Colorado Neuroscience Symposium

TECHNOLOGIES TRANSFORMING NEUROSCIENCE

Symposium Program

Brought to you by The Rocky Mountain Regional Neuroscience Group, a local chapter of the Society for Neuroscience

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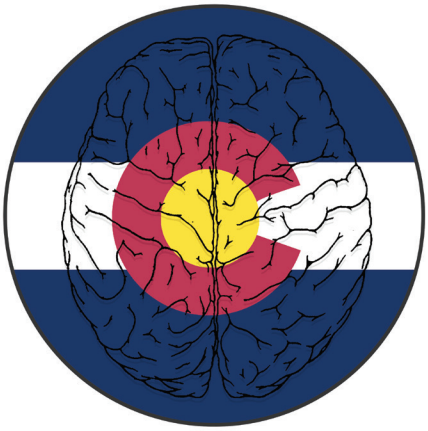
Colorado Neuroscience Symposium



All events in Education 1 Room 1500 unless otherwise noted

8:00-8:45 - **Undergraduate Welcome Breakfast**
Education 2 South Bridge (Room 2001)

8:00 - 9:00 Registration Open
Hallway outside Education 1 Room 1500

9:00 - 10:30 Session #1

Trainee talk: **Dillon McGovern**
"Recording in-vivo glutamate and GABA dynamics utilizing recently-engineered genetically encoded fluorescent indicators: SF-iGluSnFR and iGABASnFR"

Faculty talk: **Chandra Tucker, Ph.D.**
"Optogenetic tools to map and modulate neuronal circuitry and function"

Keynote talk: **Lin Tian, Ph.D.**
"Watching brain in action: creating tools for functional analysis of neural circuitry"

10:30 -10:40 Coffee Break

10:40 - 12:00 Session #2

Trainee talk: **Alex Hughes**
"Developmental myelin sheath elimination by microglia"

Faculty talk: **Matthew Taliaferro, Ph.D.**
"Decoding the regulatory language of RNA localization"

Keynote talk: **John Ngai, Ph.D.**
"Illuminating Stem Cell Trajectories and Cellular Diversity in the Nervous System"

12:00- 1:30 Lunch and Poster session in Education 2 Bridge

1:40 - 3:00 Session #3

Trainee talk: **Greg Futia**
"In vivo holographic photo-stimulation and two photon GCaMP6 imaging of vagus nerve axons"

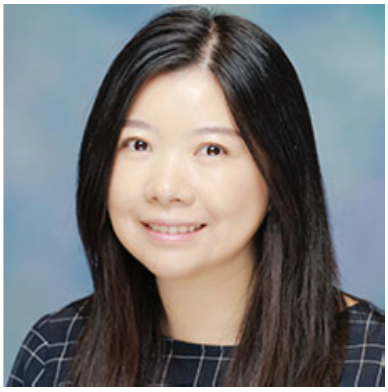
Faculty talk: **Cristin Welle, Ph.D.**
"The living interface: neurotechnology drives structural and functional plasticity in the nervous system"

Keynote talk: **John Rogers, Ph.D.**
"Soft Optoelectronic and Microfluidic Systems for Neuroscience Research"

3:00 - 4:00 Keynote Speaker Panel & Awards

4:00 - 5:00 Happy Hour at Ursula Brewery

Keynote Speakers:



After graduating from University of Science and Technology of China, Dr. Tian joined a interdisciplinary PhD program at Northwestern University, where she studied the mechanisms of protein processing via ubiquitin-proteasome pathway in Dr. Andreas Matouschek's lab. she then moved to HHMI Janelia Farm as a postdoc. The highly collaborative environment at Janelia resulted in multidisciplinary training under three principle investigators, Dr. Loren Looger, Dr. Karel Svoboda and Dr. Luke Lavis. There, her research focused on engineering optical probes for monitoring and controlling neural circuitry in living behaving animal. The imaging techniques developed there have greatly impacted the field of neuroscience, facilitating new types of biological experiments that address previously intractable questions. She started at UC Davis in 2012 where her lab has continued to develop novel biosensors to explore neural interactions.

John Ngai, Ph.D. is the Coates Family Professor of Neuroscience and Director of the QB3 Functional Genomics Laboratory at the University of California, Berkeley. He received his B.A. in Chemistry and Biology from Pomona College and his Ph.D. in Biology from the California Institute of Technology. Following a postdoctoral fellowship at Columbia University, in 1993 Dr. Ngai joined the faculty at UC Berkeley, where he currently studies the regulation of adult neural stem cells and the diversity of neuronal cell types in the brain using molecular, genetic, and genomics approaches. Dr. Ngai was previously Head of the Neuroscience Graduate program and Director of the Helen Wills Neuroscience Institute at UC Berkeley.

Professor John A. Rogers is the Louis Simpson and Kimberly Querrey Professor of Materials Science and Engineering, Biomedical Engineering and Medicine at Northwestern University, with affiliate appointments in Mechanical Engineering, Electrical and Computer Engineering and Chemistry, where he is also Director of the newly endowed Center for Bio-Integrated Electronics. He has published more than 650 papers, is a co-inventor on more than 100 patents and he has co-founded several successful technology companies. His research has been recognized by many awards, including a MacArthur Fellowship (2009), the Lemelson-MIT Prize (2011), and the Smithsonian Award for American Ingenuity in the Physical Sciences (2013) – and most recently the Benjamin Franklin Medal from the Franklin Institute (2019). He is a member of the National Academy of Engineering, the National Academy of Sciences, the National Academy of Inventors and the American Academy of Arts and Sciences.

Local Faculty Speakers

Chandra Tucker

Chandra Tucker is an Associate Professor of Pharmacology at the University of Colorado School of Medicine. Dr. Tucker received her PhD in Biochemistry from the University of Washington, working with Dr. James Hurley to study signal transduction in mammalian photoreception. She carried out postdoctoral work with Dr. Stanley Fields at the University of Washington in yeast genetics and technology development. Her research focuses on developing novel optogenetic tools for probing cellular function and protein interactions. She pioneered the use of the photoreceptor cryptochrome as an optogenetic tool, and has developed systems to modulate protein-protein interactions and protein oligomeric state with light.

