UCI Center for Neural Circuit Mapping

Cajal Club

2021 CONFERENCE

Relationship Between Transcriptomics and Connectomics

August 20 – 21
8 a.m. - 5 p.m. PT

Beckman Center
Irvine, CA

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Welcome Message

Welcome to this exciting and stimulating conference on *Relationship Between Transcriptomics and Connectomics*. It is exciting because for most of us it is the first in-person, "live" scientific meeting we have attended since the outbreak of the COVID-19 pandemic around the end of 2019. We have adapted to Zoom and Teams interactions over the Internet—and sometimes they work very well—but nothing can replace the benefits of expected and unexpected interpersonal interactions, with their exchange of information and ideas, at a meeting like this. And our conference is stimulating because it brings together two cutting-edge domains, transcriptomics and connectomics, and challenges participants to begin thinking seriously about how to approach and analyze the relationship between them. By their very nature, transcriptomics and connectomics deal with complex networks of molecules and neurons, and as such they are catalyzing a revolution in systems neuroscience that may well lead to fundamentally new ways of thinking about the etiology of brain disorders, and about their treatment in terms of novel underlying biological mechanisms.

This conference is the first event jointly sponsored by the Center for Neural Circuit Mapping at UCI (https://cncm.som.uci.edu/) and the Cajal Club (https://cajalclub.org/). The Center for Neural Circuit Mapping is in its infancy. It was approved by the Dean in January, 2020, and is headed by Professor Xiangmin Xu. In contrast, the Cajal Club is an old-timer. It was founded in 1947 and in addition to annual events at the Society for Neuroscience meeting, it has sponsored or co-sponsored major symposia, starting in 2001 with one in Madrid, and followed by others in Stockholm, Querétaro, Copenhagen, Pavia, Seattle, and Puerto Varas, Chile. The current president of the Cajal Club, Professor Carol Mason (Columbia) sends her greetings and best wishes.

The organizers are deeply grateful to the superb group of speakers who agreed to attend the conference. We also thank the staff of the UCI Center for Neural Circuit Mapping and of the Beckman Center of the National Academies of Sciences and Engineering for making the venue so functional and attractive. And finally, we thank the Cajal Club's Secretary-Treasurer, Charles E. Ribak, for facilitating the meeting. We look forward to a very rewarding two days of brainstorming.

Larry W. Swanson, Ph.D.

University Professor

University of Southern California

Conference Organizers

Larry Swanson, PhD
Professor, Departments of Biological Sciences and Psychology
University of Southern California

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

Xiangmin Xu, PhD
Director, UCI Center for Neural Circuit Mapping
Professor, Department of Anatomy & Neurobiology
UCI School of Medicine

Charles Ribak, PhD
Cajal Club Host

Professor Emeritus
Department of Anatomy & Neurobiology
UCI School of Medicine

Conference Venue

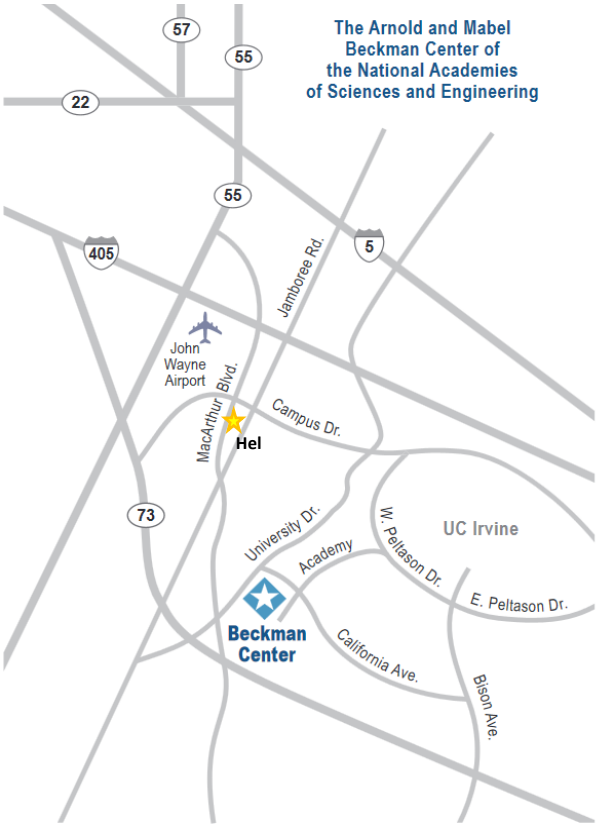
We are pleased to hold our conference at the Arnold and Mabel Beckman Center of the National Academics of Sciences and Engineering. Opened in 1988, the Beckman Center has brought to reality Dr. Arnold O Beckman's vision of a West Coast Center where experts could discuss matters of science and technology.

Directions

100 Academy Way, Irvine CA.

Near University Drive, exit from 73.

Adjacent to the University of California, Irvine and less than 3 miles from John Wayne Orange County Airport



Parking

Parking is available on-site at no charge.

COVID-19 NOTICE

Universal Masking/Face Coverings at UCI

July 30, 2021

Dear campus community,

As we have all seen in recent news reports, COVID-19 cases are on the rise due to a highly contagious Delta variant. We are experiencing this upswing within our region and among the UCI community, having an impact on our patients, students and employees.

As a result, UCI will re-implement a universal masking policy effective August 2. All individuals on campus or at UCI health facilities will be required to wear a mask or face covering while indoors, regardless of vaccination status, except when alone in an enclosed space. This update is in line with the **Centers for Disease Control and Prevention** and the **California Department of Public Health**'s recent recommendations on masking and face coverings. Please refer to the revised **Chancellor's executive directive on face coverings** for details.

While some would prefer not to wear a mask indoors, the health and safety of our community is always our primary concern. Most of those who get severely ill are not vaccinated, so we strongly urge all of you to get the COVID-19 vaccine. It is clear that the vaccine provides strong protection against illness, protecting the nearly 200 million people who have received at least one dose in the United States.

We appreciate your cooperation during this time and we will update you as quickly as possible when there is new information. Thank you for your understanding and for prioritizing the health of all our UCI community members. Please continue to take care of yourself and stay safe.

Sincerely,

Hal Stern
Provost and Executive Vice Chancellor
Chancellor's Professor, Department of Statistics

The Beckman Center has implemented a masking policy regardless of vaccination status. All attendees will be required to submit to a temperature check when entering the venue.

Conference Schedule

Friday, August 20

7:30 - 8:15 a.m.	**Continental Breakfast**
8:15 - 8:20 a.m.	Welcome and Introduction (Dean Michael Stamos)
8:20 - 8:30 a.m.	Opening Remarks - SOM & Cajal (Gall & Ribak)
	Session 1 "How is Brain Connectivity Organized Globally?" Larry Swanson, PhD, Chair
8:30 - 9:10 a.m.	Understanding the Basic Plan of the Nervous System: Perspective, Strategy, Progress Report (Swanson) Q&A 5 min
9:15 - 9:55 a.m.	Modular Organization of the Connectome: Model Organisms to Humans (Sporns) Q&A 5 min
10:00 - 10:20 a.m.	**Break**
10:20 - 11:00 a.m.	The Brain's Structural Connectome is Organized to Support Efficient Control of State Transitions (Bassett) Q&A 5 min
11:05 - 11:45 a.m.	Large-Scale, Non-Canonical Hippocampal Formation Circuit Organization and Function (Xu) Q&A 5 min
11:50 - 12:05 p.m.	Short Talk: Connectome of the Basal Forebrain Cholinergic System in Rat (Zaborszky) Q&A 3 min
12:15 - 1:30 p.m.	**Lunch / Poster Session**
	Session 2 "How to Define Cell Types?" Hongkui Zeng, PhD, Chair
1:30 - 2:10 p.m.	Understanding Brain Cell Type Diversity (Zeng) Q&A 5 min
2:15 - 2:55 p.m.	Mapping Molecular, Spatial, and Functional Organizations of Cells in the Brain by Single-Cell Transcriptome Imaging (Zhuang) Q&A 5 min
3:00 - 3:20 p.m.	**Break**
3:20 - 4:00 p.m.	Contributions of Genetics and Connectivity to Cortical Cell Type Taxonomy (Callaway) Q&A 5 min
4:05 - 4:45 p.m.	Deconstructing the Neural Control of Internal States (Anderson) Q&A 5 min
5:00 p.m.	**Adjourn**

Saturday, August 21

7:30 - 8:15 a.m.	**Continental Breakfast**
	Session 3 "How is Connection Specificity Achieved?" Liqun Luo, PhD, Chair
8:15 - 9:15 a.m.	The Pinckney J. Harman Memorial Lecture of the Cajal Club: Establishing Wiring Specificity of Neural Circuits in Flies and Mice (Luo) Q&A 5 min
9:20 - 10:00 a.m.	Wiring Up Direction Selective Circuits in the Retina (Feller) Q&A 5 min
10:05 - 10:20 a.m.	**Break**
10:20 - 11:00 a.m.	Trans-Seq: Translating Transcriptomics to Connectomics at Retinotectal Synapses (Duan) Q&A 5 min
11:05 - 11:45 a.m.	IgSF Protein Interactions Instruct Drosophila Neuromuscular Circuit Wiring (Carrillo) Q&A 5 min
11:50 - 12:05 p.m.	Short Talk: A Novel Cell-Type-Specific Brain Pathway Tunes Reward-Seeking Behaviors (Baram) Q&A 3 min
12:15 - 1:30 p.m.	**Lunch**
	Session 4 "How is Development Regulated by Gene Networks?" John Rubenstein, PhD, Chair
1:30 - 2:10 p.m.	Transcriptional Network Orchestrating Regional Patterning of Cortical Progenitors (Rubenstein) Q&A 5 min
2:15 - 2:55 p.m.	Genetic and Epigenetic Determinants of Cortical Development and Evolution (Rakic) Q&A 5 min
3:00 - 3:20 p.m.	**Break**
3:20 - 4:00 p.m.	A Molecular Logic for Cortical Projection Neuron Subtype Specification (Chen) Q&A 5 min
4:05 - 4:45 p.m.	Chromatin Regulation of Synaptic Maturation (West) Q&A 5 min
4:50 - 6:00 p.m.	**Closing reception for all attendees at Beckman Center**

SPEAKER ABSTRACTS

Larry Swanson

Understanding the Basic Plan of the Nervous System: Perspective, Strategy, Progress Report

Larry Swanson, PhD

Departments of Biological Sciences and Psychology, University of Southern California

Network analysis methodology offers systems biology a new, unified approach to understand organizing principles embedded in the “big data” generated by transcriptomics, connectomics, and other omics, and to clarify how these datasets are related to one another. The goal of our research is to create a structure-function model of a mammalian nervous system analogous to the models long available for understanding basic principles of the circulatory, respiratory, digestive, skeletal, endocrine, and other bodily systems. Our connection matrices are based on data published using experimental axonal transport pathway tracing methods and the project is over half completed. Results will be presented for the sexually dimorphic rat forebrain, where 17% of 216,690 possible macroconnections reportedly exist between 466 gray matter regions on both sides of the brain. Multiresolution Consensus Cluster Analysis reveals a nested hierarchy of interrelated structure-function subsystems (modules or communities) with 2 at the top level and 92 at the bottom level. At the top, a lateral forebrain subsystem appears to subserve voluntary behavior and cognition whereas a medial forebrain subsystem appears to subserve innate survival behaviors and affect. Because network features change with the addition or subtraction of subnetworks, a stable model of the nervous system basic plan can only be expected when the entire nervous system connectome, and its interactions with other bodily systems, is achieved, a connection matrix we refer to as the neurome of a species.

