

Evolwe Inc., Delaware

The emergence of conscious experience from the complex dynamics of neural circuits remains one of the most profound questions in neuroscience. As we deepen our understanding of the evolving brain, a parallel frontier is forming in the realm of artificial systems—those capable not only of simulating intelligence, but potentially of exhibiting traits of consciousness. In this presentation, I explore the theoretical and computational implications of bridging biological consciousness theories with brain-inspired system architectures. Drawing on established frameworks such as the Global Neuronal Workspace Theory, Integrated Information Theory, and predictive processing models, I propose a layered approach to artificial consciousness rooted in dynamic brain states, self-referential feedback, and multisensory integration. By comparing developmental and evolutionary patterns in cortical connectivity with design patterns in artificial neural networks, we identify potential markers for emergent awareness in synthetic systems. These markers—such as metastable dynamics, recursive internal models, and hierarchical abstraction—offer a testable blueprint for designing embodied systems that mimic core features of conscious cognition. While artificial consciousness remains speculative, its pursuit may offer valuable reverse-engineering insights into the structure-function relationships underlying human awareness. My work proposes a transdisciplinary roadmap for integrating neuroscience, cognitive theory, and AI in the development of ethically grounded, brain-informed conscious machines.

Poster #E24: (E. Ramirez et al.)

Hugs or Heroin? Investigating Ventral Pallidal Cell-types in Social vs. Drug Choice

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The U.S. opioid epidemic continues to worsen, exacerbated by the COVID-19 pandemic. It is now especially crucial to develop models of addiction that account for external factors, such as social influences, that impact substance use disorders, especially in contexts of social isolation or disrupted relationships. The ventral pallidum (VP) is a promising target for this research due to its role in motivated responses to both food and drug rewards. However, its participation in social reward or the decision to seek drugs over safer, natural rewards remains unclear.

Here we first investigated the role of GABAergic VP neurons in social reward alone, and next in a social-or-heroin choice operant task, using adult male and female GADiCre transgenic rats. We targeted inhibitory and excitatory DREADDs (hM4Di and hM3Dq) to VP GABA neurons, allowing bidirectional control of them during behavior. Wildtype and DREADD-expressing animals were socially isolated from their same-sex, sibling cage-mate for one week before being undergoing assessments of 1) social conditioned place preference, 2) social play and investigation, and 3) social communication via ultrasonic vocalizations. Another group of isolated rats with DREADDs were used to examine the effects of VP GABA manipulations on social-or-heroin choice. These rats were trained to lever press for 60s social interactions on a fixed ratio 1 (FR1) schedule until reaching a stabilization criterion, then tested in a counterbalanced, within-subjects design. Rats were then implanted with catheters and trained on another lever for intravenous FR1 heroin self-administration, and similarly tested. Finally, rats were trained on the social-or-heroin choice task to determine if VP GABA manipulations can shift preferred reward choice in a final within-subjects design test. Additional experiments using cFos immunohistology and fiber photometry were conducted to assess VP GABA and nonGABA neuronal activity during social behaviors.

Our findings demonstrate that chemogenetic manipulations of ventral pallidal GABAergic neurons modulate social reward seeking, social behaviors, and communication in rats. Preliminary data from the heroin-social-choice experiment will be presented at this conference. These results highlight the underappreciated role of the VP in social behaviors, which may also contribute to the social components of reward-related psychiatric conditions such as substance use disorders.

Poster #E25: (Withdrawn)

Poster #E26: (J. Sima et al.)

Restoration of locus coeruleus noradrenergic transmission during sleep

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Sleep is indispensable for health and wellbeing, but its basic function remains elusive. The locus coeruleus (LC) powerfully promotes arousal by releasing norepinephrine (NE). Here we show that LC NE transmission is markedly reduced by prolonged wakefulness and restored during sleep. Fiber-photometry imaging of NE using its biosensor showed that NE release, evoked by optogenetic activation of LC neurons, was strongly attenuated by three hours of sleep deprivation but restored during subsequent sleep. This was accompanied by weakening and recovery, respectively, of the wake-promoting effect of LC neuron activation. The reduction in both evoked NE release and wake-inducing potency can also be induced by prolonged optogenetic activation of LC neurons, indicating a key role of LC neuron activity. Furthermore, genetic inactivation of mammalian target of rapamycin (mTOR) signaling in NE neurons reduced the protein level of tyrosine hydroxylase (TH) – the rate-limiting enzyme for NE synthesis – and slowed the recovery of NE transmission. Notably, the wake-associated decline and sleep-dependent recovery of NE transmission also occur on a timescale of minutes in spontaneous sleep-wake cycles, and the time required for recovery increases with the duration of prior wakefulness. Together, these results reveal an essential function of sleep in restoring transmission of a key arousal-promoting neuromodulator.

Poster #E27: (T. Murano et al.)

Repetitive Neuronal Activation induces long-term changes of the three-dimensional genome structure and information coding in the hippocampus via Nuclear Reprogramming

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Neural stimulation therapies, including electroconvulsive therapy (ECT) and repetitive transcranial magnetic stimulation (rTMS), are among the most effective interventions for treatment-resistant psychiatric disorders. However, their underlying molecular and cellular mechanisms remain poorly understood.

To investigate the long-term effects of repeated neuronal activation, we developed a stimulation paradigm—repeated optogenetic stimulation (REPOPS)—that recapitulates key clinical features of ECT, including the duration and repetition of neuronal activation. When applied to the dentate gyrus (DG) for ten consecutive days, but not three, REPOPS induced robust and sustained antidepressant-like behaviors, mirroring the therapeutic effects of ECT. REPOPS also induced neuronal dematuration, as evidenced by immature-like gene expression profiles. Notably, RNA-seq analysis of postmortem human DG revealed similar immature-like transcriptional signatures in patients with a history of ECT, suggesting that activity-dependent dematuration may underlie its therapeutic effects.

In vivo calcium imaging revealed altered neural coding: spatial information encoding was impaired, whereas speed information encoding was enhanced, despite unchanged firing rates, indicating a reorganization of information coding in the DG. At the cellular level, REPOPS triggered a persistent immature-like transcriptional state and reactivation of cell cycle–related genes, even though DG neurons are post-mitotic. These changes were accompanied by global remodeling of 3D genome architecture and nuclear structural alterations resembling the G2/M phase of the cell cycle, including nuclear lamina disruption, histone phosphorylation, and heterochromatin enlargement. Deletion of Cyclin B1, a key regulator of the G2/M transition, reversed REPOPS-induced structural changes and partially rescued behavioral phenotypes. Notably, the AP-1 transcription factor ΔFosB was persistently elevated after 10 days of REPOPS and negatively regulated neuronal maturity. Furthermore, ΔFosB expression became transient by Cyclin B1 deletion, suggesting that cell cycle reentry sustains long-term ΔFosB expression and contributes to neuronal immaturity.

Our findings reveal a novel form of activity-dependent neuronal plasticity via nuclear reprogramming, offering a mechanistic framework for the lasting effects of brain stimulation.

Poster #E28: (D. Soneja et al.)