Matthew Taliaferro

Dr. Taliaferro received his PhD in Molecular and Cell Biology from the University of California, Berkeley in 2012, completed an NIH-funded postdoctoral fellowship at MIT, then started his lab at the University of Colorado Anschutz Medical Campus in 2017. He has primarily explored the regulation of gene expression through post-transcriptional mechanisms with a focus on alternative splicing and subcellular RNA localization. Recent work has focused on how specific RNA transcripts are trafficked to distinct subcellular locations.

Cristin Welle

Dr. Cristin Welle is an Associate Professor in University of Colorado Departments of Neurosurgery and Physiology & Biophysics faculty, where she investigates circuit-level structure and function in the context of translational neurotechnology. Using chronic in vivo electrophysiology, in vivo multiphoton imaging and advanced histological techniques, her lab examines the electrode/tissue interface of high-density recording electrodes for brain-computer interface systems. In addition, the lab is working to assess the potential for peripheral neuromodulation to influence cortical plasticity. Prior to her time at the University of Colorado, she spent five years as the principal investigator of the Neural Implant Lab in the Division of Biomedical Physics, Center for Devices and Radiological Health, Food and Drug Administration, exploring safety and performance of invasive neural recording electrodes used in neuroprosthetic systems and novel electrode technology for the detection of traumatic brain injury.

Trainee Speaker Abstracts

RECORDING IN-VIVO GLUTAMATE AND GABA DYNAMICS UTILIZING RECENTLY-ENGINEERED GENETICALLY ENCODED FLUORESCENT INDICATORS: SF-IGLUSNFR AND IGABASNFR

Dillon J McGovern, Alysabeth G Phillips, David H Root

University of Colorado, Boulder

Genetically encoded fluorescent indicators have advanced our understanding of how distinct brain structures and cell-types signal different aspects of motivated behavior. However, due to the slow timescale of neurotransmitter-identifying methods (i.e., microdialysis), identifying how specific neurotransmitters underlie neuronal activity dynamics during motivated behaviors has remained challenging. We utilized two recently-engineered genetically encoded fluorescent indicators to record the dynamics of glutamate (SF-iGluSnFR) and GABA transmission (iGABASnFR), in multiple brain regions and tasks. The present work demonstrates differential neurotransmitter activity in the lateral habenula, ventral pallidum, and ventral tegmental area across rewarding and aversive tasks. We first recorded from LHb neurons that are well-characterized to increase firing by aversive cues and stimuli and decrease firing by rewarding cues and stimuli. Preliminary data suggests LHb glutamate transmission is decreased following the presentation of a reward-paired cue and the consumption of sucrose reward. LHb glutamate transmission is increased by presentation of a shock-paired cue followed by a scalar increase in glutamate transmission until the offset of shock. After shock cessation, a rapid and temporally constrained decrease in glutamate was observed. We next recorded glutamate and GABA neurotransmission onto VTA glutamate neurons. We find that reward decreases both glutamate and GABA transmission onto VTA glutamate neurons. In contrast, footshock increases glutamate transmission, while simultaneously decreasing GABA transmission onto VTA glutamate neurons. We conclude that these novel neurotransmission-sensors rapidly extend our understanding of how the firing patterns or calcium-related neuronal activity patterns of discrete brain structures and specific cell-types dynamically signal different motivated behaviors.

DEVELOPMENTAL MYELIN SHEATH ELIMINATION BY MICROGLIA

Alexandria N Hughes[1], Bruce Appel[2]

[1]Neuroscience Graduate Program and [2]Department of Pediatrics, University of Colorado Anschutz Medical Campus

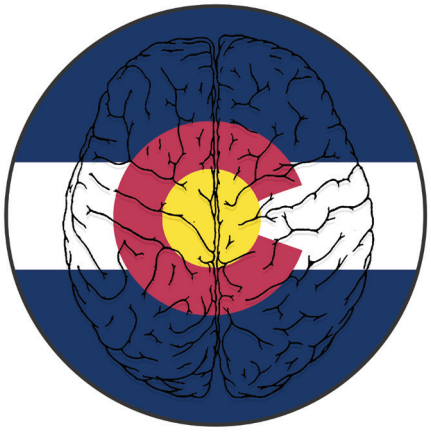
Oligodendrocytes ensheathe neuronal axons with myelin, a proteolipid-rich membrane that increases conduction velocity and provides trophic support. During development, oligodendrocytes form myelin sheaths on axons but some sheaths are later eliminated. What is the mechanism of sheath elimination? We recently found that myelin sheath formation is mediated by synaptic-like structures in axons and nascent oligodendrocyte sheaths. Because of this similarity between myelin sheath formation and synaptogenesis, we hypothesized that myelin sheath elimination also is similar to synaptic pruning. To test whether microglia eliminate developing myelin sheaths, we use zebrafish to combine pharmacological and genetic manipulations of cell and gene function paired with live imaging of microglia-oligodendrocyte-neuron interactions. We have found that microglia in the spinal cord frequently contact and engulf some nascent myelin sheaths but withdraw from others. By depleting microglia during myelination, we observed that oligodendrocytes form both excessive and ectopic myelin sheaths, consistent with a role for microglia in regulating appropriate myelination. How do microglia target specific myelin sheaths for elimination? We are currently testing the possibility that neuronal activity directs microglia to eliminate sheaths formed on less active neurons by sparsely silencing spinal cord neurons with botulinum toxin (BoNT/B). Our data support a model by which microglia-oligodendrocyte-neuron interactions regulate myelin sheath number and appropriate axon targeting during development.