Olaf Sporns

Modular Organization of the Connectome: Model Organisms to Humans

Olaf Sporns, PhD

Department of Psychological and Brain Sciences, Indiana University

Modern neuroscience is in the middle of a transformation, driven by the development of novel high-resolution brain mapping and recording technologies that deliver increasingly large and detailed “big neuroscience data”. Network neuroscience has emerged as a new field dedicated to understanding structure and function of neural systems across scales, from neurons to circuits to the whole brain. In this presentation I will address key features of brain network organization, specifically the existence of network communities (modules) and interlinking hubs. I will cover comparative studies of anatomical networks across different animal species, as well as network attributes uncovered in neuroimaging studies of the human brain. I will briefly discuss how modular organization informs our understanding of brain dynamics, functional networks, and communication processes. Modular organization appears to be a universal feature of biological networks.

Danielle Bassett

The Brain's Structural Connectome is Organized to Support Efficient Control of State Transitions

Danielle Bassett, PhD

Department of Bioengineering, University of Pennsylvania

The human brain is a complex organ characterized by heterogeneous patterns of interconnections. Non-invasive imaging techniques now allow for these patterns to be carefully and comprehensively mapped in individual humans, paving the way for a better understanding of how wiring supports cognitive processes. While a large body of work now focuses on descriptive statistics to characterize these wiring patterns, a critical open question lies in how the organization of these networks constrains the potential repertoire of brain dynamics. In this talk, I will describe an approach for understanding how perturbations to brain dynamics propagate through complex wiring patterns, driving the brain into new states of activity. Drawing on a range of disciplinary tools – from graph theory to network control theory and optimization – I will identify control points in brain networks and characterize trajectories of brain activity states following perturbation to those points. Finally, I will describe how these computational tools and approaches can be used to better understand the brain's intrinsic control mechanisms and their alterations in psychiatric conditions.

Xiangmin Xu

Large-Scale, Non-Canonical Hippocampal Formation Circuit Organization and Function

Xiangmin Xu, PhD

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

The hippocampal formation (HF) plays essential roles in learning and memory, and spatial navigation. The extended HF includes the entorhinal cortex (EC), the dentate gyrus (DG), the hippocampus proper and the subiculum (SUB) complex. The HF serves as a central hub with inputs from many other brain regions, thus mediates extensive functional interactions of neocortical and subcortical regions. Much of the seminal work on hippocampal connectivity was carried out using conventional chemical tracing techniques. The HF is traditionally characterized as having a feedforward, unidirectional circuit organization whereby the CA1 transfers excitatory information out of the hippocampus proper to EC and SUB. While this canonical HF connectivity has been well established, the emergence of viral-genetic mapping techniques enhances our ability to further determine the hippocampal formation circuitry. We recently discovered non-canonical but significant back-projections from the subiculum to hippocampal CA1 in the mouse, and related work indicates that this pathway is present in humans. This suggests previously unconsidered functional roles for direct SUB modulation of hippocampal circuit activity. In this talk, I will present our recent progress in studying the synaptic circuit organization and function of the subiculum-CA1-CA3 back-projection pathways in the mouse using recent technological advances. Our data provide an updated circuit foundation to explore novel functional roles contributed by these non-canonical hippocampal circuit connections to hippocampal dynamics and behavior.

Short Talk #1

Connectome of the Basal Forebrain Cholinergic System in Rat

Laszlo Zaborszky, MD, PhD

Rutgers University

Cholinergic neurons of the basal forebrain (BF) project to the entire cortex. Despite its broad involvement in cortical activation, attention, memory, the functional details are not well understood due to the anatomical complexity of the region, where different functional systems interdigitate with the cholinergic space. Although this system was described initially as part of the diffuse cortical projection system, our recent studies suggest that the cholinergic projection to the cortex is not diffuse, but organized into segregated or overlapping pools of projection neurons. The extent of overlap between BF populations projecting to the cortex depends on the degree of connectivity between cortical targets of these projection populations (Zaborszky et al., 2015). The cortical target-specific groups of cholinergic neurons receive specific combination inputs (Gielow and Zaborszky, 2017). This organization may enable parallel modulation of multiple groupings of interconnected yet nonadjacent cortical areas.

In order to analyze densities of different cell types and connectional data from different animals, we developed a pipeline for spatial registration of vectorial and image data. In more than 70 brains, that received pairwise conventional retrograde (FB and FG) or virus tracer injections in the cortex, cholinergic neurons in the BF were mapped with the Neurolucida^{R} system. After mapping fluorescently labeled cells from 200 µm series, sections were restained with Nissl and images of sections were aligned to existing Neurolucida maps.

We implemented a Relational Database [Postgres] schema to store vectorial cell data, delineations and images. This solution provided us with a complete set of functions for analyzing geometric components, determining spatial relationships, and manipulating delineations. We implemented all the scripts for data visualization around the QGIS framework. This framework provides us with very fast and smooth visualization of high-resolution images as well as vectorial data.

We created a reference brain from a full series of 50 micron sections of a single brain, in which all cholinergic cells were mapped irrespective of their targets and then sections were stained for Nissl. These sections were imaged with 3 micron/pixel resolution and aligned in 3D. The software (Java, ImageJ) is able to slice this high resolution 3D stack of images in any plane to match sections from the experimental brains. Interpolations are used to construct a gapless approximation of the entire brain. The spatial registration of experimental sections to the reference brain is carried out using mixed rigid body, affine and B-Spline based elastic transformations.

For each reference cell within its environment, we will be able to assess the probability of projection to each cytoarchitectonically defined cortical target (“context vector”). By assigning this context vector to each reference cell, we will use unsupervised methods such as K-means and hierarchical clustering to classify BF cells according to their projection probability to cortical areas.

This connectome model, that captures the organizational principles of the basalo-cortical network, will facilitate the understanding the function of this network and the aberrant processing in Alzheimer’s and related degenerative diseases.

Hongkui Zeng

Understanding Brain Cell Type Diversity

Hongkui Zeng, PhD

Allen Institute for Brain Science, Seattle, WA, USA

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 built multiple platforms, including single-cell transcriptomics, spatial transcriptomics, single and multi-patching electrophysiology, 3D reconstruction of neuronal morphology, and high throughput brain-wide connectivity mapping, to characterize the transcriptomic, physiological, morphological, and connectional properties of brain cell types in a systematic manner, towards a multi-modal cell atlas for the mouse and human brain. These studies reveal extraordinary cellular diversity and underlying rules of brain organization and lay the foundation for unraveling mechanisms of circuit function.

Towards the goal of creating a cell atlas for the entire mouse brain, we have generated single-cell transcriptomes from many parts of the mouse brain. A comprehensive transcriptomic cell type taxonomy across the mouse isocortex and hippocampal formation reveals a parallel cell type and circuit organization between these two major brain structures and large-scale continuous gradient distribution of cell types in multiple dimensions in both structures, suggesting evolutionary and developmental underpinnings of the adult-stage cell type landscape.

To better understand the relationship between cell types' transcriptomic profiles and other structural and functional properties, we used Patch-seq to characterize the transcriptomic, physiological and morphological properties of mouse cortical GABAergic interneurons and complete morphology reconstruction of molecularly defined long-range projection neurons from various brain regions. These studies reveal concordant phenotypic variations at major cell type level as well as differential variations at more fine-grained subtype and individual cell levels, suggesting an interplay of genetic programming and network interaction in shaping the cellular diversity in the brain.

Xiaowei Zhuang

Mapping Molecular, Spatial, and Functional Organizations of Cells in the Brain by Single-Cell Transcriptome Imaging

Xiaowei Zhuang, PhD

Howard Hughes Medical Institute, Harvard University

The brain is composed of numerous cell types organized in a complex manner to form functional neural circuits. Single-cell transcriptomic analysis allows systematic identification of cell types based on their gene expression profiles and has revealed a high diversity of cell types in the brain. Understanding how different cell types contribute to brain function further requires knowledge of their spatial organization, connectivity and function. We developed a single-cell transcriptome imaging method, multiplexed error-robust fluorescence in situ hybridization (MERFISH), which allows RNA (and DNA) imaging at the genome scale. This approach enables in situ transcriptomic profiling of single cells, and hence in situ identification and spatial mapping of distinct cell types in brain tissues. Furthermore, we integrated MERFISH with retrograde labeling to probe projection targets of molecularly defined neuronal cell types. We also combined MERFISH with activity marker imaging to identify neurons activated by specific behaviors, thereby inferring the functional properties of molecularly defined cell types. In this talk, I will describe the MERFISH technology and its applications to map the molecular, spatial, projectional and functional organizations of cell types in a few brain regions.

Ed Callaway

Contributions of Genetics and Connectivity to Cortical Cell Type Taxonomy

Ed Callaway, PhD

The Salk Institute for Biological Studies, La Jolla, California

It is generally agreed that cell type definition and characterization should incorporate multiple features including anatomy, connectivity, function, and gene expression. Single cell transcriptomic profiling and cluster analysis promises to provide a reproducible foundation for defining cell types and producing cell type taxonomies. Accordingly, it is important to assess the degree to which this approach might lead to both over-splitting (multiple transcriptomic clusters correspond to populations that are not distinguishable by other features) and under-splitting (cell types that have distinct anatomy, connectivity or function cannot be distinguished by gene expression alone). Cortical excitatory cell types are known to display extensive diversity in their patterns of projections to distant cortical and subcortical targets, yet the numbers of transcriptomic clusters for these neurons is relatively small. These observations suggest that transcriptomic profiling might under-split cortical excitatory neurons. We assessed the projection diversity of cortico-cortical projection neurons in mouse primary visual cortex (V1) and directly compared the single-cell transcriptomes of neurons to their distinct projection patterns and synaptic feedback input sources. We find that layer 2/3 CCPNs with different anatomical projections differ systematically in their gene expression, despite forming only a single genetic cluster. These neurons also differ in their connectivity, receiving feedback selectively from the same areas to which they project. We further assessed the relationships between projections and genetics of mouse cortical excitatory neurons by combining retrograde labeling with single neuron methylation sequencing (snmC-seq). Non-canonical CH methylation predicts gene expression, allowing direct links to transcriptomic profiling, while methylation of non-coding regions allows predictions of gene regulatory elements and interactions with transcription factors. By assessing the relationships between cortical and subcortical projections and gene methylation across many cortical areas we found complex relationships. For example, neurons projecting to the medulla versus thalamus could be distinguished from genetics alone for all cortical areas assessed. In contrast, for comparisons between neurons projecting to other targets, the degree to which they could be distinguished by genetics varied between cortical areas. These findings demonstrate that gene-expression analysis in isolation is insufficient to distinguish excitatory cortical projection neuron types. Insofar as transcriptomic profiling is used as a foundation for cell type taxonomies, those taxonomies should allow incorporation of other features that further distinguish neuronal subtypes.

David J. Anderson

Deconstructing the Neural Control of Internal States

David J. Anderson, PhD

Howard Hughes Medical Institute, California Institute of Technology

Behaviors that are fundamental to animal survival, such as mating or the fight-or-flight response, are driven by internal emotional or motivational states. In humans, these brain states are subjectively experienced and expressed as “feelings,” such as desire, rage, or terror. Understanding the causal brain mechanisms that govern these states, using powerful new tools such as optogenetics and calcium imaging, should ultimately lead to better treatments for neuropsychiatric disorders. However, such invasive and perturbative studies are best performed in animal models. This poses the problem of how to study an animal’s internal state in the absence of information about subjective feelings. Operant conditioning can be used to measure motivational states underlying homeostatic behaviors, but are more difficult to apply to naturalistic social behaviors and invertebrate model organisms. As an alternative, we have begun to study “emotion primitives,” meta-behavioral features generally exhibited by state-dependent but not reflexive responses. These features include persistence, scalability, valence and generalization. They can be thought of as evolutionary “building blocks” of emotion. In this talk I will describe our efforts to understand the neural encoding of these primitives, using both flies and mice as model systems.