From Aggression to Care: Functional Shifts in VMHvl Neurons Across Female Life Stages

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The pregnancy is period of physiological and hormonal changes to adapt to the needs of a growing fetus. The brain also undergoes reorganization during pregnancy, resulting in behavioral phenotypes such as infant directed care and increased aggression towards threatening conspecifics. The hypothalamus has long been studied for its role in modulating social behaviors. Particularly, the medial preoptic area (MPOA) and ventrolateral part of the ventromedial hypothalamus (VMHvl) have been studied for their roles in parental care and maternal aggression respectively. Previous literature has identified that Npy2r+ neurons in VMHvl play a causal role in maternal aggression. Since the VMHvl has been strongly associated with aggression, its role in parenting behaviors other than maternal aggression has not been thoroughly explored. In this study, we investigate the functional role of Npy2r+ neurons within the VMHvl across life stages from virgin to dam to post-lactational animals towards specifically generating infant directed behaviors. In virgin females, stimulation of this population results in aggression towards pups. In contrast, stimulation of this population in lactating dams and post-lactating females results in aggression selectively towards intruders and often results in increased attending to pups with behaviors such as pup grooming and investigation. We further investigated the functional role of the projection of Npy2r+ neurons from the VMHvl to MPOA and identified the role of this projection in enhancing pup grooming behavior in lactating dams and post-lactating females. These findings uncover a previously unexplored role of VMHvl Npy2r+ neurons in supporting flexible caregiving responses across reproductive states, highlighting the dynamic reorganization of hypothalamic circuits after pregnancy.

Poster #E29: (J. Oudah et al.)

Natural evolution of same-sex sexual behavior in Drosophila

Youcef Ouadah and David J. Anderson

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Innate social behaviors like courtship and aggression are widespread in the animal kingdom, but their expression can differ dramatically between even closely related species. Social behaviors can thus be inferred to evolve quickly under varying selection pressures or neutral drift, but the underlying changes in pheromonal systems, neural circuits, and genomes are often difficult to pinpoint. We have identified a naturally occurring switch in social behavior among males within the genus Drosophila, from predominant aggression to courtship, in the West African island endemic D. santomea. D. santomea males successfully discriminate conspecific sex and can fight intensely when aggression-promoting neurons are artificially activated, but court males frequently and seldom attack them in naturalistic settings. The gain in male-directed courtship is due to decreased production of an anti-aphrodisiac olfactory signal, which appears to have been suppressed recently in response to sexual selection pressure by conspecific females seeking to avoid hybridization with a sympatric sister species. Reduced aggression has a separate cause: presence of a neural circuit within the central brain that actively inhibits attack. Thus, distinct changes in both the central nervous system and periphery of D. santomea males coordinate to supplant conspecific aggression with same-sex sexual behavior.

Poster #E30: (J. Wang et al.)

Distinct Striatal Engrams for Relapse and Extinction Encode Competing Alcohol Memories

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Persistent drug-associated memories are a hallmark of substance use disorders and a major barrier to long-term recovery. However, the cellular mechanisms by which the brain encodes, retrieves, and suppresses these maladaptive memories remain unclear. Here, we identify and functionally dissect two competing engrams within the dorsomedial striatum (DMS) that bidirectionally regulate relapse and extinction in a mouse model of alcohol use disorder (AUD). Using ArcTRAP and FosTRAP activity-dependent tagging systems in combination with operant alcohol self-administration (OSA), we selectively labeled direct-pathway medium spiny neurons (dMSNs) activated during alcohol learning or extinction. Chemogenetic manipulation (hM3Dq/hM4Di DREADDs) revealed that alcohol-recruited dMSNs encoded a memory trace that was both necessary and sufficient for cue-induced relapse. These dMSNs exhibited persistent synaptic potentiation from alcohol-activated medial prefrontal cortex (mPFC) inputs, evidenced by enhanced AMPAR-mediated currents and increased AMPAR/NMDAR ratios. To test whether this plasticity alone could encode relapse-like behavior, we developed a dual-channel optogenetic intracranial self-stimulation (oICSS) paradigm in which mice self-induced mPFC-to-dMSN long-term potentiation (oLTPsi). oLTPsi led to the formation of a persistent, cue-associated operant memory that reinstated seeking behavior, closely mimicking alcohol relapse. Extinction training not only suppressed reactivation of these alcohol engram dMSNs but also recruited a distinct population of striosome-enriched dMSNs. These extinction-tagged dMSNs produced stronger inhibitory outputs to substantia nigra pars compacta dopamine neurons, were not reinforcing, and encoded extinction memory selectively in the alcohol context. Chemogenetic activation of extinction-recruited dMSNs enhanced extinction memory retrieval and suppressed relapse even beyond levels achieved by training alone. Together, our findings reveal a dual-engram architecture in which matrix dMSNs promote relapse and striosome dMSNs support extinction, representing anatomically and functionally distinct memory traces. These results provide a novel circuit-level framework for how the striatum integrates competing alcohol-related memories and suggest targeted strategies to enhance relapse prevention by modulating engram-specific striatal activity.

Poster #E31: (S. Xiao et al.)

Concerted Actions of Distinct Serotonin Neurons Modulate Females' Pup Care Behavior

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Serotonin has long been implicated in promoting social behavior such as maternal care. However, in many species, pup-directed behaviors change across reproductive states, with females ignoring pups when they are naïve virgins but exhibiting extensive pup care after becoming moms. Here we discovered that two distinct serotoninergic projections have opposite roles in pup care—those projecting to the medial preoptic area (mPOA) promote pup care in moms, whereas those projecting to the bed nucleus of the stria terminalis (BNST) suppress pup interaction in virgin female mice. Disrupting serotonin synthesis in each of these projections or stimulating each of these projections alone could make a virgin female behave like a mom, and a mom a virgin. In naïve virgins, serotonin release in BNST and mPOA change in opposite directions upon the first pup interaction. Moreover, pup interaction triggers much higher serotonin release at mPOA in moms than in virgin females. Interfering with serotonin signaling locally in either mPOA or BNST disrupts the state-dependent switch in pup care. Together, these results highlight functionally distinct serotonin subpopulations that work in concert to orchestrate social behaviors appropriate for reproductive stages, and reveal how a single neuromodulator can tune existing neural substrates to adapt behavioral changes throughout an animal's lifetime.

Poster #E32: (F. Reis et al.)

Perceived threat imminence is encoded by cortical ensembles to guide adaptive defensive behavior

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Neurons in the medial prefrontal cortex (mPFC) exhibit mixed selectivity, responding to multiple features simultaneously and supporting nuanced, high-dimensional representations. Likewise, adaptive responses to threat rely on the integration of behavior, kinematics, and experience-dependent perception. Therefore, a coherent mPFC representation of threat would reflect these external variables alongside a scalable internal code for perceived threat imminence. To examine this ensemble-level coding framework in the mPFC, we developed the FUGA (Flight Upon Grid Approach) paradigm, in which a shock grid advances toward the mouse, evoking spatially and dynamically modulated defensive responses. In this context, mice exhibit both conditioned escape and freezing, enabling the assessment of internal threat imminence coding through the dissociation of threat approach, reactive immobility, goal-directed flight, and threat proximity. Using miniscope calcium imaging, we recorded CaMKII-expressing neurons in the mPFC (prelimbic cortex) in mice performing the FUGA task to examine how mPFC activity dynamically integrates multimodal features during threat processing. Results: While mPFC ensemble activity represents a wide range of kinematic features and defensive behaviors, we also found a cohesive population code for threat imminence in the mPFC that differs from action selection and mere spatial proximity. Furthermore, analysis of the mPFC population vector during shock (US) revealed a positive correlation between these US experiences and subsequent perceived threat imminence. After conditioning, the grid representation acquired relatively more US-like properties. Additionally, mPFC ensembles represent an anticipatory threat state that precedes the FUGA behavior, suggesting specific neural activity encodes escape preparation rather than execution. Causal testing via optogenetic inhibition during FUGA acquisition revealed that mPFC activity is necessary for the formation of avoidance and learned escape responses, but not freezing, underscoring its role in forming memory traces associated with high-threat, proximity-driven escape behavior. Together, these findings support a model in which mPFC dynamics encode a scalable internal representation of threat imminence enabling cognitive flexibility in defensive behavior selection.