IN VIVO HOLOGRAPHIC PHOTO-STIMULATION AND TWO PHOTON GCAMP6 IMAGING OF VAGUS NERVE AXONS

Gregory L. Futia[1], Arjun Fontaine[1], Samuel Littich[1], Connor McCullough[1], Diego Restrepo[2,3], Richard Weir[1], John Caldwell[2,3], and Emily A. Gibson[1,3]

[1]Department of Bioengineering, [2]Department of Cell & Developmental Biology, and [3]Neuroscience Program, University of Colorado Denver, Anschutz Medical Campus

Vagus nerve interfacing is of interest due to its central role in parasympathetic regulation of the visceral organs, as well as its modulatory effects on the brain, which have been shown to influence epilepsy, depression and migraines. Electrical vagus nerve stimulation (VNS) has shown therapeutic effect in humans, yet the mechanisms for these effects are unknown. Additionally, VNS lacks the specificity for controlling and studying targeted pathways. In contrast, optical interfacing techniques may enable axon-specific neuromodulation using genetically targeted opsin expression and spatial patterning of the photo-stimulus. In addition, calcium-sensitive fluorescent reporters such as GCaMP6 present a pathway for axon-specific optical recording of activity. We demonstrate in vivo photo-stimulation and two-photon GCaMP6 fluorescence imaging in the vagus nerve using a custom GRIN lens-coupled nerve cuff in the anesthetized mouse. A pulsed near-IR laser (1040 nm) was modulated by a spatial light modulator (SLM) in the Fourier plane and focused by the microscope objective through a GRIN relay lens to the cervical vagus nerve. By actuating the SLM, spatially selected regions could be differentially stimulated within the nerve. Mouse vitals were monitored with a MouseOx suite to detect physiological changes in response to photo-stimulation patterns. We measured differential modulations of heart rate, respiratory rate, and blood-oxygen saturation upon photo-stimulation of selective spatial regions of the nerve. Additionally, we recorded two-photon GCaMP6 Ca2+ transients in vagal axons in response to both photo-stimulation and electrical stimulation.



Poster Abstracts

*alphabetically by presenting author (underlined)

1. CONDUCTIVE 3D SCAFFOLDS FOR NEURONAL TISSUE REGENERATION

Nuria Alegret[1,2,3], Antonio Dominguez-Alfaro[1,2], Jose M. González-Domínguez[4,5], Blanca Arnaiz[1], Unai Cossío[1], Susanna Bosi[6], Ester Vázquez[4], David Mecerreyes[2,7], and Maurizio Prato[1,6,7]

[1]Carbon Nanobiotechnology Group, CIC biomaGUNE, Paseo de Miramón 182, 2014 Donostia-San Sebastián, Spain [2]POLYMAT Universit of the Basque Country UPV/EHU, Avenida de Tolosa 72, 20018 Donostia-San Sebastián, Spain [3]Cardiovascular Institute, University of Colorado Denver Anschutz Medical Campus, School of Medicine, Division of Cardiology, 12700 E. 19th Avenue, Bldg. P15, Aurora, Colorado 80045, United States [4]Departamento de Química Orgánica, Facultad de Ciencias y Tecnologías Químicas-IRICA, Universidad de Castilla-La Mancha, 13071 Ciudad Real, Spain [5]Current address, Group of Carbon Nanostructures and Nanotechnology, Instituto de Carboquímica ICB-CSIC. C/ Miguel Luesma Castán 4, 50018 Zaragoza (Spain). [6]Department of Chemical and Pharmaceutical Sciences. University of Trieste, Via L. Giorgieri 1, 34127 Trieste, Italy [7]Ikerasque, Basque Foundation for Science, 48013 Bilbao, Spain.

Three-dimensional cellular organization was demonstrated to be able to induce cellular network outputs that strongly differ from the 2D constructs. The morphology, shape and porosity are critical parameters, and electrical conductivity is an important asset when dealing with electroactive cells, such as neurons or cardiac cells.

Carbon nanotubes (CNTs) are one of the most promising materials to interface with electrically active tissues. Their combination with polymers has been extensively studied, and the materials produced showed a great potential in tissue regeneration. On the other side, the design of electrodes based on conductive polymers (CPs) in brain-machine interface technology offers the opportunity to reduce gliosis, improve adaptability and increased charge-transfer efficiency. However, very little is reported about the combination of CPs and CNTs, and only 2D films have been synthesized and tested in vitro.

Here, we construct 3D porous and conductive composites, composed exclusively of CNTs and polypyrrole (PPy) or PEDOT, conjugated polymers demonstrated to reduce gliosis, improve adaptability and increase charge-transfer efficiency in brain-machine interfaces. We developed a new and easy strategy, based on the Vapor Phase Polymerization (VPP) technique. The resulting material is a very promising scaffold, with very low density, high and homogeneous porosity, electrical conductivity and Young Modulus similar to the in vivo tissue. Its high biocompatibility was demonstrated by incubation of astrocytic and cardiac cells, thus paving the way for the development of new conductive 3D scaffolds by following a yet unexploited approach.

2. SHEATH DYNAMICS OF PRE-EXISTING MATURE OLIGODENDROCYTES IN REMYELINATION AND LEARNING

Clara M. Bacmeister[1], Helena J. Barr[1], Crystal R. McClain[1] and Ethan G. Hughes[1]

[1]Department of Cell and Developmental Biology, University of Colorado, School of Medicine

The contribution of pre-existing mature oligodendrocytes to remyelination remains controversial. Traditionally, rodent models of demyelination indicate no participation of mature oligodendrocytes in remyelination (1–3), but recent evidence in large animal models and MS patients suggests otherwise (4-6). Using longitudinal in vivo two-photon imaging of oligodendrocytes expressing EGFP in a cuprizone model of demyelination, we show that surviving oligodendrocytes can contribute to remyelination in the adult mouse motor cortex through the rare addition of small numbers of new myelin sheaths. Oligodendrocytes that survive a demyelinating insult lose a quarter of their sheaths and rarely add new sheaths. However, after mice are trained in a skilled forelimb reach task, ~90% of surviving cells add new sheaths at a rate that compensates for sheath loss. Two weeks after training, the number of sheaths on a given cell is restored to the starting number of sheaths and is maintained for at least two additional weeks. Furthermore, half of the new sheaths added

after training are placed in previously unmyelinated locations, suggesting that learning generates a novel pattern of myelination. In addition, training also promotes retraction of pre-existing sheaths, with less sheaths maintaining their length after training. Therapies designed to engage mature oligodendrocytes in remyelination may therefore be beneficial in demyelinating disorders and could promote adult neuroplasticity.