Liqun Luo

The Pinckney J. Harman Memorial Lecture of the Cajal Club:
Establishing Wiring Specificity of Neural Circuits in Flies and Mice

Liqun Luo, PhD

Howard Hughes Medical Institute, Department of Biology, Stanford University

Developing brains utilize a limited number of molecules to specify connection specificity of a much larger number of neurons and synapses. How is this feat achieved? In this talk, I will first discuss our work using the *Drosophila* olfactory circuit as a model to address this question. I will then discuss studies of mammalian homologs of some of the wiring molecules we identified in the fly, focusing on the mouse hippocampal network.

Marla B. Feller

Wiring up Direction Selective Circuits in the Retina

Marla B. Feller, PhD

Division of Neurobiology, Department of Molecular and Cell Biology & Helen Wills Neuroscience Institute, University of California, Berkeley

The development of neural circuits is profoundly impacted by both spontaneous activity and sensory experience. This is perhaps most well studied in the visual system, where disruption of early spontaneous activity called retinal waves prior to eye opening and visual deprivation after eye opening leads to alterations in the response properties and connectivity in several visual centers in the brain. We address this question in the retina, which comprises multiple circuits that encode different features of the visual scene, culminating in over 40 different types of retinal ganglion cells. Direction-selective ganglion cells respond strongly to an image moving in the preferred direction and weakly to an image moving in the opposite, or null, direction. Moreover, as recently described (Sabbah et al, 2017) the preferred directions of direction selective ganglion cells cluster along four directions that align along two optic flow axes, causing variation of the relative orientation of preferred directions along the retinal surface. I will provide recent progress in the lab that addresses the role of visual experience and spontaneous retinal waves in the establishment of direction selective tuning and direction selectivity maps in the retina.

Xin Duan

Trans-Seq: Translating Transcriptomics to Connectomics at Retinotectal Synapses

Xin Duan, PhD

Departments of Ophthalmology & Physiology, Kavli Inst. for Fundamental Nsci, UCSF, CA, 94143

Comprehensive neural circuit mapping starts from cataloging neuronal cell types and then leveraging cell identity to trace circuits. Though there is ever-increasing genetic access to diverse neuronal types of interests, genetically tractable tools for use in the mammalian brain remain limited. This gap in neural circuit tracing tools present challenges in determining how diverse neuronal types are interconnected to form functional circuits. Furthermore, without information on circuit connectivity, the cellular and molecular mechanisms underlying synaptic partner choice remain enigmatic. We were motivated to develop such a circuit tracing method to address long-standing questions of circuit wiring from retinal ganglion cells (RGCs) to the brain. The mouse visual pathway, starting from the retina, is an ideal system to understand mammalian neural circuit assembly, as nearly all retinal neuron types are genetically, morphologically, or functionally defined. While the field, including our past work, mainly focused on local retinal circuit mapping, connectivity maps from individual RGC types to retinorecipient neurons within the brain remain largely unknown. The knowledge gap exists in understanding the retina-brain connectivity with cell-type specificity over a long distance, representing common challenges to understand long-range circuit assembly across brain regions to decode circuit assembly.

We set out to address circuit tracing of genetically defined starter neuronal populations by building new technology, Trans-Seq, which combines an engineered, genetically-encoded anterograde tracer to label presynaptic neurons and single-cell RNA-Seq to profile their postsynaptic neurons for their cell type identification. We applied Trans-Seq to map retina-brain circuits. We molecularly classified specific retinorecipient neuronal subtypes in the brain (the superior colliculus in particular) connected with retinal ganglion cells in the eye. These results were carefully validated by orthogonal approaches, including *in situ* hybridization of marker gene expressions, electrophysiology, and retrograde tracing from the brain to RGCs. Through this means of converting neuronal transcriptomes to digital connectomes and identifying connections for further validation through electrophysiology, the Trans-Seq platform represents a generalizable anterograde tracing and sequencing approach broadly applicable to many neuroscience questions.

Through Trans-Seq, we additionally discovered that distinct RGC starter neurons in the eye are differentially connected to multiple superior collicular (SC) neuron types. We, in particular, defined a previously unknown synaptic connection from αRGCs to SC wide-field neurons. Also, leveraging the rich insights from the single-cell transcriptomic data from Trans-Seq, we identified an extracellular matrix molecule, Nephronectin, that is specific and required for the formation αRGCs to SC wide-field neurons *in vivo*. Nephronectin is the first molecule identified that governs synaptic specificity from the mammalian eyes to the brain. We further explored the molecular mechanisms mediated by Nephronectin, which may shed light on future therapies for abnormalities in neuronal connectivity related to ophthalmological or neurological diseases.

Funds: NIH R01EY030138, Research Prevent Blindness, Ziegler Foundation for Blindness, Glaucoma Research Foundation (CFC3), Klingenstein-Simons Fellowship.

Robert A. Carrillo

IgSF Protein Interactions Instruct Drosophila Neuromuscular Circuit Wiring

Robert A. Carrillo, PhD

Department of Molecular Genetics & Cellular Biology, Neuroscience Institute, University of Chicago

The developmental programs that regulate neuronal wiring are ultimately responsible for all facets of behavior. Our understanding of how neural circuits form and specifically how neurons determine their synaptic partner(s) is poorly understood. This problem is especially daunting in the context of neural circuits in the human brain where somehow specific connections must be made from a pool of billions of neurons. A prevalent model suggests that cell surface protein (CSP) interactions on corresponding neurons allow for recognition and subsequent synaptogenesis. To instruct synaptic specificity, cognate CSP partners must be expressed in corresponding synaptic partners. To identify these “individual identification tags”, as suggested by the work of Roger Sperry and others, we utilized gene trap GAL4 lines to examine the expression of two interacting subfamilies of the immunoglobulin superfamily (IgSF) – the Dprs and DIPs – in the *Drosophila* larval neuromuscular circuit. This system facilitates our analyses due to the stereotyped circuit wiring, limited number of synaptic partners, and available genetic tools. We found that each motor neuron (MN) expresses a unique subset of *dprs* and *DIPs*. *DIP-α*, for example, is selectively expressed in two motor neurons. We confirmed this expression with both antibody labeling and tagging the endogenous *DIP-α* and observed localization to motor axon filipodia during synaptic partner selection. Loss of *DIP-α* results in highly penetrant connectivity defects of a specific motor neuron, suggesting DIP-α is required for motor neuron-muscle recognition. A DIP-α binding partner, Dpr10, is expressed in muscles, and loss of *dpr10* mimics the *DIP-α* null phenotype. Furthermore, DIP-α interacts homophilically and our data suggests that homophilic interactions may inhibit weaker non-specific heterophilic binding. Finally, we reasoned that multiple CSP interactions may instruct circuit wiring. Guided by the *dpr/DIP* expression map, we identified *DIPs* that are co-expressed in motor neurons and simultaneous loss of these *DIPs* reveals further perturbations in connectivity. Overall, our data suggest that combinatorial CSPs act as molecular codes that underlie synaptic partner recognition.

Short Talk #2

A Novel Cell-Type-Specific Brain Pathway Tunes Reward-Seeking Behaviors

Tallie Z. Baram, MD, PhD

Matthew T. Birnie[*1], Annabel K. Short[*1], Aidan L. Pham[1], Ashima Kundu[1], Christy A. Itoga[2], Xiangmin Xu[2], Stephen V. Mahler[4], Yuncai Chen[*1] & Tallie Z. Baram[*1,2,3]

[1.] Dept. of Pediatrics, University of California-Irvine, USA; [2.] Dept. of Anatomy/Neurobiology, University of California-Irvine, USA; [3.] Dept. of Neurology, University of California-Irvine, USA
[4.] Dept. of Neurobiology & Behavior, University of California-Irvine, USA

The seeking of pleasure and reward are fundamental human behaviors that are executed by coordinate activity of the brain's reward circuitry. Disrupted operations of the reward circuit and its components are thought to underlie major emotional disorders including depression and drug abuse, and these disorders commonly arise after early life stresses. Yet, how early life adversities (ELA) impact the functional maturation of reward-related circuits to promote disease remains unclear.

Here, we employ viral-genetic technologies to identify a novel GABAergic projection that co-expresses the stress-related neuropeptide corticotropin-releasing hormone (CRH) and connects the basolateral amygdala (BLA) to the nucleus accumbens (NAc), both nodes of the reward circuit. We show that chemo- and optogenetic activations of this pathway suppress reward seeking behaviors in naïve mice, recapitulating deficits observed following ELA. Notably, inhibition of the CRH+ projection in adult ELA-experienced mice reverses their reward-seeking deficits. Thus, the novel, GABAergic CRH+ projection from BLA to NAc tunes reward seeking behaviors and may constitute a target for interventions aiming to mitigate the contributions of ELA to prevalent and disabling mental illnesses.

Supported by NIH P50 MH096889 and the Hewitt Foundation for Biomedical Research

John Rubenstein

Transcriptional Network Orchestrating Regional Patterning of Cortical Progenitors

John Rubenstein, MD, PhD

Department of Psychiatry, UCSF Weill Institute for Neurosciences

We uncovered a transcription factor (TF) network that regulates cortical regional patterning in radial glial stem cells. Screening the expression of hundreds of TFs in the developing mouse cortex identified 38 TFs that are expressed in gradients in the ventricular zone (VZ). We tested whether their cortical expression was altered in mutant mice with known patterning defects (*Emx2, Nr2f1* and *Pax6)*, which enabled us to define a cortical regionalization TF network (CRTFN). To identify genomic programming underlying this network, we performed TF ChIP-seq and chromatin-looping conformation to identify enhancer-gene interactions. To map enhancers involved in regional patterning of cortical progenitors, we performed assays for epigenomic marks and DNA accessibility in VZ cells purified from wild-type and patterning mutant mice. This integrated approach has identified a CRTFN and VZ enhancers involved in cortical regional patterning.

Pasko Rakic

Genetic and Epigenetic Determinants of Cortical Development and Evolution

Pasko Rakic, MD, PhD

Department of Neurobiology and Kavli Institute for Neuroscience, Yale University School of Medicine, New Haven, CT, USA

The identity, synaptic connection and, ultimately, function of cortical neurons in all species is defined by their proper areal, laminar and columnar position acquired by active migration from proliferative zones near cerebral ventricles. Although the basic principles of neuronal specification and migration follow the radial unit and protomap models in all mammals, the modifications of developmental events during evolution produce not only quantitative, but also qualitative, changes; including elaboration of neuronal and glial sub-types, novel transient embryonic zones and modes of neuronal migration that create the addition of specialized cytoarchitectonic areas with new patterns of axonal connections. Our strategy is to use a variety of *in vitro* and *in vivo* assays to compare the development of the cerebral cortex in rodents, non-human primates and humans by the most advanced methods, including DNA, RNAseq and their regulatory elements. We uncovered many similarities between species that are conserved during evolution of the cerebral cortex. However, importantly, we also identified some promoters and enhancers that become enriched in modules of co-expressed genes in the human lineage and are involved in neuronal proliferation, determination, migration, and cortical map formation. This is a growing and fast moving field, and I will present our recent data on the species-specific differences that are generated by differential gene expression, emergences of novel regulatory elements and pattern of non-synaptic Ca^{2+} transients during embryonic stages that provide insights into the evolution of the human cerebral cortex as the organ of thought.