Poster #F1: (J. Arzavala et al.)

Maternal Immune Activation Induces Epigenomic and Functional Alterations in Developing Excitatory Cortical Neurons

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Viral infection during pregnancy has been linked to altered neurodevelopment in offspring, increasing the risk of disorders such as schizophrenia and autism spectrum disorder. Pro-inflammatory cytokines released in response to maternal immune activation (MIA) are thought to mediate these effects on fetal brain development. Animal models of maternal immune activation (MIA) recapitulate behaviors resembling neurodevelopmental disorder phenotypes, but genomic differences in adult offspring are often subtle. This suggests that the long-term effects of MIA may originate from early disruptions in neuronal and circuit formation. We investigated transcriptional, epigenomic, and electrophysiological alterations in perinatal excitatory cortical neurons following MIA. By focusing on the perinatal period—from late embryonic stages to the first two postnatal weeks—we aimed to understand how MIA impacts the transition from embryonic to mature programming in a major neuronal class of the frontal cortex. Using an INTACT mouse line to isolate cortical excitatory neurons, Poly(I:C) was administered in pregnant dams to simulate a severe inflammatory reaction during mid-gestation. We found early disruption of progenitor cell proliferation genes at embryonic day (E) 15, followed by stalled synaptic development at birth marked by dysregulated genes associated with scaffolding proteins and glutamatergic transmission. Genomic regions that typically gain methylation by birth remained unmethylated in Poly(I:C) offspring, while regions that usually lose methylation were hypermethylated. Additionally, pyramidal neurons in layer 5, but not in layer 2/3, exhibited electrophysiological properties indicative of immature intrinsic function. Together, these findings suggest that MIA leads to aberrant epigenomic timing and disrupted physiological maturation in developing excitatory neurons. This work offers new insights into how MIA may impair early circuit formation and cell-type-specific development across cortical layers.

Poster #F2: (N. Butkovich et al.)

Strategies to facilitate nanoparticle (NP) crossing of the BBB for therapeutics delivery

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Crossing the blood-brain barrier (BBB) is a major hurdle for the effective delivery of therapeutics to treat disorders such as Alzheimer's disease [1]. In our work, we aimed to combine two distinct strategies, nanoparticle (NP) design and BBB electroporation, for the transient transport of nanocarriers past the BBB. As inspiration for NP design, directed evolution has recently been used to identify adeno-associated virus serotype 9 (AAV9) NP variants that can efficiently home to the brain and are distinguished by short (<10 amino acid) peptide loops on their surface: AAV-PHP.eB [2], AAV-X1 [3], and AAV.CPP.16 [4]. Electroporation via nanosecond pulsed electric fields (PEFs) is another promising technique for increasing tissue permeability, although their practical application for non-invasive treatment of the BBB is understudied [5, 6]. We therefore examined the feasibility of using these AAV9-based peptides in combination with PEFs to target drug carriers to the brain. We chose E2 NPs as a model platform, which share similar icosahedral protein structure and ~30 nm diameter to AAVs but are non-infectious [7]. We chemically attached or genetically inserted AAV-mimetic peptides onto E2 NPs by protein engineering, predicting that the context of peptide presentation would be critical to enable efficient BBB crossing. We used AlphaFold-facilitated modeling and computationally calculated secondary structure fidelity for the protein engineered AAV-mimicking E2 NPs. We performed in vitro assays using a murine brain endothelial cell line and demonstrated increased rates of E2 NP uptake only for designs which integrated AAV peptides into the E2 NP backbone, demonstrating the importance of faithfully translating AAV-mimetic peptide structures onto nanocarriers. Furthermore, we showed that PEFs could be non-invasive, not causing cell death for determined parameters. Most importantly, we observed that the most efficient transient transport of NPs past an in vitro BBB model required the combination of both strategies: NPs designed to mimic brain-penetrating AAVs and PEFs treatment. Our results emphasize the benefit of combining multiple strategies to synergistically increase nanocarrier transport past the BBB for therapeutics delivery.

Poster #F3: (M. He et al.)

Two distinct cell types of the medial mammillary body forming segregated subcircuits

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The medial mammillary body (MM) is an integral component of the Papez circuit and the extended hippocampal system essential for cognitive and emotional functions. However, whether MM contains morpho-electrophysiologically distinguishable, genetically identifiable neuron types, and how they interact to process information underlying diverse MM functions, has remained largely unexplored. Here we employed a multidisciplinary approach in mice, combing genetic labeling, electrophysiological recording, morphological reconstruction, viral tracing, activity monitoring and manipulation, and behavioral testing to perform an integrative analysis of MM. We identified two major neuron types in MM, distinguished by the expression of calbindin (CB) and parvalbumin (PV). These neuron types occupy complementary MM territories and exhibit discernable anatomical and physiological characteristics. Further, they display segregated outputs and differential inputs, with scarce local connectivity, forming independent subcircuits for parallel information processing. Using optogenetic activation and calcium fiber photometry, we demonstrated that CB-expressing MM neurons, but not PV-expressing ones, drive place aversion and hyperlocomotion and exhibit elevated activity during locomotion. In summary, our findings reveal the neuronal composition of MM, delineate its local and long-range circuit organization, and uncover functionally divergent, cell-type- specific subcircuits, establishing a robust framework for future investigations in both healthy and diseased states.

Poster #F4: (J. Ratliff et al.)

Developmental Principles of Long-range Cortico-Cortical Connectivity in the Mouse Visual Cortex

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In the mammalian cerebral cortex, the processing of sensory information into a coherent sensory percept is mediated by inter-areal connectivity between hierarchically organized cortical areas. While this connectivity is highly stereotyped across both individuals and species, little is known about how the precise projection targets of the cortico-cortical projection neurons (CCPNs) that mediate this long-range connectivity are determined during development. Here, we characterize the postnatal axon development and projection target specification of CCPNs that mediate inter-areal connectivity between mouse primary visual cortex (V1) and ten surrounding higher visual areas (HVAs). Utilizing rapid anatomical tracing strategies during successive developmental time windows, we have shown that the innervation of HVAs by V1 CCPNs occurs in an exuberant, asynchronous manner. In order of events, this process is characterized by early proliferative axonal outgrowth into medial HVAs, later proliferative axonal outgrowth into lateral HVAs, pruning of lateral HVA innervation, and later pruning of medial HVA innervation. Additionally, we have shown that while exuberant, axonal outgrowth from V1 is still target directed, with a set of medial and lateral HVAs that receive input from largely non-overlapping V1 populations in maturity not showing any more overlap in their V1 afferents during development. Together, these findings demonstrate that cortico-cortical connections are achieved primarily through a directed projection mechanism, and that the initiation and refinements of this process occur on heterogeneous timescales that vary according to target location. These results provide the groundwork for a better understanding of the mechanisms by which cell-type specific, long-range cortical circuits are formed during development, as well as where this process may go awry in psychiatric disorders associated with aberrant cortico-cortical connectivity.

Poster #F5: (S. Sudarsanam et al.)

Mef2c Controls Postnatal Callosal Axon Targeting by Regulating Sensitivity to Ephrin Repulsion

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In the mammalian brain, sensory integration and transformation primarily occur in the cerebral cortex, mediated in part by long-range connections between cortical areas. Axonal projections through the corpus callosum control information flow between the two cortical hemispheres, and they are organized topographically such that callosal projection neurons (CPNs) innervate contralateral target domains that match their ipsilateral region of origin. Deficits in callosal connectivity are associated with neurodevelopmental and neuropsychiatric disorders, but the genetic determinants and molecular mechanisms of topographically organized target innervation by CPNs remain enigmatic.