3. STRESS-DEPENDENT PLASTICITY OF GLUTAMATERGIC SYNAPSES IN THE LOCUS COERULEUS

Kelsey Barcomb & Chris Ford

Department of Pharmacology, University of Colorado Anschutz Medical Campus

The locus coeruleus (LC) is the primary source of norepinephrine (NE) in the mammalian brain. These NE neurons play an important role in broadcasting information throughout the neuraxis. In particular, LC-NE cells are strongly activated by stressful stimuli. Stress-dependent LC activation is at least in part modulated by glutamatergic signaling, however the landscape of glutamatergic afferents coming in to the LC is not well understood. The current study aims to fill this gap in knowledge by using a mouse model to determine if stress modulates the strength of glutamatergic synapses in the LC. A restraint stress paradigm was used to induce acute stress, which was shown previously to activate LC-NE cells in mice. After stress, slice electrophysiology was used to perform whole cell recordings in the LC in order to determine synaptic properties. Stress was not found to alter glutamatergic synaptic strength globally, as measured by the amplitude and frequency of spontaneous excitatory postsynaptic currents. However, when afferents from the prefrontal cortex (PFC) were optogenetically isolated, stress was found to increase the strength of those selected synapses. In contrast, afferents from the central amygdala and the periaqueductal gray were unchanged. Taken together, these results suggest that stress does not induce a global change in glutamatergic strength but rather selectively targets specific synapse types, notably those originating in the PFC. Future studies will determine the mechanism of this effect, as well as the functional consequences.

4. TIMING OF BEHAVIORAL INTERVENTIONS FOLLOWING DEMYELINATING INJURY MODULATES THE DYNAMICS OF MYELIN REPAIR

Helena J. Barr[1], Clara M. Bacmeister[1], Crystal R. McClain[1], Michael A. Thornton[1,2,] and Ethan G. Hughes[1,2]

[1]Department of Cell and Developmental Biology, University of Colorado School of Medicine,
[2]Neuroscience Graduate Program, University of Colorado School of Medicine

Loss of oligodendrocytes, the myelin-producing cells of the central nervous system (CNS), critically affects axonal health and leads to neurological disability, as in the demyelinating disease Multiple Sclerosis (MS). Multiple immunotherapies target MS-attack prevention, yet mechanisms to stimulate the generation of new oligodendrocytes remain limited. Although robust oligodendrogenesis and white matter changes occur in response to motor learning, rehabilitation models have had variable and limited success in MS patients, perhaps due to the critical importance of timing for the efficacy of motor rehabilitation in neurological injury. Here, we used longitudinal two-photon in vivo imaging of myelinating oligodendrocytes expressing EGFP in transgenic mice (MOBP-EGFP) throughout learning and rehearsal of a skilled, single-pellet forelimb reach task to characterize the effects of learning on oligodendrocyte lineage cell behavior. We report that learning initially suppresses, then subsequently increases the rate of oligodendrogenesis. These effects are mirrored in oligodendrocyte precursor cells (OPCs), where learning initially suppresses cell proliferation, then increases cell differentiation. We then demyelinated mice using cuprizone, resulting in significant cortical oligodendrocyte loss and motor impairments characteristic of MS. To examine the effects of learning-induced oligodendrocyte modulation on myelin repair, we trained demyelinated mice in the forelimb reach task. While rehearsal of the task had no effects on remyelination, learning strongly shaped oligodendrogenesis in a timing-dependent manner. Learning occurring immediately after injury impeded generation of oligodendrocytes, resulting in delayed and decreased repair. However, learning occurring after the onset of initial remyelination increased maximum oligodendrogenesis. Together, our results suggest that the correct timing of motor circuit signals shapes recovery from demyelinating disease such as MS.

5. CONSISTENCY IN THE EFFECTS OF COMBINED SLEEP RESTRICTION AND CIRCADIAN MISALIGNMENT ON INTERLEUKIN-6

Alivia B. Blumenstein[1], Kate E. Sprecher[1], Emily Hay-Arthur[1], Austin J. Schreiber[1], Tina M. Burke[1,2], Christopher M. Depner[1], Pieter C. Dorrestein[3,4,5], Monika Fleshner[6,7], Rob Knight[3,8,9], Christopher A. Lowry[7,10], Fred W. Turek[11], Martha H. Vitaterna[11], and Kenneth P. Wright Jr[1]

[1]Sleep and Chronobiology Laboratory, Department of Integrative Physiology, University of Colorado, Boulder [2]Behavioral Biology Branch, Walter Reed Army Institute of Research [3]Skaggs School of Pharmacy and Pharmaceutical Sciences, [4]Collaborative Mass Spectrometry Innovation Center, and [5]Department of Pediatrics, School of Medicine, University of California, San Diego [6]Stress Physiology Laboratory, Department of Integrative Physiology and [7]Center for Neuroscience, University of Colorado, Boulder [8]Center for Microbiome Innovation and [9]Department of Computer Science and Engineering , University of California, San Diego [10]Behavioral Neuroendocrinology Laboratory, Department of Integrative Physiology, University of Colorado, Boulder [11]Center for Sleep and Circadian Biology, Department of Neurobiology, Northwestern University

Introduction: Interleukin (IL)-6 is important for mediating inflammation. Previous research shows that sleep restriction and circadian misalignment, independently, alter IL-6 levels. The effects of combined sleep restriction and circadian misalignment on IL-6 and the consistency of this response, however, is unknown. Methods: Twenty healthy adults, age 25.65(±4.2), BMI 21.97 (±2.2) kg/m2, completed two 18-day protocols consisting of 2 weeks of self-selected 8h sleep schedules at home followed by a 4-day laboratory visit. During visits, participants were given a baseline 8hr sleep opportunity on night 1, 3h opportunity on night 2, and 3h opportunities during mornings 3 and 4. Ten blood draws occurred during each visit: baseline after the 8hr sleep opportunity, then after the nighttime 3hr sleep opportunity, and draws every 6hr thereafter. Plasma IL-6 concentrations were measured using a multiplex immunoassay. Changes in IL-6 between and throughout visits were assessed using mixed model ANOVA and t-tests for individual time points. Intra-class correlation coefficients (ICC) were used to quantify the stability of individual differences in IL-6 at baseline and during sleep restriction and circadian misalignment. Results: IL-6 was increased compared to baseline at all time points ($p<0.025$ adjusted for multiple comparisons). IL-6 levels showed almost perfect trait-like stability (ICC 0.85) between visits at baseline and showed moderate consistency (ICC 0.44) between visits during sleep restriction and circadian misalignment (average samples 2-10). Conclusion: Combined sleep restriction and circadian misalignment produces a significant and moderately stable inflammatory response. The implications and nature of this response remains unclear and further research is required.