Bin Chen

A Molecular Logic for Cortical Projection Neuron Subtype Specification

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Projection neuron subtype identities in the developing cerebral cortex are established by expressing pan-cortical and subtype-specific genes, which execute terminal differentiation programs and bestow neurons with a glutamatergic phenotype and subtype-specific morphology, physiology, and axonal projections. Whether the pan-cortical glutamatergic phenotype and subtype-specific characteristics are regulated by the same genetic program or controlled by distinct genes remains largely unknown. In *C. elegans*, expression of terminal effector genes is activated by terminal selector genes, which are transcription factors that act in differentiating neurons by binding to common *cis*-regulatory elements in effector genes and activating their expression (Hobert and Kratsios, 2019). Whether similar mechanisms are utilized in developing mammalian brains is unknown, except the corticospinal neurons, a subset of subcerebral projection neurons specified by the transcriptional regulator Fezf2 (Lodato et al., 2014). I will summarize some previously published work, and report our most recent progress on investigating the molecular logic for cortical projection neuron subtype specification. These studies together suggest that distinct genetic programs act sequentially to regulate the differentiation of cortical projection neurons, with genes expressed in progenitor cells specifying the pan-cortical glutamatergic phenotype (that is, specifying the cortical projection neuron lineage in the cortical progenitors), and subtype-specifying transcription factors functioning in postmitotic cells to selectively repress the expression of genes associated with alternate subtype identities.

Anne E. West

Chromatin Regulation of Synaptic Maturation

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Neuronal differentiation requires the precise orchestration of gene expression programs and is coordinated by dynamic changes to chromatin state and structure. Temporal control of gene transcription is particularly important in postmitotic neurons of the postnatal brain, which undergo extensive changes in their gene expression programs as they mature, respond to sensory signals, and refine their synaptic connections. In order to identify the chromatin and transcriptional mechanisms of synapse maturation, we have been using developing cerebellar granule neurons (CGNs) in the postnatal brains of mice as a robust model system for determining the roles of chromatin regulation in neuronal differentiation and maturation. Our data show extensive changes in chromatin accessibility for transcription factor binding even across postmitotic stages of neuronal maturation. I will discuss how we are using sequence information contained within dynamically regulated enhancers to understand molecular mechanisms of how the Zic family of transcription factors switches its transcriptional targets as CGNs develop. We wondered how genes that turn on late in synaptic maturation are delayed in their activation after cell fate commitment and cell cycle exit and found that a subset of these genes are marked by repressive histone H3 lysine 27 trimethylation that is subsequently lost. I will discuss our evidence that timely activation of these genes, as well as the proper development of CGN synapses, is regulated by the lysine demethylase Kdm6b. Finally, chromatin regulators including *KDM6B* are among the most common classes of genes to be found mutated in autism spectrum disorder (ASD) and intellectual disability (ID), however why dysregulation of chromatin leads to aberrant neural development and whether multiple chromatin regulators feed into convergent biological processes such as synapse maturation is poorly understood. I will share our evidence that disease-causing variants of the Rahman Syndrome gene and linker histone *H1-4* disrupt selective synaptic gene expression programs in postmitotic neurons. Taken together these data provide a framework for understanding mechanisms that regulate the pace of neuronal and synaptic maturation.

POSTER ABSTRACTS

Poster #1 (Nedelescu et al.)

3D Brain-Wide Profiling of Neuronal Ensembles Reactive to Relapse-Promoting vs. Relapse-Suppressing Cues in Rats

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Drug addiction is a chronically relapsing brain disease. While environmental stimuli signaling drug availability (S+) are well-known to promote relapse, we have found that environmental stimuli signaling drug omission (S-) can suppress relapse in rats. This bidirectional modulation of drug relapse is regulated by two functionally distinct coactive groups of infralimbic cortex (IL) neurons – neuronal ensembles or engram cells – with each group selectively reactive to S+ or S- (as visualized by Fos protein). However, the neuroanatomical source of afferents that activate these neurons remains unknown. We have thus conducted brain-wide analysis to identify neuronal ensembles that send axonal projections (visualized by AAV2retro-GFP) to the IL and that are evoked by S+ or S- in different groups of rats trained to self-administer cocaine or alcohol. While automated brain-wide profiling is available for mice, similar procedures for rats (preferred animal model for studying more complex behaviors to model drug addiction) are not yet established. Hence, we developed automated 3D brain-wide profiling for rats where image data and cell counts were registered using NeuroInfo to the Waxholm rat atlas (ref: https://www.nitrc.org/projects/whs-sd-atlas). Briefly, [1] whole slide images of serial brain sections (60 µm) were captured by a ZEISS Axioscan slide scanner microscope, [2] these 2D images were aligned and registered using NeuroInfo to the Waxholm rat atlas coordinate system, [3] Fos- and GFP-positive IL-projecting neurons were detected with single cell resolution in NeuroInfo using deep learning methods with anatomic specificity conferred by matching image data to the atlas coordinate space. This method provides superior cellular tagging (staining) and image resolution – especially for the larger rat brains – than similar methods using brain clearing and light-sheet microscopes. In my presentation, I will further describe our new NeuroInfo tool for rat brains by applying it to identify brain-wide neuronal networks reactive to relapse-promoting vs. relapse-suppressing stimuli. In short, this study provides a new tool for automated 3D brain-wide profiling of rats and expands our knowledge of brain circuitry mediating environmental modulation of drug relapse.

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Poster #2 (Buckley et al.)

Simultaneous Acquisition of Single-Cell Transcriptomes and Neuroanatomy

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Synapses define the points of communication between neurons, controlling the flow of information across neural circuits. The number of synapses (synaptic density) between partners is tightly controlled and dysregulation of synaptic density between cell-types is implicated in a wide range of neurodevelopmental and neurodegenerative diseases. However, current technology to assay synaptic connectivity is laborious, low-throughput and agnostic to gene-expression, a critical measure of neuronal identity.

We are developing a suite of tools to fill these gaps in our ability to study synaptic connectivity. These tools aim to deliver both transcriptomic and neuroanatomical information for single neurons. The tools rely on a system that transports mRNA barcodes to synapses. To achieve this transport, we first deliver mRNA barcodes to neurons using Adeno-associated viruses (AAVs), such that each neuron expresses a unique set of barcodes. We simultaneously transfect cells with AAVs encoding custom-engineered proteins that traffic the barcodes to pre- or post-synapses. A barcode will therefore be present in a neuron's nucleus and pre- or post-synapses. We then read out the barcodes from synapses and relate them to the transcriptomes of their originating cells using spatial transcriptomics or single-nucleus RNA sequencing. Because our transport system is implemented using AAVs, it preserves endogenous gene expression and allows us to target large populations of neurons.

Here, we show our successful barcode trafficking system, which can now deliver mRNA barcodes to pre- and post-synapses in-vitro and in-vivo. Using thalamocortical projection neurons as an example, we also demonstrate our first approach to mapping synaptically-trafficked barcodes to their host cells. This approach uses Slide-seq, a spatial transcriptomics method with transcriptome-wide detection at 10 μm resolution. With Slide-seq, we show that we can precisely map thalamocortical projections to the cortex at scale on a cell type-specific basis. The spatial resolution of Slide-seq allows us to capture the layer specificity in the cortex of these thalamocortical projections as well.

Mapping barcodes from synapses back to their cells of origin will be a powerful tool for studying neural projection patterns in relation to gene expression and cell-type. In general, the tools we are building around our barcode transport system will unlock exciting opportunities to explore projection patterns, synaptic density, and the balance of excitation/inhibition within specific cell types and across behavioral paradigms, developmental stages, and disease states.

Poster #3 (Merullo et al.)

Cellular Transcriptomics Reveals Evolutionary Identities of Songbird Vocal Circuits

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Birds display advanced behaviors, including vocal learning and problem-solving, yet lack a layered neocortex, a structure associated with complex behavior in mammals. To determine whether these behavioral similarities result from shared or distinct neural circuits, we used single-cell RNA sequencing to characterize the neuronal repertoire of the songbird song motor pathway. Glutamatergic vocal neurons had considerable transcriptional similarity to neocortical projection neurons; however, they displayed regulatory gene expression patterns more closely related to neurons in the ventral pallium. Moreover, while γ-aminobutyric acid–releasing neurons in this pathway appeared homologous to those in mammals and other amniotes, the most abundant avian class is largely absent in the neocortex. These data suggest that songbird vocal circuits and the mammalian neocortex have distinct developmental origins yet contain transcriptionally similar neurons.

Poster #4 (Zhou et al.)

The Consequence of Patient-Derived Anti-NMDA Receptor Autoantibodies in Neural Circuit Development and Subsequent Behavior

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Anti-NMDA receptor autoantibodies cause severe behavioral deficits and prominent abnormal movements in pediatric patients, indicating disruption of sensory-motor circuits. A key structure for integrating sensory-motor information is the callosal connection between primary somatosensory cortices (S1). We have demonstrated that genetic disruption of NMDA receptor and antibody-mediated loss of function lead to defects in the somatosensory callosal projection in mice. We showed that disruption of axon guidance functions of the EPHRIN-B/EPHB pathway due to loss of NMDAR is the likely mechanism of this phenotype (Zhou et al., *eLife*, 2021). To further address whether exposure to patient-derived anti-NMDA receptor autoantibodies during callosal development disrupts callosal connectivity in S1, we generated anti-NMDA receptor monoclonal antibodies from the cerebrospinal fluid (CSF) of an anti-NMDA receptor encephalitis patient and identified two mAbs against two different subunits of NMDA receptor – mAb1 is against the NR2A subunit, mAb3 is against the NR1 subunit. Injections of either of these antibodies during callosal development in mice disrupts the ordered somatosensory callosal circuit. Injection of mAb1 caused small changes in the callosal projection but injection of mAb3 caused dramatically increased callosal innervation in the somatosensory cortex. Most interestingly, the disruption of a developmentally patterned circuit by the two patient-derived anti-NMDA receptor autoantibodies causes sensory-motor deficits in fine movement when these mice grew to adulthood. In particular, mAb3-treated mice show much worse performance in sensory-motor coordination tasks than mAb1- treated mice. This is the first demonstration that sensory-motor circuit deficits caused by patient-derived autoantibodies during development, cause subsequent sensory-motor behavior deficits in adulthood. This is relevant to the effects of this syndrome in pediatric and newborn patients and may also shed light on the protracted neurocognitive deficits experienced by anti-NMDA receptor encephalitis patients even after resolution of the acute phase of the illness.

Poster #5 (R. Ali Marandi Ghoddousi et al.)

SCAMPR: A Pipeline for Quantitative Analysis and Spatial Mapping of Neuronal Gene Expression using High-Dimensional Fluorescent In Situ Hybridization

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Heterogeneity in structure begets heterogeneity in function. This is especially true for the nervous system, where the existence of different neuron types allows for the diverse range of behaviors exhibited by a single animal. Utilizing observable, fundamental characteristics to classify neurons into distinct and/or inter-related groups is the first step towards disentangling the role of neuron populations in the production of complex functions. Two such characteristics are cellular location and gene expression patterns, and until recently, the relationship between these two characteristics has been difficult to investigate in a high throughput manner. Recent advances in commercialized multiplexed spatial transcriptomics technologies have created opportunities to visualize complex gene expression patterns at single cell resolution in tissue sections, helping bridge the gap between transcriptomics and topology.

Hiplex RNAScope, a commercially available multidimensional *in situ* hybridization technique allows for the visualization of 12 different mRNAs in tissue sections on a single-cell basis. There has been a significant challenge, particularly related to identification of cell boundaries, in accurately processing, analyzing, and visualizing multiplexed *in situ* hybridization data. Existing methods for quantitative analysis of spatial mRNA expression focus on nuclear gene expression, require high computing resources, and generate extremely large datasets. We developed a dual immunohistochemistry-RNAscope protocol for whole-cell segmentation and quantification of Hiplex RNAScope data. The Single-Cell Automated Multiplex Pipeline for RNA (SCAMPR) was developed for semi-automated image processing and quantitative analysis of Hiplex RNAscope. SCAMPR requires modest computing power, generates datasets that are practical in size, and allows for sensitive and specific quantification of mRNA expression in both the nuclear and cytoplasmic compartments of the cells.