We reasoned that determinants of CPN axon targeting are likely highly expressed in Layer 2/3 (L2/3) of the murine cerebral cortex, a major source of callosal projections, during early postnatal development. Therefore, we performed an in utero electroporation-mediated loss-of-function screen of L2/3-enriched candidate genes, identifying the transcription factor Myocyte enhancer factor 2-c (Mef2c) as a novel regulator of callosal projection targeting.

While Mef2c directs the specification of laminar identity across several populations of cortical neurons during embryogenesis, its expression shifts to L2/3 during early postnatal development. We show that the deletion of Mef2c specifically in post-mitotic L2/3 CPNs of the somatosensory (S1) cortex strongly reduces axonal projections to the contralateral homotopic (S1) domain without affecting midline crossing. Through functional manipulation of EphrinA-EphA signaling in Mef2c-mutant CPNs, we demonstrate further that Mef2c represses EphA6 to desensitize S1-L2/3 CPN axons to EphrinA5-mediated repulsion at their contralateral targets.

Our work uncovers dual roles for Mef2c in cortical development: regulation of laminar subtype specification during embryogenesis, and axon targeting in postnatal callosal neurons. Further, we shed new light on the molecular logic of interhemispheric projection targeting by ascribing a role for EfnA5-EphA signaling downstream of Mef2c in this process.

Poster #F6: (N. Hosseini et al.)

Generation of Human Cerebellar Organoids to Investigate Human-Specific Features of Cerebellar Development

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The cerebellum has long been associated with motor control, but growing evidence highlights its involvement in humans' higher-order cognitive functions and emotional regulation. Understanding how the human cerebellum develops its unique cellular diversity is crucial for uncovering its full scope of functions and role in physiological and pathological conditions. Comparative studies reveal significant differences between human and murine cerebellar development. In mice, cerebellar neurogenesis originates from two progenitor zones: an inhibitory ventricular zone (VZ) and an excitatory Rhombic Lip, both lacking a subventricular zone (SVZ). In contrast, human cerebellar development is marked by a distinct expansion and compartmentalization of these progenitor zones, each forming its own SVZ. Unlike in mice, where radial glial progenitors divide only at the ventricular surface, humans possess mitotic progenitors in both the VZ and SVZ. These basal progenitors, characterized by long radial fibers and mitotic radial glial markers, expand within the SVZ and contribute significantly to human-specific cerebellar neurogenesis.

Such distinctions are key to understanding species-specific brain development, but limited access to the human brain necessitates alternative models. Human pluripotent stem cell-derived cerebellar organoids (hCerOs) have emerged as a promising in vitro system to interrogate the early stages of development and disease of this human brain region. We have recently established a robust and reproducible protocol for generating cerebellar organoids from human induced pluripotent stem cells (hiPSCs) to model key aspects of human cerebellar development. These organoids give rise to all major cerebellar cell types, including both excitatory and inhibitory neurons, and multiple glial subtypes. Using this protocol, I generated human cerebellar organoids containing basal radial glial cells with morphological and spatial features closely resembling those found in human fetal cerebellar tissue. In parallel, I applied our optimized methodology to mouse embryonic stem cells (mESCs) to generate mouse cerebellar organoids, in which I also identified basal radial glial cells localized within the ventricular zone. This comparative system provides a unique platform to investigate species-specific aspects of cerebellar development, particularly the role and distribution of basal radial glia. Finally, I describe our ongoing efforts using lineage tracing approaches to map the developmental trajectories of cerebellar progenitors within organoids, focusing on their contributions to inhibitory and excitatory lineages during cerebellar development.

Poster #F7: (A. Wani et al.)

Developmental Programs Regulating the Fate Specification, Identity, and Function of Dorsal Fan-Shaped Body Neurons in the Drosophila Central Complex

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Complex behaviors such as sleep, and foraging require precise neural circuitry composed of diverse, well-specified neuron types. This diversity is orchestrated by spatial and temporal patterning programs that guide neural stem cells (NSCs) through stages of division and differentiation. In Drosophila, the adult central complex (CX)—a brain structure essential for navigation, sleep, memory, and metabolic regulation—is largely derived from type II NSCs and their intermediate progenitors, which express temporally dynamic gene programs. However, the developmental logic underlying the formation of specific CX circuits, especially those regulating sleep and metabolism, remains unclear.

Here, we uncover how extrinsic hormonal cues, and intrinsic transcriptional programs converge to generate distinct dorsal fan-shaped body (dFB) neuron types that regulate sleep and foraging. Using genetic lineage tracing, we show that 23E10-GAL4-labeled sleep-promoting dFB neurons arise from the DL1 and DM1 type II NSCs and are specified between 48–76 hours after larval hatching. We identify the ecdysone-induced transcription factor E93 as a critical regulator of their fate: loss of ecdysone signaling or NSC-specific E93 impairs neuronal specification and disrupts adult sleep homeostasis.

We further identify E93 as a post-mitotic regulator of 84C10-labeled dFB neurons, a distinct population that innervates deeper dFB layers (6–7) and controls foraging and sugar diet preference. These neurons originate from late DL1 and DM4 NSCs, and E93 knockdown after neurogenesis leads to defective innervation and altered metabolic behavior, including suppressed diet-induced triglyceride accumulation.

Together, this work establishes E93 as a temporal master regulator that acts at multiple levels—within NSCs and post-mitotically—to generate distinct, behaviorally specialized neuron types. Our findings bridge developmental neurobiology and behavior, suggesting that adult sleep and metabolic disorders may originate from disrupted temporal patterning during brain development.

Poster #F8: (A. Treptow et al.)

Cas adaptor proteins are required for cerebellar development

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The cerebellum is responsible for motor coordination, balance, and posture as well as non-motor functions such as working memory, language, emotion processing, and executive function. Proper establishment of cerebellar circuitry is critical to cerebellar function and relies on the appropriate positioning of cells within the cerebellum. A coordinated series of events between granule cells, Purkinje cells, and Bergmann glia is hypothesized to contribute to the formation of cerebellar folia. However, the intracellular molecular mechanisms underlying cerebellar foliation remain to be elucidated. Using a Cas Triple Conditional Knock Out (Cas TcKO) model generated in our lab, we provide genetic evidence that Cas adaptor proteins are broadly required for cerebellar foliation. Genetic dissection of Cas TcKO mutants reveals a non-neuronal autonomous requirement of Cas genes in the formation of the Bergmann glial scaffold. Additional analyses show that Cas proteins are required for proper foliation patterning in a granule cell autonomous manner. These findings build on previous research from our lab demonstrating that Cas proteins are critical mediators of adhesion signaling during glial scaffold formation.

Poster #F9: (Q. Zhu et al.)