6. THE C2A DOMAIN OF SYNAPTOTAGMIN IS AN ESSENTIAL COMPONENT OF THE CALCIUM SENSOR FOR SYNAPTIC TRANSMISSION

Matthew R Bowers[1] and Noreen E Reist[1]

[1]Department of Biomedical Sciences, Colorado State University, Fort Collins, Co

The synaptic vesicle protein, synaptotagmin, is the principle Ca2+ sensor for synaptic transmission. Ca2+ influx into active nerve terminals is translated into neurotransmitter release by Ca2+ binding to synaptotagmin's tandem C2 domains, triggering the fast, synchronous fusion of multiple synaptic vesicles. The Ca2+-dependent membrane insertion of these C2 domains is required for this process. Previous research suggested that one of its tandem C2 domains (C2B) is critical for fusion, while the other domain (C2A) plays only a facilitatory role. However, the function of Ca2+-dependent membrane insertion by C2A had not been adequately tested in vivo. Here we show that membrane insertion by the C2A domain is absolutely required for synaptotagmin to trigger vesicle fusion. Using in vivo electrophysiological recording at the Drosophila larval neuromuscular junction, we found that loss of hydrophobicity at two key C2A residues almost completely abolished neurotransmitter release. In fact, mutation of both hydrophobic residues resulted in more severe deficits than seen in synaptotagmin null mutants. Thus, we report the most severe phenotype of a C2A mutation to date. Our results now demonstrate that membrane penetration by the C2A domain of synaptotagmin is an essential effector interaction for synaptotagmin's function as the electrostatic switch.

7. CHOLINERGIC NEUROMODULATION CONTRIBUTES TO VNS-MEDIATED CHANGES IN THE LEARNING OF A SKILLED FORELIMB REACH TASK

Spencer Bowles[1], Jordan Hickman[1], Ryan Williamson[1], Cristin Welle[1]

[1]University of Colorado Anschutz Medical Campus

Vagus nerve stimulation (VNS) improves the acquisition of sensory discrimination and skilled motor tasks when paired with the successful execution of the task. However, the mechanisms by which this stimulation increases the acquisition of these tasks are poorly understood. Here we use acute and chronic optogenetic manipulations, paired with VNS to demonstrate that activation of the basal forebrain (BF) cholinergic system is necessary for VNS-mediated learning increases to occur. ChAT-Cre mice werebilaterally injected in BF with an AAV2 viral construct containing either an excitatory or inhibitory light sensitive opsin(AAV-EF1a-DIO-hChR2(H134R)-EYFP or AAV-EF1a-DIO-eNpHR3.0-EYFP) implanted with 200µm fiberoptic cannulas. An additional cohort with a flexible silicone cuff on the left cervical vagus nerve (Microleads). After a recovery period, mice were trained on a novel forelimb reach task for 14 days and the measured on successful reach completion. Across all days of training, successful completion of the task was followed by stimulation through a fiberoptic, the cuff, or both. A subset of these mice were video recorded during their training sessions and their reaches were tracked using a machine learning algorithm in order to obtain reach kinematics data which is currently being analyzed. Inhibition of cholinergic signaling impaired learning compared to both VNS-stim and control animals. Furthermore, VNS paired with simultaneous BF inhibition eliminated VNS-mediated enhanced learning. Taken together, these experiments suggests that VNS-mediated learning depends on basal forebrain cholinergic neuromodulation.

8. SEXUALLY DIMORPHIC CARDIO-METABOLIC ALTERATIONS INDUCED BY SPINAL CORD INJURY IN RATS

Adel B Ghnenis[1], W Osimanjiang[1], KC Santos Roballo[1], DT Burns[1,2], CJ Asher[1], JS Bushman[1,2]

[1]School of Pharmacy and [2]Program in Neuroscience, University of Wyoming

Background

Spinal cord injury (SCI) can disrupt neural signals to vital organs in the body. In the US, the prevalence of SCI is higher than in other countries, with more injuries occurring in males than in females. Despite incidences of long-term complication associated with SCI, the cardio-metabolic consequences and gender related responses remain unexplored.

Aims

This study was designed to examine the effects of SCI on cardiac function, body composition, and glucose metabolism on adult female and male rats.

Methods

Sprague-Dawley rats were randomly assigned to either sham or SCI groups. SCI was induced via contusions using an impactor device with a force of 350 kilodyne.

Body composition was periodically measured by dual-energy X-ray absorptiometry (DEXA scans). Echocardiography was used to evaluate cardiac structure and function and glucose metabolism was periodically measured by intra peritoneal glucose tolerance test.

Data are mean ± SEM estimated by one-way ANOVA using mixed procedures in SAS.

Conclusions

SCI decreased body weight in males significantly in males at weeks 8 and 12, but not in females. SCI decreased LVID in females at 4 and 8 weeks and increased LVEF and LVFS in both males and females at 4 weeks. No differences in glucose metabolism at up to 12 weeks.

Significant cardio-metabolic differences were seen as a result of SCI, with sex being an underlying factor in the differences seen.