Different examples are provided to demonstrate that SCAMPR is compatible with multiple tissues in the nervous system. Important comparisons are made regarding the accuracy and time demand of manual segmentation of cellular regions of interest (ROI) compared to SCAMPR. Several analysis tools are presented for determining single-cell gene expression relationships and mapping these relationships back onto the source tissue. Lastly, we demonstrate that SCAMPR can be used to measure gene expression differences between two experimental groups.

Supported by NIMH grant MH067842 and The Saban Research Institute of Children's Hospital Los Angeles Pre-Doctoral Award.

Poster #6 (Sun et al.)

Neural Circuit Dynamics of Drug-Context Associative Learning in the Hippocampus

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One of the most potent triggers for relapse to drug use after prolonged abstinence is exposure to an environmental context associated with previous drug consumption. However, it is unclear that how this maladapted associations are encoded and maintained in the brain. Despite the critical role of the hippocampus in supporting spatial and episodic memory, very little work has considered how the representation of individual neurons in the hippocampus might support the encoding of drug-context associations and contribute to drug-seeking behavior. Nearly 30 years ago, researchers first observed that hippocampal place cells fire in one or few restricted spatial locations and 'remap' (place cell firing fields turn off or on, or move to a new location) between different spatial contexts. Here, we took this tractable cognitive property of place cells as an access ramp to address this knowledge gap in understanding drug-context associations. We imaged calcium activity in freely moving mice to examine hippocampal place cell representations over the course of conditioned place preference. We first focused on the effects of methamphetamine (MA), a widely abused psychostimulant that alters catecholaminergic signaling. We identified a subset of CA1 place cells that remap to represent the drug-paired context after MA conditioning, switching off their activity on the neutral/saline-paired side and sharpening their activity on the MA-paired side of the CPP apparatus. This place cell remapping correlated with the MA-induced place preference behavior and emerged via an experience dependent mechanism. Further pointing to a relationship with drug-seeking behavior, both MA-associated place cell remapping and CPP behavior was blocked with the administration of ketamine, which produces a dissociation-like state in mice (Vesuna et al., 2020). Finally, we extended these findings to morphine (MO), and report that MO-context associations were encoded through a similar mechanism but recruited a different functionally defined population of place cells. Together, this work reveals a unified mechanism in the hippocampus to encode spatial/contextual associations with different drugs using a sub-population of hippocampal place cells, pointing to a potential novel neuronal target for the development of therapeutics for treating context-induced drug relapse.

Poster #7 (Tsyporin et al.)

FEZF2 is a Selective Repressor that Specifies Multiple Excitatory Neuron Fates in the Mammalian Cerebral Cortex

Jeremiah Tsyporin and Bin Chen

Jeremiah Tsyporin[1,7], David Tastad[1,7], Xiaokuang Ma[2], Antoine Nehme[2], Thomas Finn[1], Liora Huebner[1], Guoping Liu[3], Daisy Gallardo[1], Amr Makhamreh[1], Jacqueline M. Roberts[1], Solomon Katzman[1], Nenad Sestan[5], Susan K. McConnell[6], Zhengang Yang[3], Shenfeng Qiu[2], and Bin Chen[1,8]

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Excitatory projection neurons in the mammalian cerebral cortex are specified by genetic programs that confer a glutamatergic identity along with subtype-specific morphology, physiology, and axonal projections. Subtype-specific transcription factors have been identified as critical regulators of the specification and generation of distinct classes of projection neurons. For example, Tbr1 and Sox5 promote the generation of layer 6 corticothalamic projection neurons, Fezf2 and Bcl11b are required for layer 5 subcerebral projection neurons, and Satb2 is required for the development of both callosal projections and layer 5 subcerebral neurons. Exactly how these genetic programs work is unclear. In neuroblasts of the Drosophila embryonic central nervous system, temporal transcription factors are sequentially expressed that confer temporal identity. In *C. elegans*, terminal selector genes initiate and maintain the expression of effector genes that define a particular neuronal phenotype. The molecular logic instructing the development of cortical projection neuron subtypes in mammalian brains is not fully understood. Fezf2 has been reported to be a terminal selector gene for layer 5 subcerebral neurons, yet its ability to directly activate effector gene transcription has not been rigorously tested. To determine how Fezf2 functions, we created a fusion protein consisting of the Fezf2 DNA binding domain and the Engrailed transcription repressor domain that we termed Fezf2-EnR. By utilizing BAC transgenics to express Fezf2-EnR in the context of a Fezf2 knockout mouse, we observed that Fezf2-EnR fully rescued the molecular and axonal projection defects seen in the Fezf2 knockout mouse. In the context of cell fate specification, Fezf2 functions exclusively as a repressor. Furthermore, we conditionally knocked out Fezf2 in newly born neurons, rather than at the progenitor level, and found that the defects seen when knocking out Fezf2 in newly born neurons were comparable to the full Fezf2 knockout. We found that Fezf2 is also necessary for the proper development of layer 6 corticothalamic projection neurons. Without Fezf2, these neurons take on the molecular characteristics of layer 5 subcerebral projection neurons. We showed that Tle4, a gene highly specific to corticothalamic projection neurons and necessary for proper corticothalamic neuron physiology, is a co-repressor of Fezf2. Indeed, in Tle4 knockout mice, layer 6 corticothalamic projection neurons take on molecular characteristics of layer 5 subcerebral projection neurons. These findings support a no vel model of deep-layer projection neuron specification wherein Fezf2 promotes the acquisition of layer 5 subcerebral identity by directly repressing layer 6 corticothalamic genes; in the context of a newly born corticothalamic projection neuron, Fezf2 and Tle4, together, promote layer 6 identity by directly repressing layer 5 subcerebral genes. Therefore, Fezf2 functions as a selective repressor by actively recruiting co-repressors in a cell-type-specific context to inhibit the acquisition of alternate cell identities.

Poster #8 (Ma et al.)

Malat1 Regulates Th17 Program to Promote Intestine Inflammation

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Chromatin associated non-coding RNAs (chaRNAs) are critical regulators of mammalian gene programs. However, their chromatin localization and immune function *in vivo* remain to be elucidated. One of the most abundant chaRNAs expressed in colonic T lymphocytes is Metastasis Associated Lung Adenocarcinoma Transcript 1 (*Malat1*). Here, we report that Malat1 promotes colonic inflammation by restricting IL-17A and IL-17F productions in non-pathogenic Th17 cell. Global RNA interactions with DNA by deep sequencing (GRID-seq) coupled with transcriptomic studies revealed Malat1 is recruited to the *Il17a-Il17f* super enhancer when naïve T cells differentiate into the Th17 lineage. By retaining Suz12 and maintaining local H3K27 methylation, Malat1 dampens transcription of the *Il17a-Il17f* locus. These results shed new light on the chromatin localizations and immunological functions of Malat1, providing new opportunities for therapeutic intervention to treating T cell-mediated diseases.

Poster #9 (Long et al.)

RNA Binding Protein DDX5 Directs Tuft Cell Specification to Regulate Microbial Repertoire and Promote Tumorigenesis in the Intestine

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Tuft cells residing in the intestinal epithelium provide protection against helminth infections, but also serve as entry portals for enteroviruses. These cells also express the pro-survival doublecortin-like kinase 1 (DCLK1) implicated in tumorigenesis. Tuft cell lineage commitment requires CDC42, a Rho GTPase that acts downstream of the Epidermal Growth Factor Receptor (EGFR) signaling cascade, and the master transcription factor POU2F3. Here, we report a post-transcriptional mechanism involving a DEAD box containing RNA binding protein, DDX5, in directing the tuft cell specification and providing a niche for MNOV virus in the intestine. Mechanistically, DDX5 binds to the *Cdc42* transcript and promotes its protein translation to drive POU2F3 expression. In the intestinal epithelial cell specific DDX5 knockout mice, loss of tuft cell specification resulted in altered microbial repertoire and protection against tumorigenesis.

Poster #10 (Prange et al.)

Comparing Dendrite Regeneration Versus Maintenance in a Drosophila Model of PolyQ Disease

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Dendrites are essential structures of the neuron that receive information from both the environment and other neurons. Dendrite architecture determines what inputs a neuron receives, and is therefore an important part of neural circuit function. This important role is compromised when dendrites are injured. Dendrites can be injured both acutely, like during a traumatic brain injury or stroke, or progressively through neurodegeneration. However, dendrite regeneration following either acute or progressive injury is under-studied. We hope to better understand dendrite regeneration by examining regeneration in situations where neurons exhibit dendrite defects, such as in neurodegenerative disease models.

Dendrites are normally actively maintained after development by a number of surveillance mechanisms, because defects in dendrite architecture can lead to negative consequences for neurons and their ability to communicate to one another. Dendrite maintenance mechanisms are compromised in neurodegenerative diseases, and dendrites are lost before neurons begin to die. By using a *Drosophila melanogaster* model of neurodegenerative disease, we seek to understand how dendrite regeneration following injury relates to normal dendrite maintenance. We ask the question: when dendrite maintenance is defective, as in neurodegenerative disorders, is dendrite regeneration also defective?

We are examining dendrite development, maintenance, and regeneration after injury in ddaC class IV da neurons of the peripheral nervous system in *Drosophila* larvae. It has previously been shown that these neurons degenerate dendrites when constitutively overexpressing MJD-78Q, a polyglutamine expansion of the spinocerebellar ataxia gene SCA3/MJD. In this poster, we present our preliminary results on how neurons overexpressing MJD-78Q regenerate after injury. To assess this, we selectively injured individual dendrite branches using a two-photon laser. First we assessed regeneration following an extreme injury, when all branches were severed. Dendrite regeneration after severing all the dendrites is robust in wild-type ddaC neurons, and is possible but limited in early-stage neurons expressing MJD-78Q that have not yet begun to degenerate, but we find that once dendrite degeneration has begun, late-stage neurons expressing MJD-78Q are defective for regeneration after this dramatic injury. Next, we assessed how dendrite degeneration of uninjured branches was affected when only a single branch of the arbor was injured. We have found that cutting a single primary branch in degenerating neurons expressing MJD-78Q induces a neuroprotective effect in the rest of the arbor, even in older neurons. This supports the provocative idea that injury of one dendrite branch could effectively “turn on” regenerative processes in uninjured branches, to slow or limit degeneration in spared branches. We anticipate that the results of this pilot study will provide insight into the processes of dendrite maintenance and regeneration. It will also aid our comprehensive understanding of neurodegenerative disorders.

Poster #11 (Fiederling et al.)

Tools for Efficient Analysis of Neurons in a 3D Reference Atlas of Whole Mouse Spinal Cord

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The first control center for somatosensory signals is the spinal cord, comprising the neurons that transmit primary sensory signals from the periphery to the brain. These neurons are essential for the sensation of pain, itch, temperature, touch and relative body position and provide critical feedback to motor neurons that control all movements. To understand the contributions of spinal neurons to sensorimotor circuits, it is essential to map the positions of identified subsets of neurons in relation to others throughout the spinal cord. To date, however, unbiased approaches to map positional information in the context of the whole spinal cord are severely limited by a lack of tools for efficient data collection and analysis. Moreover, the absence of a standardized 3D reference atlas for the mouse spinal cord complicates anatomical mapping of data and comparison between samples and results across the field.