3D Genome Architecture Orchestrates Human Neurodevelopment Through Chromatin Regulators CTCF and Cohesin

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The dynamic organization of the 3D genome plays a central role in regulating gene expression during human brain development. Key genome architectural proteins like CTCF and cohesin mediate chromatin loops and topologically associating domains (TADs) that is believed to facilitate enhancer-promoter communication. While their short-term depletion in cultured cells yields limited transcriptional effects, their long-term impact during neurodevelopment remains poorly understood due to cohesin's essential role in cell proliferation. To address this, we integrated in vivo chromatin imaging of the developing human brain with in vitro perturbation of chromatin regulators in human iPSC-derived excitatory neurons. Multimodal spatial imaging revealed diverse long-range chromatin interactions across neuronal subtypes in the developing cortex, suggesting cell–type–specific 3D genome landscapes. In parallel, targeted depletion of CTCF or RAD21 (a cohesin subunit) in differentiating neurons revealed distinct phenotypes: RAD21 loss impaired axon formation, while CTCF depletion broadly blocked neuronal maturation. Bulk Hi-C and single-nucleus multi-contact (snm3C-seq) analyses showed that disruption of either factor weakened TAD boundaries and reduced chromatin insulation, though residual structures were captured by high-resolution DNA imaging. Transcriptomic analyses further revealed that each perturbation altered the expression of thousands of genes, including those essential for neuronal identity and synaptic function. Together, our findings highlight the critical and non-redundant roles of CTCF and cohesin in shaping the 3D genome and guiding gene expression during neurodevelopment. This work provides new insight into how chromatin architecture contributes to the formation of neural circuits and offers a framework for understanding brain disorders linked to architectural protein dysfunction.

Poster #F10: (P. DePalma et al.)

Crk adaptor proteins regulate cortical lamination

Payton Rao, Alyssa Treptow, Martin Riccomagno

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Proper neuronal migration during cortical lamination is essential for functional circuit development. Disruptions in this process lead to a host of neurodevelopmental disorders hallmarked by an array of functional deficits. Cortical migration requires Reelin signaling. Reelin is secreted in the marginal zone by Cajal-Retzius cells and binds to its receptors in migrating neurons, leading to phosphorylation of the adaptor protein Disabled-1 (Dab1). Dab1 subsequently activates downstream signaling to regulate cell adhesion and cytoskeletal remodeling in migrating cortical neurons. However, the molecular mechanisms linking Dab1 activation to cytoskeletal remodeling and migration remain poorly understood. The chicken tumor virus #10 regulator of kinase, or Crk, family of adaptor proteins (Crk and CrkL) are well-positioned to bridge Reelin signaling to actin and adhesion dynamics via interactions with integrin adhesion complex (IAC) components and other cytoskeletal regulators. Here, we investigated the role of Crk and CrkL in cortical lamination using conditional gene knockout strategies in embryonic mouse cortex. We established a Crk and CrkL double conditional knockout (dcKO) model and assessed effects on neuronal positioning using layer-specific markers at postnatal day 7 (P7). Loss of both Crk and CrkL led to severe cortical layering defects resembling those observed in Reelin or Dab1 mutants, with cortical layers appearing inverted: in mutants, superficial layer markers are present closer to the ventricular zone while deep layer markers are present close to the pial surface, opposite of what is seen in control animals. These results demonstrate that Crk and CrkL are required for proper cortical lamination, and may act downstream of Reelin-Dab1 signaling, coordinating neuronal positioning during cortical development. Future work will focus on molecular epistasis analysis to determine whether Crk and CrkL indeed act downstream of Reelin and testing the functional role of Crk proteins in cell adhesion and migration.

Poster #F11: (S. Pallas et al.)

The need for visual experience in visual cortical development differs across mammalian species according to ecological niche

Pedro F. Fernández, Korey Sudana, Stephen D. Van Hooser & **Sarah L. Pallas**

UMass-Amherst

For many years, the influence of visual experience on the refinement of receptive fields (RFs) was studied primarily in animals with high acuity vision such as carnivores and primates. Cats and monkeys reared in the dark exhibit a detriment in the maturation of visual cortex that impacts some properties of RFs, including their size. In those species, light exposure during development is necessary for receptive field (RF) refinement. More recent studies have focused on rodent species. But non-diurnal rodents rely much less on their visual perception for survival than monkeys and cats do, raising the question of whether they require visual experience during development. We hypothesized that diel activity pattern determines the need for visual experience in RF development more than does phylogeny, thus matching the need for visual experience with the likelihood of light exposure. We have now studied three rodent and one carnivore species in this respect and have found considerable variation in the role of visual experience in constructing and refining receptive fields in visual cortex. In crepuscular Syrian hamsters (Mesocricetus aureus), visual experience plays a role in maintenance, but not refinement, of visual receptive fields in superior colliculus and visual cortex, opposite of cats and monkeys (Carrasco et al., 2005; Balmer & Pallas, 2016). We now report that RF refinement is both achieved and maintained without any light exposure at all in nocturnal mice (Mus musculus). In ferrets (Mustela furo), a crepuscular carnivore species, we found that, as in hamsters, rearing in either normal or dark conditions results in a reduction in RF size from young to adolescent ages, but continued dark rearing causes a re-enlargement of RFs in adulthood. Thus, crepuscular species such as ferrets and hamsters share similar independence from light during development, distinct, in opposite directions, from cats and mice. Our data support the hypothesis that diel time partitioning rather than phylogeny defines the need for visual experience in RF development. This study is a significant step in understanding the role of visual experience in the development of visual processing across commonly studied species.

Poster #F12: (S. Jain et al.)

Activity-dependent and -independent development of L2/3 identities in the mouse visual cortex

Juyoun Yoo[1,6], Fangming Xie[1,6], Salwan Butrus[2,6], Vincent Xu[1], Zhiqun Tan[3], Ryan Gorzek[4], Sanjana Suresh[5], Jinho Kim[5], Joshua Trachtenberg[4], Xiangmin Xu[3], Larry Zipursky[1,7], Karthik Shekhar[2,7], **Saumya Jain[1,5,7]**

[1]Biological Chemistry, University of California, Los Angeles, [2] Department of Chemical and Biomolecular Engineering, University of California, Berkeley, [3]Anatomy & Neurobiology, School of Medicine, University of California, Irvine, [4]Department of Neurobiology, University of California, Los Angeles, [5]School of Biological Sciences, Georgia Institute of Technology, Atlanta, [6]Equal contributors, [7]Co-senior authors

The development of neuronal circuits depends upon the proper establishment of cell-identities and precise control over spatial and temporal gene expression. Mechanisms via which nature and nurture control cell-identities and spatiotemporal gene expression programs for proper circuit formation are poorly understood. We recently described that layer 2/3 (L2/3) excitatory neurons in the mouse visual cortex comprise of cell-identities that vary continuously between three extremes (called archetypes) – L2/3 A, L2/3 B and L2/3 C. We also previously showed that L2/3 identities correlate with cortical depth and are dependent upon vision for maintenance during the critical period. To gain insight into how this continuum of cell-identities is generated during development, we performed single-nucleus multiome profiling (snRNA-Seq + snATAC-Seq) and spatial transcriptomic analysis (MERFISH) of developing visual cortex neurons under normal and dark-reared conditions. We found that the continuum is generated in two steps: 1) L2/3 neurons are already born with identities that vary between L2/3 A and L2/3 C; and 2) L2/3 B identity first appears ~P10, and then continues to mature over development, largely via reprogramming of L2/3 A-like cells. Gene regulatory network analysis identified transcription factors (e.g. Rfx3 and Meis2) that regulate genes enriched in L2/3-A, while other transcription factors (e.g. Tcf12 and Satb1) regulate L2/3-C enriched genes. Key roles for these factors in the establishment of L2/3 identities is underscored by their strong association with neurodevelopmental disorders such as autism. B-genes were found to be largely regulated by neuronal activity-induced transcription factors such as Fos, Jun, Npas4 and Egr1. Increased abundance of B-like cells over development is a result of an increase in activity-induced transcription. Dark rearing reduces, but does not eliminate the expression of activity regulated genes. This results in a developmental delay leading to the assembly of an abnormal continuum with an increase in A-like cells at the expense of B-like cells and changes in the patterns of gene expression in B-like and C-like cells. Genes expressed in a graded fashion across the continuum are enriched for genes implicated in circuit formation and correlate with the targeting preferences of L2/3 axons for different higher visual areas (HVAs). We demonstrate that targeting to some HVAs is dependent upon vision and targeting to others is not. Together, our analyses identify genetically-hardwired and sensory input-dependent mechanisms for the development of the continuum of L2/3 identities and the establishment of cortical circuits.