9. ANIMAL BEHAVIOR CORE

Nicolas Busquet, Stacey Zander, Michael Mesches

University of Colorado - Anschutz Medical Campus

A presentation of the services offered by the Animal Behavior Core, a joint operation of the Center for NeuroScience (CNS) and the newly formed NeuroTechnology Center (NTC). The Animal Behavior Core can help investigators at all the stages of behavioral testing of rodent models, from establishing the testing strategy (selection of the behavioral assays, establishment of the protocol) to performing the experiment (training on-site, coordination with the animal facility) and analyzing the data (in-depth behavioral analysis, visualization and representation of the results, and statistical analysis). The poster will present an overview of the behavioral tasks available and their significance, as well as the tools to analyze behavioral data (video-tracking system, automated apparatus). Some examples of behavioral testing results obtained by the Animal Behavior Core will be presented.

10. DOPAMINE CELLS DIFFERENTIALLY REGULATE STRIATAL CHOLINERGIC TRANSMISSION ACROSS REGIONS THROUGH CORELEASE OF DOPAMINE AND GLUTAMATE

Yuan Cai[1], Christopher P Ford[1]

[1]Department of Pharmacology, University of Colorado School of Medicine, Anschutz Medical Campus

The balance of dopamine and acetylcholine in the dorsal striatum is critical for motor and learning functions. Midbrain dopamine cells and local cholinergic interneurons (ChIs) densely innervate the striatum and have strong reciprocal actions on one another. It's known previously that dopamine neurons can inhibit cholinergic activity via D2 dopamine receptors. Dorsal striatum can be anatomically and functionally separated into dorsolateral (DMS) and dorsomedial (DLS) striatum. It's unclear whether dopamine neurons differentially modulate ChIs in the DMS and DLS, and how this modulation is altered in different sub-regions in Parkinson's disease. Here, we find that midbrain dopamine neurons drive pauses in the firing of DMS ChIs but robust bursts in DLS ChIs. Pauses are mediated by dopamine D2-receptors, while bursts are driven by glutamate co-release and activation of mGluR. By injecting a moderate dose of 6-hydroxydopamine (6-OHDA, 1ug/uL, 1uL) into the medial forebrain bundle, we develop a mouse model mimicking the early-stage Parkinson's disease. We find that in the DMS of 6-OHDA treated mice, activating dopamine axons drives prolonged pauses in firing in ChIs compared with that in control mice. This even augmented inhibition of ChIs by dopamine neuron is due to the down-regulation of dopamine re-uptake in Parkinsonian DMS. However, in the DLS of 6-OHDA injected mice, the glutamate-driven potentiation of cholinergic activity is totally lost. Taken together, this suggests that the modulation of cholinergic transmission is differentially altered across sub-regions of the dorsal striatum in the early-stage Parkinson's disease.

11. IMPACT OF DEEP BRAIN STIMULATION OF THE SUBTHALAMIC NUCLEUS ON NEUROPSYCHOLOGICAL OUTCOMES AND VOXEL-BASED MORPHOMETRIC ANALYSIS IN PARKINSON'S DISEASE

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[1]Modern Human Anatomy Program and [2]Department of Neurosurgery, University of Colorado Anschutz Medical Campus

Parkinson's Disease (PD) is a movement disorder characterized by tremor, rigidity, and bradykinesia. Deep brain stimulation to the subthalamic nucleus (STN-DBS) is presently a common treatment for PD motor symptoms. Historically, the majority of clinical research on STN-DBS has focused on motor outcomes; fewer studies have explored post-surgical cognitive outcomes. Moreover, the relationship between cognitive outcomes and post-surgical neuroanatomical changes in PD patients with STN-DBS is unknown. The goal of this study was to correlate cognitive outcome changes, with neuroanatomical structure volumes before and after STN-DBS. Ten patients met inclusion criteria for this Colorado Multiple Institutional Review Board (COMIRB) approved study (#16-1060). Voxel-based-morphometry and segmentations of T1-weighted MR were used to assess whether volumes of cortical and subcortical structures correlated with post-surgical changes in performance on standard neuropsychological exams including: California Verbal Learning Test 2nd edition (CVLT-II); Brief Visuospatial

Memory Test – Revised (BVMT-R); Controlled Oral Word Association Test (COWA); and Animal Naming Test (ANT). The present analyses found post-surgical decreases in raw scores for COWA, ANT, CVLT-II List Learning Total Free Recall (Learning Trials 1-5), and CVLT-II List Learning Delayed Free Recall. Strong correlations were observed between percent change in these neuropsychological measures and several pre-surgical structure volumes. This study better characterizes neuroanatomical and neurofunctional changes that occur with STN-DBS in a small cohort. Future studies on larger samples may lead to predictive measures which allow physicians to better screen for patients who will benefit most from STN-DBS surgery based on both neuropsychological and neuroanatomical pre-testing.

12. PRELIMINARY IDENTIFICATION AND VALIDATION OF A PLASMA METABOLOME-BASED BIOMARKER FOR CIRCADIAN PHASE IN HUMANS

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Introduction: Identifying a reliable predictor of circadian phase is important to diagnose circadian disorders and support personalized medicine. The potential utility of metabolomics to predict circadian phase is unknown. Here, we analyzed the plasma metabolome during adequate and insufficient sleep to identify a circadian phase biomarker. Methods: 16 (8M/8F) healthy participants aged 22.4±4.8y (mean±SD) completed a randomized cross-over in-laboratory study with 3 baseline days (9h sleep opportunity/night), followed by control (9h sleep) and insufficient sleep (5h) conditions. Circadian phase was determined by dim-light melatonin onset (DLMO). Blood was collected every 4 hours across 24 hours on the fifth day of each condition. Models were built using Partial Least Squares Regression using the full dataset and a rhythmic metabolite-only dataset. Each model was created with 66% of the data used to train the model, and 33% used as validation samples. Results: Using Leave-One-Out Cross-validation (LOOCV), R-squared for the full dataset was 0.58 using 7 components and 20 features. When validated on the 33% holdout samples, R-squared was 0.37 with a median error of 3.5hr±4.2 (median±IQR). Using LOOCV, R-squared for the rhythmic metabolite-only dataset was 0.62 using 11 components and 50 features. When validated, R-squared was 0.53 with a median error of 2.4hr±3.9. During insufficient sleep, the rhythmic data set was non-significantly (P = 0.051, Wilcoxon t-test) better at predicting DLMO. Conclusion: Our preliminary findings show promising trends for metabolomics, however additional analyses with more subjects are required.