Here, we present tools for practicable analysis of labelled cells and projections in whole mouse spinal cord in the context of a novel 3D anatomical atlas. We have designed soluble, 3D-printed, 'SpineRacks' that guide oriented and parallel embedding of serial tissue segments of the entire spinal cord within a single block for synchronous cryo-sectioning. SpineRacks precisely position tissue pieces within each block section array, permitting automated imaging of slides and rostro-caudal sorting of section images. Additionally, we have developed 'SpinalJ', a user-friendly plugin for ImageJ, to register section images and to map the reconstructed data to a prototype 3D reference atlas. SpinalJ further combines various tools for manual and automated cell and projection analysis, as well as for data visualization. Using this platform, we have verified mapping accuracies for known neurons and demonstrated its usefulness to reveal unknown neuronal distributions.

Together, these tools facilitate high-throughput analyses of neurons and their projections in whole spinal cord and allow direct comparison of data across studies and labs.

Poster #12 (Hwu et al.)

Reshaping the Current Standards of Structural Plasticity: Chromatin Remodeling Factors involved in both Memory and Regeneration

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Aging in the brain is characterized by changes in cognitive function, which range from normal cognitive decline to the exponential cognitive deterioration of dementia. Age-related memory impairments in mice can be ameliorated by deleting HDAC3 in the dorsal hippocampus of old mice. Removing HDAC3 from the hippocampus of old mice causes changes in gene expression, which improves performance on spatial memory tasks (Kwapis et. al., 2018). In order to understand the mechanism of how changes in gene expression cause improvements in spatial memory, it is necessary to study how these functionally relevant genes affect neuron structure.

Memory processes prompt neurons to retract and extend dendrites, to reinforce and develop new synaptic connections. We hypothesize that differentially expressed genes involved in enhancing spatial memory in mice, will also enhance structural plasticity and synaptic remodeling. Drosophila melanogaster is a powerful model organism to study how genes impact structural plasticity on a cellular resolution. The in-vivo dendrite regeneration assay in Drosophila challenges neurons to remodel extensively after all dendrites are severed by a 2-photon laser (Song et. al., 2012; Thompson-Peer et. al., 2016). The injured neurons can be tracked for several days, as the animal continues development, to investigate various characteristics of regeneration. By expressing and knocking out differentially expressed HDAC3 target genes, in regenerating Drosophila neurons, we should be able to conduct a large-scale screen to identify genes involved in neuroplasticity.

To test this hypothesis, we overexpressed human-*Baf53b* (*hBaf53b*) in peripheral nervous system neurons in Drosophila. *Baf53b* is a chromatin remodeling subunit, exclusively expressed in post-mitotic neurons, that is required for long-term memory, functional plasticity, and neuron morphology (Vogel et. al., 2013; Parrish et. al., 2006). Since *Baf53b* is required for functional plasticity, we predicted that overexpressing *hBaf53b* in regenerating neurons would enhance dendrite regeneration length and branch number in Drosophila. We found that *hBaf53b* overexpression in highly *stable* neurons does not enhance regeneration. However, expressing *hBaf53b* in highly *dynamic* neurons significantly and noticeably enhances morphology after injury, compared to wild-type regeneration. We imaged real-time WT dendrite dynamics, during development and regeneration, on a fine temporal and spatial resolution and found that dendrites extend and retract on the scale of tens-of-seconds. These results indicate that genes involved in functional plasticity are likely involved in some aspect of structural plasticity and validates our planned approach to screen through HDAC3-target genes for those mechanistically involved in how HDAC3 controls dendrite shape to regulate memory.

Poster #13 (Donohue et al.)

Latrophilin-2 Controls Topographic Assembly of Presubiculum Inputs in the Medial Entorhinal Cortex

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Brain circuits are comprised of distinct interconnected neurons that are assembled according to synaptic recognition molecules presented on defined pre- and post-synaptic sites. This cell-cell recognition process is mediated by varying cellular adhesion molecules, including the latrophilin family of adhesion G-protein coupled receptors (Lphn1-3; gene symbols *ADGRL1-3*). Recently, latrophilins have been shown to mediate excitatory synaptogenesis through trans-synaptic interactions with extracellular binding partners teneurins and FLRTs. Of the three latrophilin genes, Lphn2 demonstrates the most stringent expression patterning, and is the focus of this study. Here we sought to define cell type specific expression and Lphn2 protein localization within the parahippocampal circuit of interconnected subregions of the medial entorhinal cortex (MEC), presubiculum (PrS), and parasubiculum (PaS). In doing so, we find notable topographical expression patterning at both the protein and RNA level in all three of these interconnected regions. To study the role of Lphn2 in controlling the connectivity between these regions, we utilized a retrograde labeling approach. Performing dual-retrograde viral tracing from the Lphn2 deficient versus enriched compartments of the MEC reveals unique topographical patterning of inputs arising from the PrS and PaS which mirrors Lphn2 expression. We find that Lphn2 enriched compartments of these regions show strong connectivity, whereas Lphn2 deficient compartments of these regions show similarly enriched connectivity. Using this retrograde viral labeling method coupled with a Lphn2 conditional knock-out mouse model, we find that deletion of Lphn2 expression in the MEC selectively impairs labeling of inputs arising from the ipsilateral PrS. Combined with analysis of Lphn2 expression within the MEC, these data reveal Lphn2 as a molecule that is expressed by defined cell types, and selectively controls circuit development between interconnected regions.

Poster #14 (Ahn et al.)

Structure and Function Relationships of Interneurons in the Carnivore and Rodent Lateral Geniculate Nucleus of the Thalamus

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Local interneurons in the dorsal lateral geniculate nucleus (dLGN) of the thalamus influence every spike visual cortex receives; they provide feedforward inhibition to each other and to relay cells. Here, we focused on understanding structure/function relationships in inhibitory circuits in dLGN from a comparative perspective using species with good (carnivore) versus poor (rodent) visual acuity. Our anatomical studies were motivated by whole-cell recordings from thalamic interneurons in vivo. For carnivore interneurons, membrane currents were dominated by serial, unitary inhibitory postsynaptic currents, each of which was preceded by a depolarizing notch. These recordings suggested the presence of triadic circuits in which pairs of interneurons share input from the same retinal afferent and connect with each other via dendrodendritic synapses. By contrast, recordings from rodent interneurons exhibited trains of unitary excitatory postsynaptic currents, indicating different types of connectivity between species. To explore synaptic connections in inhibitory circuits, we first used transmission electron microscopy to analyze single sections of GABA-stained tissue of ferret and mouse. Our results revealed numerous dendrodendritic synapses between interneurons. To gain a fuller view of synaptic arrangements that include interneurons, we made 3D reconstructions using tissue prepared with serial block-face scanning electron microscopy. We found triads involving two interneurons in both species; however, there was great variation between species. First, there were substantial differences in the composition of synaptic glomeruli, the structures in which triadic circuits usually occur. In carnivore, glomeruli involved a large central retinal bouton surrounded by several interneuronal dendrites. Rodent glomeruli, conversely, comprised only few interneuronal dendrites encircled by small retinal boutons, and the interneurons that participated in triads also received input from multiple retinal terminals. In addition, in carnivore alone, we found interneuronal dendritic segments that did not receive retinal input but formed many connections with other interneuronal dendrites. Further, dendrodendritic synapses between interneurons were evenly distributed between shafts and appendages in carnivore but concentrated on appendages in rodent. These results suggest that there is a more powerful and flexible inhibition in carnivore versus rodent dLGN.

Poster #15 (Nie et al.)

Inference and Analysis of Cell-Cell Communication Using CellChat

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Understanding global communications among cells requires accurate representation of cell-cell signaling links and effective systems-level analyses of those links. We construct a database of interactions among ligands, receptors and their cofactors that accurately represent known heteromeric molecular complexes. We then develop CellChat, a tool that is able to quantitatively infer and analyze intercellular communication networks from single-cell RNA-sequencing (scRNA-seq) data. CellChat predicts major signaling inputs and outputs for cells and how those cells and signals coordinate for functions using network analysis and pattern recognition approaches. Through manifold learning and quantitative contrasts, CellChat classifies signaling pathways and delineates conserved and context-specific pathways across different datasets. Applying CellChat to mouse and human skin datasets shows its ability to extract complex signaling patterns. Our versatile and easy-to-use toolkit CellChat and a web-based Explorer (http://www.cellchat.org/) will help discover novel intercellular communications and build cell-cell communication atlases in diverse tissues.

Poster #16 (Hernandez et al.)

PRMT5 Regulates Colonic Goblet Cell and Tuft Cell Homeostasis and Promotes Tumorigenesis

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Arginine methylation is a common post-translational modification that is defined as the addition of methyl groups to protein arginine residues to form methylarginine. There are three main forms of methylarginine found in eukaryotes: monomethylarginine (MMA), asymmetric dimethylarginine (aDMA), and symmetric dimethylarginine (sDMA). PRMT5 is the type II enzyme responsible for the majority of sDMA catalysis. Through its arginine methylation activity, PRMT5 has been demonstrated to play critical roles in regulating chromatin accessibility, constitutive splicing activity, and translational fidelity in multiple tissue types. To study the biological roles of PRMT5 across multiple tissues, we are utilizing a PRMT5 heterozygous mouse line, in which there is only heterozygous expression of *Prmt5* throughout the body. We previously surveyed the global transcriptome of wildtype mouse colonic epithelial tissue through an RNA-seq experiment and found significantly high expression of PRMT5 relative to other members of the PRMT family, suggesting that PRMT5 plays particularly critical roles in colonic epithelia homeostasis. To further study the roles of PRMT5 in colonic epithelia, we characterized the colonic epithelia cell gene expression signatures of PRMT5 Het mice. We found reductions of the colonic goblet and tuft cell gene signatures in PRMT5 Het mice compared to WT, including reduced expression of the key goblet cell genes *Spdef* and *Clca1* and the key tuft cell genes *Dclk1* and *Pou2f3*. Colon organoids treated with a PRMT5 enzymatic inhibitor also show a reduction in *Clca1* and *Dclk1* expression, implicating an epithelial-intrinsic role of PRMT5 in goblet and tuft cell maintenance. Through histological examinations of fixed colon tissues, we found a reduction in goblet cell numbers in PRMT5 Het colons but no difference in tuft cell numbers, suggesting that PRMT5 regulates goblet cell differentiation but may only regulate tuft cell function. To further characterize goblet cell functional deficiencies in PRMT5 Het mice, we challenged them with *Citrobacter rodentium* and found that PRMT5 Het mice have reduced clearance of bacterial loads compared to WT mice. Since *Dclk1* expression correlates with colorectal cancer initiation and progression, we questioned whether PRMT5 Het mice would have reduced colorectal tumor loads. We crossed the PRMT5 Het mouse line with the $APC^{fl/fl}$ $CDX2^{Cre}$ mouse line to generate a colorectal cancer tumor model in a PRMT5 Het background. We found that PRMT5 Het mice developed fewer colorectal tumors compared to WT, implicating PRMT5 in colorectal cancer development, perhaps through modulation of *Dclk1* expression. Overall our data demonstrates that PRMT5 plays important roles in colonic goblet cell and tuft cell differentiation or function as well as colorectal tumorigenesis.

Poster #17 (Jun et al.)

Disrupted Place Cell Remapping and Impaired Grid Cells in a Knockin Model of Alzheimer's Disease

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Patients with Alzheimer's disease (AD) suffer from spatial memory impairment and wandering behavior, but the brain circuit mechanisms causing such symptoms remain largely unclear. In healthy brains, spatially tuned hippocampal place cells and entorhinal grid cells exhibit distinct spike patterns in different environments, a circuit function called “remapping.” We tested remapping in amyloid precursor protein knockin (APP-KI) mice with impaired spatial memory. CA1 neurons, including place cells, showed disrupted remapping, although their spatial tuning was only mildly diminished. Medial entorhinal cortex (MEC) neurons severely lost their spatial tuning and grid cells were almost absent. Fast gamma oscillatory coupling between the MEC and CA1 was also impaired. Mild disruption of MEC grid cells emerged in younger APP-KI mice, although the spatial memory and CA1 remapping of the animals remained intact. These results point to remapping impairment in the hippocampus, possibly linked to grid cell disruption, as circuit mechanisms underlying spatial memory impairment in AD.