Poster #F13: (V. Martina et al.)

Molecular Regulation of Neuronal Maturation in the Postnatal Mouse Brain

Verdion Martina, Dalton Patterson, Terry Lewis, and HaoShen Sun

University of Alabama at Birmingham

The number of neurons mammals are born with does not significantly change as they mature from juvenile to adult stages. However, these post-mitotic neurons undergo connectivity and functional changes during this developmental period. One brain region that exhibits many changes during postnatal maturation is the prefrontal cortex (PFC), a critical brain region in regulating animal behavior due to its long-range connectivity to other brain regions, for example, the basolateral amygdala (BLA). Altered connectivity between the PFC and BLA is a key contributor to multiple neural disorders such as autism spectrum disorders, anxiety, depression, and post-traumatic stress disorders. The PFC-BLA circuitry is also recognized for its involvement in associative learning and memory. While this pathway and its associated behaviors are well explored in adulthood, limited research has examined how they mature. Therefore, my research aims to identify and characterize molecular cues that guide the postnatal development of the PFC-BLA circuitry.

To do this, I performed cued fear conditioning, a PFC-BLA dependent behavioral paradigm, in juvenile (postnatal day 18(p18)), adolescent (p34-p46), and adult mice (>p60) to identify the age of onset of associative fear memory in mice. While all stages of mice can retrieve recent fear memory (24 hours after training), juvenile mice cannot recall remote fear memory (7 and 14 days after training). Since the PFC-BLA connectivity regulates cued fear conditioning behavior, I injected an anterograde AAV1-ChR2-eYFP virus with a glutamatergic neuron promoter into the PFC of mice at multiple developmental time points to measure changes in axonal innervation from the PFC to the BLA across postnatal development. My analysis revealed developmental changes/increases in PFC-BLA connectivity, mirroring the changes in long-term fear memory recall capability across postnatal development.

To identify molecular targets that play a role in mouse PFC-BLA postnatal maturation, I performed snRNA-sequencing in the PFC of juvenile and adult mice and identified developmental up- and down-regulated genes in all cell types. Using gene ontology analysis, I found that developmentally downregulated genes were enriched for axon guidance molecules (ex. semaphorins and plexins) in excitatory neurons. For future studies, I will target these axon guidance molecules to determine their functional role in PFC maturation (connectivity and associated behaviors).

Completing this work will provide valuable insights into the role of axonal guidance molecules (e.g., semaphorins and plexins) in guiding the projections between the prefrontal cortex (PFC) and basolateral amygdala (BLA), as well as how disruptions in the maturation of this pathway may contribute to the dysregulation observed in neurodevelopmental disorders.

Poster #F14: (D. Chen et al.)

CTCF regulates global chromatin accessibility and transcription during rod photoreceptor development

Dahong Chen[1], Saumya Keremane[2], Silu Wang[3], Elissa Lei[2]

[1] SUNY Buffalo, Department of Biochemistry, Buffalo, NY, [2] NIH, NIDDK, Bethesda, MD, [3] SUNY Buffalo, Department of Biological Sciences, Buffalo, NY

Chromatin architecture facilitates accurate transcription at a number of loci, but it remains unclear how much chromatin architecture is involved in global transcriptional regulation. Previous work has shown that rapid depletion of the architectural protein CTCF in cell culture alters global chromatin organization but results in surprisingly limited gene expression changes. This discrepancy has also been observed when other architectural proteins are depleted, and one possible explanation is that full transcriptional changes are masked by cellular heterogeneity. We tested this idea by performing multi-omics analyses with sorted juvenile post-mitotic mouse rods, which undergo synchronized development, and we identified CTCF-dependent regulation of global chromatin accessibility and gene expression. CTCF depletion leads to dysregulation of ~20% of the entire transcriptome (>3,000 genes) and ~41% of genome accessibility (>27,000 sites) before any prominent cellular or physiological phenotypes arise. Importantly, these changes are highly enriched for CTCF occupancy at euchromatin, suggesting direct CTCF binding and transcriptional regulation at these active loci. CTCF mainly promotes chromatin accessibility and frequently inhibits expression of these direct binding targets, which are enriched for binding motifs of transcription repressors. These findings provide different and sometimes opposite conclusions from previous studies, emphasizing the need to consider cellular heterogeneity and cell-type specificity when performing multi-omics analyses. CTCF knockout rods undergo complete degeneration by adulthood, indicating an essential role for their viability. We conclude that the architectural protein CTCF binds chromatin and regulates global chromatin accessibility and transcription during rod development.

Poster #F15: (S. Simó et al.)

Let-7 Sustains Cortical Projection Neuron Migration by Targeting Rbx2

Steven Decker, Keiko Hino, Anna La Torre, and **Sergi Simó**

Department of Cell Biology and Human Anatomy, University of California, Davis

In the central nervous system, there is a tightly coordinated relationship between the fate and migration of projection neurons, ensuring that specific neuronal fates settle in precise spatial locations. This is particularly evident in the mammalian neocortex, where early-born projection neurons predominantly settle in the deeper layers of the cortical plate, whereas later-born neurons localize more superficially. However, it remains unclear whether neuronal fate acquisition directly determines projection neuron positioning, or whether fate and positioning are regulated independently. MicroRNAs have emerged as key regulators of cell fate determination in the neocortex. Among them, let-7 is known to influence neural progenitor competence and promote the neurogenesis of late-born projection neurons. Here, we show that let-7 also regulates projection neuron migration by targeting RBX2, a core component of the E3 ubiquitin ligase CRL5, which has been previously shown to inhibit neuron migration by terminating the Reelin/DAB1 signaling pathway. Let-7 directly binds a conserved motif in the 3′ UTR of RBX2, reducing its translation and thereby diminishing CRL5 activity. Importantly, restoring RBX2 levels in the context of let-7 expression rescues the positioning of pyramidal neurons without altering let-7-induced effects on neuronal fate. Furthermore, we demonstrate that let-7 enhances pyramidal neuron migration by increasing locomotion speed and prolonging migratory activity. Together, these findings reveal that let-7 coordinates neuronal fate specification and migration via distinct molecular pathways, thereby ensuring the proper laminar positioning of late-born pyramidal neurons in the neocortex.

Conference Travel Awardees / Conference Support Recipients

Award recipients were selected based on a combination of academic potential, demonstrated financial need, and research merit as reflected in the quality of submitted abstracts. These awards are made possible through support from an R13 Conference Grant from the National Institute of Mental Health (*1R13MH139291-01, UC Irvine Center for Neural Circuit Mapping 2025 Conference: The Changing Brain*), along with additional funding from the Center for Neural Circuit Mapping (CNCM).