13. LTD REQUIRES ENGAGEMENT OF TWO DISTINCT MECHANISMS FOR SUPPRESSION OF CAMKII SYNAPTIC TARGETING

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Learning, memory, and cognition are mediated by the long-term potentiation (LTP) and depression (LTD) of synaptic strength. These plastic processes require the Ca2+/calmodulin-dependent protein kinase II (CaMKII) and its auto-phosphorylation at T286. LTP requires CaMKII targeting to excitatory synapses, mediated by its binding to NMDA receptors. In contrast, during LTD, CaMKII instead targets inhibitory synapses. Once there, CaMKII promotes inhibitory potentiation, however, mechanisms directing LTD-induced CaMKII synaptic targeting are largely unknown. Here, we explore this differential CaMKII synaptic targeting and find that LTD requires suppression of CaMKII targeting to excitatory synapses by engagement of two distinct mechanisms: the death associated protein kinase 1 (DAPK1) and CaMKII T305/306 auto-phosphorylation (pT305/306). To study the regulation of CaMKII movement during plasticity, we used FingR intrabodies to simultaneously live-image endogenous CaMKII and markers for excitatory and inhibitory synapses in neurons from WT vs mutant mice. Either DAPK1 KO or a phospho-null mutation of CaMKII T305/306 was sufficient to allow accumulation of endogenous CaMKII at excitatory synapses not only after LTP but also LTD. Meanwhile, only pT305/306, but not

DAPK1, was also required for the LTD-induced CaMKII accumulation at inhibitory synapses. Thus, DAPK1 and pT305/306 regulate the bi-directional targeting of CaMKII during synaptic plasticity.

14. RODENT IN VIVO NEUROPHYSIOLOGY CORE (EEG CORE)

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The Rodent In Vivo Neurophysiology Monitoring Core (EEG core) facility provides continuous rodent behavioral (video) and neurophysiological/EEG monitoring services to members of the UC Anschutz Medical Campus (AMC) community to study the neurophysiological functioning of the normal brain and to identify abnormalities such as seizures or sleep disturbances in various disease models. The core provides equipment, facilities, consultation, and technical expertise for carrying out in vivo neurophysiology experiments. The core can record EEG from neonatal, juvenile, and adult rats and mice and can video-EEG monitor 32 rats and 28 mice simultaneously 24/7. The EEG core is equipped with a robotic, digital stereotaxic apparatus for precise electrode placements and drug/virus delivery to the brain region of interest. Last year alone, the core recorded 51,000 hours of video-EEG and performed over 100 stereotactic surgeries. The core also develops and re-engineers techniques and tools to meet investigators' needs and trains and teaches students and University researchers.

15. SINGLE CELL TRANSCRIPTOMICS REVEAL NEW INSIGHTS INTO THE DEVELOPING MENINGES

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BACKGROUND: Single-cell RNA sequencing (scRNA-Seq) has rapidly emerged as a powerful tool for investigating gene expression in heterogeneous cell populations. The meninges are a heterogeneous three-layer membrane of fibroblasts surrounding the central nervous system (CNS). Meningeal cells participate in vital developmental processes, including neuronal migration, neurogenesis and ossification of the skull, yet little is known about the developmental roles of individual meningeal layers. METHODS: We dissected the cortical meninges from Col1a1-GFPGFP/+ mouse embryos (E14.5), disaggregated the tissue and sorted for GFP positive cells using fluorescent activated cell sorting to obtain embryonic telencephalic meningeal fibroblasts. We performed scRNA-Seq on these cells to create a molecular atlas of the fibroblast populations at E14.5. RESULTS: We identified distinct gene expression profiles for the pial, arachnoid and dural fibroblast subtypes, including unique and previously unknown marker genes, which we confirmed at the protein level using immunofluorescence staining. We performed geneset enrichment analysis to identify functional gene expression patterns that may correlate with differing developmental roles in each meningeal layer. We also showed that subtype specific markers for the pial and arachnoid fibroblast populations are conserved in human fetal meninges. Finally, we discovered a previously unknown meningeal fibroblast subtype that localizes to specific regions of the mouse meninges and is also present in human fetal meninges. CONCLUSIONS: Our studies provide an unprecedented view of meningeal fibroblast diversity in mouse and human development, new markers and potential functions for embryonic meningeal fibroblasts, and important insights into their roles in CNS development.

16. REACTIVE OXYGEN SPECIES SIGNALING IN THE REGULATION OF GLUTAMATE RECEPTOR TRANSPORT

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The AMPAR sub-type of ionotropic glutamate receptors (AMPARs) are fundamental for excitatory neurotransmission and synaptic plasticity that underlies learning and memory. AMPARs are primarily translated in the cell body and transported to synapses by molecular motors. This process is not well understood; however, our lab has shown that voltage-gated calcium channels (VGCCs) and calcium/calmodulin-dependent kinase II (CaMKII) are critical for transport. Recently, it has been uncovered that VGCCs are regulated by reactive oxygen species (ROS), normal byproducts of energy production. Additionally, abnormal intracellular ROS levels have been consistently reported to perturb synaptic plasticity. These findings together led us to ask: do ROS levels impact synaptic plasticity due to a ROS-mediated modulation of VGCC and/or CaMKII activity influencing AMPAR transport? To address this, we use the genetically malleable, transparent C. elegans model to visualize fluorescently tagged AMPARs in vivo in real time. Our results indicate that increased ROS levels cause decreased AMPAR transport as well as short-term learning and memory deficits. Our genetic pathway experiments suggest that ROS alter AMPAR transport by acting on or directly downstream of VGCC, but upstream of CaMKII. To further understand this signaling pathway, we will use genetically encoded tools and microscopy to measure and manipulate ROS levels. We will also optimize electrophysiology techniques for C. elegans neuronal culture to determine the effect of ROS on intracellular calcium levels. Together, these techniques will allow us to pinpoint where and how ROS are acting in this calcium signaling pathway to impact AMPAR trafficking and therefore synaptic plasticity.