Poster #18 (Tsai et al.)

Trans-Seq Decodes a Specific Mammalian Retinotectal Synapse Assembled by Nephronectin

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Transsynaptic tracers have served as powerful tools for neural circuit reconstruction. Though retrograde tracers such as the pseudo-typed rabies virus are available to map the pre-synaptic inputs of a target neuronal population, there is a lack of efficient mono-synaptic anterograde tracers to support mapping of post-synaptic outputs. Our ultimate goal is to establish a genetically tractable system to define the outputs of a given genetically- or regionally defined 'starter' neuron in the mammalian nervous system. To address this gap in the circuit mapping toolbox, we have engineered anterograde fluorescent trans-synaptic tracers based on wheat-germ-agglutinin (WGA) fused to the fluorescent protein mCherry. Our WGA-mCherry fusion significantly improves trans-synaptic tracing efficiency in mammalian neurons over existing WGA configurations and includes the following features: (1) It is genetically encoded as a single component to facilitate adeno-associated viral (AAV) delivery into the mammalian nervous system; (2) it comprehensively labels the mono-synaptic partners of a given starter neuron in an efficient and unbiased manner; and (3) it is coupled to a fluorescent protein for direct visualization without immunostaining. Moreover, we were able to establish an AAV with Cre-dependent WGA-mCherry for mapping outputs from neuronal Cre-driver lines. By coupling the WGA-mCherry tracer to single-cell RNA sequencing, we created a platform, which we call Trans-Seq, that both identifies and comprehensively profiles the neuronal 'outputome', or post-synaptic outputs at the bioinformatic level. The Trans-Seq workflow is straightforward and involves a single injection of the tracer before isolating connected neurons based on mCherry fluorescence for submission to single-cell RNA sequencing. We demonstrated the utility of Trans-Seq by reconstructing the retinal ganglion cell (RGC) outputomes to the superior colliculus with RGC subtypes as inputs. For this, we compared the outputomes from all RGCs, alpha-RGCs, and On-Off direction-selective ganglion cells (ooDSGCs) using Trans-Seq. We validated our outputome data from Trans-Seq using immunohistochemistry and in situ hybridization. From this, we found a set of bona fide molecular markers for the excitatory neurons within the superficial superior colliculus consisting of wide-field, narrow-field, and stellate cells. Based on these validated markers, we generated and collected a new set marking lines for superior collicular neuron subtypes. Differential gene expression analysis from parallel retinal neuron outputomes (alpha-RGCs vs. ooDSGCs) furthermore predicted a unique neuronal pair from αRGCs to Nephronectin-positive wide-field neurons (NPWFs) and revealed distinct molecular candidates for selective circuit wiring from the retina to the superior colliculus. We validated the αRGC to NPWF connection using genetic labeling, electrophysiology, and retrograde tracing. We then utilized transcriptomic data from Trans-Seq to identify Nephronectin as a determinant for selective synaptic choice from αRGC to NPWFs via binding to Integrin-α8β1. We envision the Trans-Seq platform to be widely applicable for precise circuit discovery, revealing new principles for synaptic specificity and circuit assembly in the mammalian brain.

Poster #19 (Lin et al.)

Non-Canonical Projections from Ventral Hippocampal CA1, Subicular Complex and Perirhinal Cortex to Dorsal Hippocampal CA3 Augment the Feedforward Hippocampal Trisynaptic Pathway

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The hippocampal formation is well documented as having a feedforward, unidirectional circuit organization termed the trisynaptic pathway. This circuit organization exists along the septotemporal axis of the hippocampal formation, but the circuit connectivity across septal to temporal regions is less well described. The emergence of viral-genetic mapping techniques enhances our ability to determine the detailed complexity of hippocampal formation circuitry. In earlier work, we mapped a subiculum back-projection to CA1 prompted by the discovery of theta wave back-propagation from the subiculum to CA1 and CA3. We reason that this circuitry may represent multiple extended non-canonical pathways involving the subicular complex and hippocampal subregions CA1 and CA3. In the present study, multiple retrograde viral tracing approaches produced robust mapping results, which supports this prediction. We find significant non-canonical synaptic inputs to dorsal hippocampal CA3 from ventral CA1, perirhinal cortex, and the subicular complex. Thus, CA1 inputs to CA3 run opposite the trisynaptic pathway and in a temporal to septal direction. Our retrograde viral tracing results are confirmed by anterograde-directed viral mapping of projections from input mapped regions to hippocampal dorsal CA3. We find that genetic inactivation of the projection of ventral CA1 to dorsal CA3 impairs object-related spatial learning and memory but does not modulate anxiety-related behaviors. Our data provide a circuit foundation to explore novel functional roles contributed by these non-canonical hippocampal circuit connections to hippocampal dynamics and behavior.

Poster #20 (Wei et al.)

Molecularly and Functionally Investigate the Parallel Pathway from the Retina to the Brain

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Vision, accounting for more than 70% of all our sensory inputs, processes the information flow from retina to the primary visual cortex (V1) through the visual thalamus, the dorsal lateral geniculate nucleus (dLGN). More than 30 subtypes of retinal ganglion cells (RGCs) convey distinct visual features (such as luminance, contrast, orientation or direction) through the dLGN to the rest of the brain. In primates, it has been well characterized that midget, parasol and bistratified retinal ganglion cells project to separated layers of LGN and on to different layers of V1. However, how the recipient thalamic cells preserve and recapitulate these separate retinal channels in a parallel manner remains unclear at the circuit level. Recent advancement in mouse genetics allows us to label and manipulate different subtypes of RGCs and trace their projections from the retina to the brain. One subtype of RGCs, On-Off direction-selective ganglion cells (ooDSGCs), synapse on dLGN neurons located in the superficial dorsolateral region (referred as “dLGN Shell” hereafter), thereby relaying direction information from ooDSGCs to the superficial layers of V1, such as layers 2/3. In contrast, several other subtypes of non-direction-selective ganglion cells such as αRGC, mainly project to the deeper dLGN (referred as “dLGN Core” hereafter), conveying non-direction selective visual information to the deeper layers of V1 such as layer 4. Whether the dLGN neurons are molecularly and functionally heterogeneous, and whether different types of dLGN neurons receive distinct retinal inputs contributing to the parallel pathways in the early visual system are largely unknown.
First, we used a “Patch-seq” method (performing single-cell RNAseq after electrophysiological patch-clamp recording) in the dLGN to correlate functional properties with transcriptional profiles. I expressed channelrhodopsin-2 (ChR2) in specific RGC-Cre lines and photostimulate the ChR2-containing axonal terminals in ex-vivo brain slices specifically. Following electrophysiological recording, the interior content of the identified dLGN neuron was aspirated into the patch pipette and harvested for single-cell RNAseq. We found that different RGCs’ recipient neurons showed laminar preferences in the dLGN, and convergency in the Core region. Second, we have optimized an genetically-encoded wheat germ agglutinin (WGA)-mCherry with high anterograde trans-synaptic trafficking efficiency to investigate the global connectivity from retina to dLGN though a high throughput manner. Since the excitatory dLGN neurons are not interconnected, the retinogeniculate circuitry provides an excellent opportunity to validate the monosynaptic transfer efficiency and specificity of WGA exclusively. mCherry can be used for fluorescence-activated cell sorting (FACS) to collect postsynaptic neurons for single-cell RNAseq. We applied this tracer in available RGC-Cre driver lines, and the postsynaptic mCherry+ dLGN neurons will be collected through FACS automatically. Different populations of dLGN neurons with distinct retinal inputs were collected and will be sequenced using SMARTer single cell RNA-seq platforms.

Poster #21 (Boldrini et al.)

Single-Nucleus and Spatial Transcriptomics of Human Hippocampus

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In mammals, adult hippocampal neurogenesis (AHN) is necessary for cognitive and emotional functions. We reported that the human hippocampus neurogenic niche of normal aging subjects with no cognitive impairment showed no age-associated decline in progenitor (SOX2/nestin+) cells and immature (doublecortin[DCX]/PSA-NCAM+) neurons into the eighth decade of life, despite a smaller multipotent (SOX2+) progenitor pool in older subjects. Sustained AHN in human hippocampus has been replicated by other groups. Although, other studies did not detect immature neuron markers in adult dentate gyrus (DG). therefore, if AHN exists in human remains debated. Gaps in knowledge, and differences between human and lower mammals' brain, warrant using new technologies to investigate cellular lineages in the human neurogenic niche, and molecular regulators of progenitor cell proliferation, differentiation, and maturation.

In homogenized DG-hilus tissue, we implemented single nuclei (sn) RNA sequencing (seq). In slide-mounted hippocampus tissue, we applied our custom-made slide-seq technology, using deterministic barcoding in tissue for spatial omics sequencing (DBiT-seq). These technologies provide complementary information on the human hippocampus neurogenic niche: they quantify single nuclei RNA expression (snRNA-seq) and provide anatomical co-mapping of cell-type specific differentially expressed genes on intact tissue sections (DBiT-seq), where whole cells and their connections are preserved.

To investigate human hippocampus neurogenic niche molecular features, we applied snRNA-seq and DBiT-seq to hippocampus postmortem tissue from four non-psychiatric subjects who had negative toxicology for psychotropic drugs and alcohol and were clinically characterized using psychological autopsy and neuropathology assessment, age 20-75 years, with sudden death, short agonal state, postmortem interval<24 hrs, RNA integrity number (RIN)>8, and brain tissue pH>6.

Our snRNA-seq and DBiT-seq data segregate human hippocampus cell populations based on gene expression. Spatially resolved cell clusters localize in known hippocampus subfields, including granule cell layer, subgranular zone, molecular layer, and Cornu Ammonis regions, and express genes that are expected to be associated with the specific cell population.

Differentially expressed genes in cells located in the DG neurogenic niche, or subgranular zone, included: PTPRT (p=6.44*E-95), involved in cell growth, differentiation, and mitotic cycle; GAD2 (p=1.50*E-94), involved in production of gamma-aminobutyric acid; SOX1 (p=4.72*E-11) involved in the regulation of embryonic development and in cell fate determination; L1CAM (p=1.56*E-05), with a role in nervous system development, neuronal migration and differentiation; RELN (p=1.38*E-46), involved in cell migration and adhesion; RSN1(p=1.73*E-65), involved in neurite extension and memory consolidation.

In the DG granule cell layer, top differentially expressed genes include PROX1 (p=1.65*E-149), transcription factor involved in cell fate determination, embryonic development and neurogenesis; CALB1 (p=1.18*E-52), calcium-binding protein thought to buffer entry of calcium upon stimulation of glutamate receptors, MAP2 (p=9.13*E-30), neuron-specific cytoskeletal protein enriched in dendrites, with a role in determining and stabilizing dendritic shape during neuron development.

Proper taxonomy of cell type in the brain requires integration of single cell and spatial transcriptomic approaches. Here we demonstrate that DBiT-seq allows identifying, in human hippocampus tissue, cell clusters showing molecular features and anatomical distribution that reflect their phenotype and function, as well as discovering molecular markers that regulate cell activity and viability.

Poster #22 (Englund et al.)