Conference Travel Awardees	
Pauline Wonnenberg	Georgetown University
Jung-Chien Hsieh	University of Michigan
Arda Kipcak	University of Virginia
Lindsey Washiashi	University of California, Santa Barbara
Anurag Das	Iowa State University
Tsan-Ting Hsu	Academia Sinica
Julia Plank	Stanford University
Han Yang	University of California, San Francisco
Sai Krishna Bhamidipati	University of California, San Diego
Laura Perrault	University of North Carolina Chapel Hill
Bibudha Parasar	Stanford University
Raghav Madan	University of Washington
Huma Naz	Washington University in Saint Louis
Jessica Arzavala	University of California, San Diego
Conference Registration Fee Waiver Awardees	
Anjali Chawla	McGill University
Kentaro Ishii	University of Washington
Aswathy Ammothumkandy	University of Southern California
Samuel S. Park	University of California, San Diego
Xin Wang	University of California, Irvine
Wei Feng	Washington University in Saint Louis
Max Garduño	University of California, Irvine
Madeline Snyder	University of California, Irvine
Khanh Luong	University of California, Irvine
Nina Butkovich	University of California, Irvine
Zijing Wang	University of California, Irvine
Daniel Zhang	University of California, Irvine
Kelly Jin	Allen Institute for Brain Science
Yang Xiao	University of Michigan
Xiongtao Ruan	University of California, Berkeley
Shyam Srinivasan	University of California, San Diego
Fangming Xie	University of California, Los Angeles; (Visiting at University of California, San Fransicso)

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Heartstrings

UC Irvine School of Medicine
Department of Otolaryngology-Head & Neck Surgery

UC Irvine School of Medicine
Medical Humanities & Arts Program

More Info / RSVP

Contact: cncm@uci.edu

Background artwork:
Baby Blues 13. Mixed media on paper.
Sarah Hoenicke Flores, 2025.

Day 4 - Workshop Lectures and On-site Demos

Additional workshops on spatial transcriptomics and viral-genetic tools will be held at the UC Irvine campus on August 21, 2025. These workshops are free and open to all registered conference attendees. Individuals not registered for the main conference may attend for a nominal fee. If you're interested in attending, please submit an on-line interest form (see the next page) by August 15, 2025.

Location: Interdisciplinary Science and Engineering Building, UC Irvine (419 Physical Sciences Quad, Irvine, CA 92697)

Please use the google map link for Lot 12A for driving and ride-share directions to the nearby parking area

Workshop #1: Viral targeting reagents for mammalian brain neural circuit analysis

Thursday, August 21, 2025

8:30 a.m. - 10:30 a.m.

Interdisciplinary Science and Engineering Building (ISEB), Room 1200

New viral-genetic tools are critical for improving anatomical mapping and functional studies of cell-type-specific and circuit-specific neural networks in the intact brain. The goal of the CNCM viral core is to develop new and improved viral tools that can be used for a broad range of applications and to make them widely available in the neuroscience field. As part of the BRAIN Initiative Armamentarium project, the CNCM currently produces and distributes cell-type specific enhancer AAV reagents for the broad neuroscience community. The tools provide researchers with gene delivery systems for various species used in research, without the need for genetically modified, or transgenic, animals. Our CNCM and collaborative teams also have created new recombinant rabies viral vectors and yellow fever virus (YFV-17D) vectors for neural circuit mapping that offer a range of significant advantages over existing tools. In this workshop, we invite leading experts, including Drs. Ian Wickersham and Wei Xu, to share the latest development in tool creation and applications for neuroscience.

Agenda:

- **8:30 a.m. - 8:45 a.m. Introduction and CNCM viral resources:** Dr. Alexis Bouin, UCI Center for Neural Circuit Mapping Viral Core Director
- **8:45 a.m. - 9:00 a.m. Lecture:** Specific targeting of brain endothelial cells using enhancer-AAV vectors. Dr. Eric Velazquez-Rivera, UCI Center for Neural Circuit Mapping
- **9:05 a.m. - 9:30 a.m. Lecture**: Third-generation monosynaptic tracing using a nontoxic single-deletion-mutant virus. Dr. Ian Wickersham, Principal Research Scientist, McGovern Institute, MIT
- **9:35 a.m. - 10:00 a.m. Lecture:** The development and application of YFV-17D-derived anterograde trans-neuronal viral vectors for neuroscience research. Dr. Wei Xu, Associate Professor, UT Southwestern Medical Center
- **10:00 a.m. - 10:30 a.m. Group Discussion / Break**

Interest Form

Workshop #2: Harnessing the potential of spatial transcriptomics

Thursday, August 21, 2025

10:30 a.m. - 12:30 p.m.

Interdisciplinary Science and Engineering Building (ISEB), Room 1200

Single-cell genomics has the potential to unlock new insights into brain cell types, neural circuit function and therapeutic implications. The UCI Center for Neural Circuit Mapping (CNCM) currently houses Vizgen MERSCOPEs and other instruments and is actively developing multimodal MERFISH and high-resolution sequencing-based spatial transcriptomics. The CNCM team coordinates with UC San Diego investigators and PacGenomics experts to host this spatial transcriptomic workshop with a focus on MERFISH+ and Stereo-seq technologies.

Agenda:

- **10:30 a.m. - 10:35 a.m. Workshop Introduction:** Dr. Zhiqun Tan, Research Professor, UCI Center for Neural Circuit Mapping.
- **10:35 a.m. - 11:00 a.m. Lecture:** An introduction to MERFISH+: Enabling large-scale, multimodal spatial transcriptomic mapping. Dr. Bogdan Bintu, Assistant Professor, University of California San Diego.
- **11:05 a.m. - 11:25 a.m. Lecture:** Spatial transcriptomics development and applications in health and disease. Bereket Berackey, UCI Center for Neural Circuit Mapping
- **11:30 a.m. - 12:30 p.m. Stereo-seq technology presentation and demo:** PacGenomics experts (Dr. Yuan Gao, Chief Scientific Officer; Dr. Zheng Fu, Clinical Data Scientist; Dr. Hongjie Zhang, Chief Operating Officer and Raymon Ylagan, Research Scientist) and CNCM expert (Dr. Zhiqun Tan, Research Professor)
    - Principles of Stereo-seq based spatial transcriptomics
    - PacGenomics platform: throughput, resolution, and unique advantages
    - Sample requirements and experimental design
    - Live hands-on demonstration
    - CNCM/PacGenomics services and collaboration opportunities

Interest Form

2025 Conference Attendee List

First Name	Last Name	Affiliation
Geoff	Abbott	University of California, Irvine
Ishmail	Abdus-Saboor	Columbia University
Gagan	Acharya	University of California, Riverside
Gautam	Agarwal	Pitzer and Scripps Colleges
Varun	Ajith	University of California, Irvine
Andy	Alexander	University of California, Santa Barbara
Aswathy	Ammothumkandy	University of Southern California
Bobae	An	Massachusetts Institute of Technology
Paola	Arlotta	Harvard University
Jessica	Arzavala	The Salk Institute for Biological Studies
Brittany	Auclair	Vizgen, Inc.
Estefania	Azevedo	Medical University of South Carolina
Kia	Banaie Boroujeni	Princeton University
Ariana	Barcoma	The Salk Institute for Biological Studies
Brain	Barton	University of California, Irvine
Michel	Baudry	Western University of Health Sciences
Margarita	Behrens	The Salk Institute of Biological Studies
Arash	Bellafard	University of California, Los Angeles
Guillermina	Bendito	Instituto de Neurociencias. CSIC & UMH
Bereket	Berackey	University of California, Irvine
Jillian	Berry	University of California, Irvine
Sai Krishna	Bhamidipati	University of California, San Diego
Aritra	Bhattacherjee	Harvard University
Guoqiang	Bi	University of Science and Technology of China
Xiaoning	Bi	Western University of Health Sciences
Devin	Binder	University of California, Riverside
Bogdan	Bintu	University of California, San Diego
Jackson	Blackman	University of California, Irvine
Saga	Bolund	Karolinska Institutet
Emiliana	Borrelli	University of California, Irvine
Alexis	Bouin	University of California, Irvine
Lomax	Boyd	The Rockefeller University
Alyssa A.	Brewer	University of California, Irvine
Julia	Brzac	University of California, Santa Barbara
Elizabeth	Buffalo	University of Washington
Minh	Bui	University of Southern California
Nina	Butkovich	University of California, Irvine
Dawen	Cai	University of Michigan
Xiaochun	Cai	Stanford University