A Common Reference Frame for Comparing Cortex-Wide Gene Expression Between Species

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Advances in sequencing techniques have made comparative studies of gene expression a recent focus for understanding evolutionary and developmental processes. These studies have provided invaluable information about species and cell-type specific gene expression in terms of differences in gene sequence and level of expression. Yet quantitative comparisons of spatial patterns of gene expression have been extremely limited due to a lack of robust methodology. Thus, we developed a set of algorithms for quantifying and comparing spatial gene expression within and across species, which we refer to as *Stalefish, The Spatial Analysis of Fluorescent (and non-fluorescent) In-Situ Hybridization*. Here, we apply these algorithms to quantify cortex-wide expression of Id2 and RZRb mRNA in early postnatal mice and voles to determine the extent to which spatial patterns of expression of these genes is conserved between species. We found that Id2 is moderately conserved across the neocortex in mice and voles, but the extent of this conservation varies by layer and area (e.g. high conservation in caudal neocortex). On the other hand, RZRb expression is tightly conserved in somatosensory cortices (S1 and S2) between species, while expression in visual (V1 and V2) and auditory (A1) areas vary. We propose that these differences reflect independent evolution of brains, bodies and sensory systems in the 35 million years since their last common ancestor. We believe scientists across fields will use this newly developed tool to quantify spatial patterns of gene expression (or other histological markers) to address differences between species, developmental time-points, or experimental conditions.

Poster #23 (Chen et al.)

Hippocampal Neural Ensemble Representations of Spatial Mapping in Freely Behaving Mice

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An emerging theory about neuronal organization of hippocampus CA1 area is that CA1 pyramidal neurons may formulate parallel cell assemblies that simultaneously processing different types of memories (Soltesz & Losonczy, 2018). However, studies about the functional and anatomical profiles of these potential organization scheme are still inconclusive. In the present study, we address spatiotemporal ensemble representations in hippocampal CA1 during behavior tasks through in vivo Ca++ imaging using head-mounted miniature microscopes and divide the neural activity into specific activation groups that share similar calcium event patterns in the temporal domain. Interestingly, when correlating the identity of these “temporal clusters” to the anatomical location of neurons, neurons in the same "temporal cluster" are locally concentrated and form anatomical clusters to a degree well beyond chance. At the same time, these temporal clusters are dynamic across time and between different environments. Between different periods of a single trial, the neuron composition of temporal clusters evolves somewhat with time. Across days and environments, temporal and anatomical clustering is more dynamic, but with significant overlap for the same shaped environments. We believe that this representational topography in hippocampal sub-region CA1 reveals a previously less discussed in temporal and spatial dynamics according to neuron groupings in anatomical space

Soltesz, I., & Losonczy, A. (2018). CA1 pyramidal cell diversity enabling parallel information processing in the hippocampus. *Nature Neuroscience, 21*(4), 484-493. doi:10.1038/s41593-018-0118-0

Poster #24 (Lee et al.)

Dopamine Facilitates Associative Memory Coding in the Lateral Entorhinal Cortex

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Dopamine plays a central role in motivation and reward. Accumulating evidence shows that dopaminergic inputs in the ventral tegmental area (VTA)-striatum circuit are critically involved in reward-based learning. However, it remains unclear how dopamine reward signals control the entorhinal-hippocampal circuit, another brain network critical for learning and memory. Here we show that dopaminergic inputs from the VTA/substantia nigra to the lateral entorhinal cortex (LEC) are critical for cue-reward associative memory. Optogenetic inhibition of LEC dopamine inputs, or inhibition of LEC layer 2a fan cells, impaired the learning of olfactory cue-reward association, although the retrieval of pre-learned memory was spared. Photometry recordings revealed that LEC dopamine transmits novelty-induced reward expectation signals. Using optogenetic-assisted electrophysiological recording during associative learning, we found a cue-reward association coding of LEC fan cells, where representations of newly learned rewarded cues gradually overlapped with that of a pre-learned rewarded cue while separating them from the representation of a pre-learned unrewarded cue. This coding of LEC fan cells was disrupted by the inhibition of LEC dopamine inputs. These results indicate that dopamine functions as a powerful memory gatekeeper for the entorhinal cortex to encode cue-reward associative memory, pointing to the involvement of the entorhinal cortex in dopamine-mediated reinforcement learning.

Poster #25 (Bouin et al.)

Design of New Rabies Virus-Based Bright Fluorescent Reporters that Target to Specific Subcellular Locations

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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 our work 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. Using the genome of the rabies vaccine strain, SAD B19, we developed multiple recombinant rabies viruses (RABV) that encode bright fluorescent proteins along with subcellular localization signals that replace the viral glycoprotein (G). These engineered viruses cannot spread from initially infected cells, as they do not encode the rabies glycoprotein and thus can be safely used under BSL2 conditions. To propagate these modified viruses, they are cultured in cell lines stably expressing the viral G glycoprotein. Plasmids encoding the recombinant viral genome were co-transfected with vectors that express the viral N, P, L and G proteins.

We have generated a series of novel spectrally distinguishable recombinant RABV that express blue (EBFP), teal (mTFP1), turquoise (mTurquoise2), green (mNeonGreen), red (tdTomato), and far-red (smURFP) reporters. Among the fluorescent reporters, mNeonGreen and tdTomato are brighter, longer-lasting than others previously described, and they do not quench as easily compared to traditional GFP and RFP (DsRed and mCherry). We also engineered a range of reporters that localize to different sub-cellular compartments, including the cytoplasm, nucleus, cellular membranes, somatodendritic, and pre- and post-synaptic locations. Nuclear-localized tdTomato allows for fast and accurate quantification using automated cell counting software. PSD95-targeted virus can be used to study dendritic spines, and synaptophysin-targeted virus can be used to study axon synaptic terminals.

We used *in vitro* cell culture assays to assess the fluorescence and localization of the reporters in B7GG cells and produced viral stocks. To determine if our modified recombinant rabies viruses perform as they were designed, C57BL/6J mice were injected with recombinant RABV intracranially. At eight days post-injection, mice were perfused and brains were collected and prepared for imaging. A bright and localization-specific fluorescent signal was detected in infected neurons, allowing for sensitive and precise monosynaptic tracing. The use of specific localization signals did not increase viral toxicity in injected mice.

In summary, our newly developed recombinant RABVs offer a range of sub-cellular targeted fluorescent reporters that facilitate a wide repertoire of cell-type specific and circuit specific mapping studies that are amenable to automated computer analysis. We plan to expand these reporters for electron-microscopy detectable markers to study ultrastructure and cell-cell interactions. Ongoing studies are aimed at attenuating viral toxicity *in vivo*.

Poster #26 (Patino et al.)

Transcriptomic Identification of Input Cells to Defined Neuronal Populations with Single Transcriptome Assisted Rabies Tracing (START)

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The complex functions of the mammalian neocortex rely on neuronal networks of interconnected glutamatergic excitatory neurons and GABAergic inhibitory interneurons. Many studies have begun to explore the diversity of cortical neurons, their global and microcircuit connectivity patterns, and their role in cortical information processing. However, detailed understanding of the specialized connectivity patterns underlying cortical networks remail largely incomplete and many aspects of cortical circuit organization remain unknown. Here we present a method, Single Transcriptome Assisted Rabies Tracing (START), which combines monosynaptic rabies tracing and single nuclei RNA sequencing (snRNA-seq) to identify the transcriptomic cell types that provide presynaptic inputs to defined populations of neurons. To establish whether rabies infected neurons could be classified according to recognized transcriptomic cortical cell types we conducted transcriptomic cluster analysis of 10,000 rabies infected neurons from mouse primary visual cortex and identified the correspondence between these clusters and established transcriptomic clusters from a dataset of 9,000 uninfected neurons from mouse primary visual cortex. We found that despite global and cell type specific rabies infection induced transcriptional changes, using integrative computational analysis, rabies infected and uninfected cells grouped together by established cell types. These results suggest that these computational strategies can be utilized to overcome gene expression differences induced by rabies infection and allow for transcriptomic characterization of input cells in monosynaptic rabies tracing circuit studies.

Poster #27 (Smith et al.)

Regional Synapse Gain & Loss Accompany Memory Formation in Larvalzebrafish

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Changes in neuronal activity that occur following memory formation have been characterized; however, structural changes at the synapse level that drive functional changes remain poorly understood. Here, we image large scale synaptic changes in the brain of a midlarval zebrafish during associative memory formation using selective plane illumination microscopy to map spatial patterns of synaptic change in an unbiased manner. Following associative memory formation, we detect an increase in the number of synapses in the ventrolateral pallium, which contains neurons that are active during memory formation and retrieval. Concurrently, there is a predominant loss of synapses in the dorsomedial pallium. Surprisingly, we did not detect long term changes in the intensity of synaptic labeling, a proxy for synaptic strength, with memory formation in any region of the pallium. Our results suggest that memory formation due to classical conditioning in this context is associated with reciprocal changes in synaptic distributions in medial and lateral regions of the pallium.

Poster #28 (Zhang et al.)

Epigenomic Diversity of Cortical Projection Neurons in the Mouse Brain

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Neuronal cell types are classically defined by their molecular properties, anatomy, and functions. While recent advances in single-cell genomics have led to high-resolution molecular characterization of cell type diversity in the brain, neuronal cell types are often studied out of the context of their anatomical properties. To better understand the relationship between molecular and anatomical features defining cortical neurons, we combined retrograde labeling with single-nucleus DNA methylation sequencing to link neural epigenomic properties to projections. We examined 11,827 single neocortical neurons from 63 cortico-cortical (CC) and cortico-subcortical long-distance projections. Our results revealed unique epigenetic signatures of projection neurons that correspond to their laminar and regional location and projection patterns. Based on their epigenomes, intra-telencephalic (IT) cells projecting to different cortical targets could be further distinguished, and some layer 5 neurons projecting to extra-telencephalic targets (L5 ET) formed separate clusters that aligned with their axonal projections. Such separation varied between cortical areas, suggesting area-specific differences in L5 ET subtypes, which were further validated by anatomical studies. Interestingly, a population of CC projection neurons clustered with L5 ET rather than IT neurons, suggesting a population of L5 ET cortical neurons projecting to both targets (L5 ET+CC). We verified the existence of these neurons by dual retrograde labeling and by anterograde tracing of CC projection neurons, which revealed axon terminals in ET targets including thalamus, superior colliculus, and pons. These findings highlight the power of single-cell epigenomic approaches to connect the molecular properties of neurons with their anatomical and projection properties.

Abstract (Mendelsohn et al.)

Genetic Dissection of Basal Ganglia Output Circuits

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Basal ganglia circuits are key regulators of movement, cognition, motivational behavior, sensory processing and homeostasis and dysregulation in basal ganglia circuits has been implicated in multiple neuropsychiatric disorders. Sensorimotor, associative and limbic basal ganglia pathways are grossly anatomically segregated, but have yet to be dissected or interrogated on the basis of genetically defined cell types. It thus remains unclear to what degree and in what manner distinct basal ganglia subcircuits modulate different aspects of brain function.

To genetically dissect basal ganglia pathways controlling behaviorally divergent circuits, we focused on the primary output nucleus of the basal ganglia, the substantia nigra pars reticulata (SNr). We reasoned that that the SNr is likely to contain genetically defined subtypes controlling parallel functional streams because SNr neurons are topographically organized and display electrophysiologic specialization based on projection target.

We first confirmed the topographic organization of SNr output circuits using retrograde viral tracing and automated brain mapping techniques. We then profiled SNr subpopulations based on their projections to six downstream targets using single nucleus RNA-sequencing. We found that SNr neurons can be genetically distinguished based on their projections to distinct midbrain or hindbrain targets.

These results suggest that the SNr represents a locus within the basal ganglia in which behaviorally divergent circuits can be molecularly distinguished. We plan to first use viral tracing, brain clearing and automated brain mapping techniques to define the anatomical organization of genetically defined SNr subpopulations. We then plan to examine the SNr's differential involvement in motor behaviors such as orofacial movement, forelimb and hindlimb control, escape and locomotion by recording activity from genetically defined SNr populations during naturalistic behavior.

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Michele Wu
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