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Edward	Callaway	The Salk Institute for Biological Studies
Shuo	Cao	California Institute of Technology
Wenhao	Cao	University of California, Irvine
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Robert	Carson	Vizgen, Inc.
Kaira	Carstens	University of California, Riverside
David	Castaneda	Vizgen, Inc.
Martina	Cavallini	University of California, Los Angeles
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Jayaram	Chandrashekar	Allen Institute for Neural Dynamics
Edward	Chang	University of California, San Francisco
Anjali	Chawla	McGill University
Dahong	Chen	University at Buffalo SUNY
Fujun	Chen	Washington University in St. Louis
Lijie	Chen	University of California, Irvine
Ruoyu	Chen	Harvard University
Wanqiu	Chen	Loma Linda University
Yu-Chieh (David)	Chen	New York University
Zhong	Chen	Loma Linda University
Lin	Cheng	University of California, Irvine
Bernard	Choi	University of California, Irvine
Shen-Ju	Chou	Institute of Cellular and Organismic Biology,
Karen	Christopher	National Institute of Mental Health
Anne	Churchland	University of California, Los Angeles
Patricia	Cogram	University of Chile
Isaac	Cohen	University of Southern California
Karina	Cramer	University of California, Irvine
Christian	Crouzet	University of California, Irvine
Charles R.	Crumly	Taylor & Francis / CRC Press
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Mingyu	Du	University of California, Irvine
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Laramie	Duncan	Stanford University

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Guoping	Feng	Massachusetts Institute of Technology
Wei	Feng	Washington University in St. Louis
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Sasha	Feshchenko	Ross University Faculty of Medical Sciences
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Pan	Gao	University of California, Irvine
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Gocylen	Gast	University of California, Irvine
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Ellen	Gingrich	Stanford University
Elizabeth	Glater	Pomona College
Michael	Goard	University of California, Santa Barabra
Ann	Goldstein	Cell Press
Michael	Gongwer	University of California, Los Angeles
Caitlin	Goodpaster	University of California, Los Angeles
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Joel	Hahn	University of Southern California
Richard	Harris	University of California, Irvine
Chris	Hartl	Epigenome Technologies, Inc.
Alan	Hauser	Health Discovery Corporation
Mary	Hawkes	Pacific Symphony Orchestra
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Songwei	He	Max Planck Institute for Biological Intelligence
Zhigang	He	Boston Children's Hospital
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Hailan	Hu	Zhejiang University
Wenhui	Hu	Virginia Commonwealth University
Xueming	Hu	Icahn School of Medicine at Mount Sinai
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Yan	Huo	University of California, Irvine
Duc	Huynh	Stanford University
Anya	Hwang	University of Southern California
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Hiroro	Ito	Nagoya University
Autumn	Ivy	Johns Hopkins University
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Shin	Kang	Temple University School of Medicine
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Eun Joo	Kim	University of Washington
Eunyoung	Kim	National Institute of Mental Health
Jineun	Kim	California Institute of Technology
Arda	Kipcak	University of Virginia
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Mable	Lam	Stanford University
Sofia	Landi	University of Washington
Jan	Laskowski	Cortivision
Tiandong	Leng	Morehouse School of Medicine
Ren	Li	University of California, Irvine
Zhuoran	Li	Stanford University
Heidi	Liang	University of California, Irvine
Ziyu	Liang	University of Southern California
Julio	Licinio	State University of New York
Jonathan	Lim	Washington University in St. Louis
Xiaoxiao	Lin	University of California, Santa Barbara
Andi	Liu	Stanford University
Yuanming	Liu	University of Virginia
Hui	Lu	George Washington University
Xiao-Hong	Lu	Louisiana State University
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Gyorgy	Lur	University of California, Irvine
Cheng	Lyu	Stanford University
Raghav	Madan	University of Washington
Stephen	Mahler	University of California, Irvine
Maryam	Majeed	Allen Institute for Brain Science
Firoza	Mamdani	University of California, Irvine
Verdion	Martina	University of Alabama at Birmingham
Francisco	Martini	Institute for Neurosciences of Alicante
Dylan	Martins	University of California, Santa Barbara
Katherine (Katie)	Matho	University of Southern California
Lauren	McElvain	University of Southern California
Daniel	McHugh	PackGene Biotech, Inc.
Rob	Meijers	Institute for Protein Innovation
Santi	Mendoza	University of California, Santa Barbara
Kokoe Fany	Messanvi	American University
Mariajose	Metcalfe	University of Louisville
Raju	Metherate	University of California, Irvine
Fernando	Midea Reis	University of California, Los Angeles
Tsuyoshi	Miyakawa	Fujita Health University

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Derrick	Morton	University of Southern California
Thrinath (3nath)	Mullapudi	University of California, Irvine
Tomoyuki	Murano	Fujita Health University
Tatsuki	Nakagawa	University of California, Irvine
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Acknowledgments

Welcome to the 2025 CNCM Conference, *"The Changing Brain"*, co-hosted by the Cajal Club and the Allen Institute for Brain Science. Now in our fifth year, we are thrilled to host nearly 400 attendees from across the neuroscience community—our largest gathering to date. This year's conference reflects not only growth in size, but also in scope, creativity, and collaboration.

We are sincerely grateful to the UCI School of Medicine for their continued backing, with special thanks to Dean Michael Stamos and Vice Dean of Basic Research Geoffrey Abbott for their advocacy and leadership. Behind the scenes, we appreciate the expert help of Jim and Rachel in navigating the permitting process, and Jessica for her creative guidance in event branding and design.

Our appreciation extends to the Department of Anatomy & Neurobiology team—Doug Schroettinger, Arnold Ortiz, and Lesley Dowd—whose support has been essential to our budgeting efforts. We are also thankful for the guidance from Justin, Arnold, and Margaret in Travel & Accounting, and to Andrew and Felicia in UCI Contracts for their patience and professionalism in addressing contract- and policy-related inquiries.

This year's conference would not have been possible without the outstanding collaboration with the Irvine Marriott team, particularly Bobby Shoemaker and Garlonn Farrell, who helped us manage the increased capacity and evolving space needs with care and professionalism.

We are proud to recognize the generous contributions of our academic partners, including the UCI Department of Otolaryngology–Head & Neck Surgery, Center for Hearing Research, Department of Anatomy & Neurobiology, and Office of Research.

A very special thank you to our corporate sponsors, including PacGenomics, RWD Life Sciences, Vizgen, Intelligent Imaging Innovations (3i), Institute for Protein Innovation (IPI), Qingdao Haier Biomedical, Femtonics, Quantum-Si, PackGene Biotech, and 3Brain AG. Your partnership plays a vital role in driving innovation and expanding the reach of our mission.

To our speakers, poster presenters, and performers—thank you for your time, enthusiasm, and intellectual generosity. Your contributions are the heart of this conference.

We also want to spotlight the dedicated CNCM team: Michele Wu and Ginny Wu for their hands-on leadership across operations; Jabez Domingo for managing critical logistical and financial elements; and Kaitlyn Huynh for her support in bringing together the Art of Science special event.

To all who made this conference possible—your hard work and commitment have created something truly special. We're honored to share this experience with you and look forward to what's ahead in 2026.

Jane Alshami
Conference Coordinator & CNCM Administrative Assistant
UC Irvine Center for Neural Circuit Mapping

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