17. DTI ANALYSIS INDICATES FOCAL IMPACT OF GLIOMA ON WHITE MATTER PATHWAYS

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Gliomas account for 26.5% of all primary central nervous system tumors. Histopathological observation and biochemical manipulation have been the gold standard for diagnosing and studying gliomas, however these methods come with several limitations. Recent studies have used diffusion tensor imaging (DTI) to extract white matter fibers and the diffusion coefficients derived from the magnetic resonance processing to provide useful, non-invasive insights into the extent of tumor invasion, axonal integrity, and gross (pathophysiological and anatomical) differentiation of glioma from metastasis. Here, we extend this work by examining whether a tract-based analysis can improve non-invasive localization of the tumor impact on white matter integrity. This study retrospectively analyzed preoperative magnetic resonance sequences highlighting pathological tissue through contrast enhancement (i.e., T2 FLAIR MRI) and DTI scans of subjects that were biopsy confirmed to have either high or low-grade glioma. Whole brain seeding, required for reconstruction of specific white matter pathways, was derived through DSI Studio's deterministic Euler Streamline Algorithm. Two major white matter pathways, the corticospinal tract and superior longitudinal fasciculus, were reconstructed for along-tract-analysis (ATA) by applying the JHU White Matter Atlas based regions of interest. Ipsilesional and contralesional hemisphere tractography were used to compute within-subject comparisons for both analyses. Diffusion parameters indicated higher levels of white matter degradation in the ipsilesional hemisphere. Novel application of ATA revealed tracts traversing the tumor region showed significant white matter degradation which decreased with distance from the tumor.

18. DETERMINATION OF BEHAVIORAL AND OPTOGENETIC PARAMETERS FOR SYNAPSE SPECIFIC STRENGTHENING IN C. ELEGANS

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Excitatory neurotransmission in vertebrate central nervous systems is accomplished primarily through the neurotransmitter glutamate. Glutamate is associated with learning and memory due to its binding to the AMPA and NMDA subtypes of ionotropic glutamatergic receptors. The trafficking of AMPA receptors (AMPARs) through

long-distance transport from the neuronal cell body to their insertion at the synaptic membrane underlie these higher-order functions by supporting synaptic plasticity. Although extensive research has been conducted on AMPAR trafficking, it has primarily focused on trafficking more locally to synaptic spines. To better understand this, the Hoerndli lab has established a microscopy platform that allows for real-time visualization of AMPAR transport in C. elegans within a single pair of of AVA locomotive neurons. According to the current literature, AVA makes distal glutamatergic synapses to a chemosensory PHB neuron, which mediates an avoidance behavior to chemical repellents that can be easily quantified. Presumably, transmission and plasticity of these synapses is dependent on AMPAR transport. In this study, we characterize the PHB/AVA synapses using genetics, optogenetics, and behavioral analysis. Surprisingly, we show that the PHB/AVA synapses seem dependent on nAChRs but not AMPARs, thereby providing a new model to study the transport of nAChRs in C. elegans.

19. FUNCTIONAL CIRCUITS FOR SPATIAL CHOICE IN THE SUPERIOR COLLICULUS

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Decision making is a fundamental process for generating goal-directed behaviors. The midbrain superior colliculus (SC) contributes to sensorimotor decision making by integrating cortical and subcortical inputs to guide orienting movements, however the underlying circuitry for how the SC selects where to orient is unknown. Multiple models of excitatory/inhibitory interactions have been proposed to describe SC function, but are largely derived from neural recordings from unknown cell types. Here, we record and manipulate the activity of GABAergic neurons in mice performing a spatial-choice task to determine the functional role of inhibition during spatial choice. We train mice to select a left or right reward port based on a binary odor mixture. Importantly, after odor delivery, mice wait for a 'go tone' before orienting to the reward port, giving us access to neural activity during the decision (i.e., the "delay epoch"). We hypothesized that GABAergic neurons would shape spatial choice locally by inhibiting SC motor output neurons promoting contralateral choice, and therefore predicted that these cells would be most active before an ipsilateral choice. However, optogenetic identification and activation of channelrhodopsin-expressing GABAergic neurons revealed that GABAergic neurons are active before contralateral choices and driving their activity during the 'delay epoch' biases mice to select the contralateral port. These findings support a role for long-range inhibitory interactions in the SC. We are incorporating these data into a bump attractor model as a framework for understanding how the dynamics of excitation/inhibition give rise to nodes of activity in the SC underlying spatial choice.

20. OPTOGENETIC STIMULATION OF PANCREATIC FUNCTION VIA VAGAL CHOLINERGIC AXONS

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Previous studies have demonstrated stimulation of endocrine pancreas function by vagal nerve electrical stimulation. While this increases insulin secretion; concomitant reductions in circulating glucose do not occur. A complicating factor is the non-specific nature of electrical nerve stimulation. Optogenetic tools enable high specificity in neural stimulation using cell-type specific targeting of opsins and/or spatially shaped excitation light. Here, we demonstrate light-activated stimulation of the endocrine pancreas by targeting vagal parasympathetic axons. In a mouse model expressing ChannelRhodopsin2 (ChR2) in cholinergic cells, serum insulin and glucose were measured in response to both ultrasound image-guided optical stimulation of axon terminals in the pancreas and optical stimulation of axons of the cervical vagus nerve, together with ultrasound-based measures of pancreas blood flow. Measurements were made in basal-glucose and glucose-stimulated conditions. Significant increases in plasma insulin occurred relative to controls under both pancreas and vagal stimulation, accompanying rapid reductions in glycemic levels. Additionally, a significant increase in pancreatic blood flow was measured following optical stimulation. Together, these results demonstrate the utility of in-vivo optogenetics for studying the neural

regulation of endocrine pancreas function and suggest therapeutic potential for the control of insulin secretion and glucose homeostasis.

21. INVESTIGATING GLIAL AND AXONAL ADAPTATIONS TO SENSORY DEPRIVATION IN THE OLFACTORY SYSTEM

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The olfactory system is unique among sensory systems in that it remains plastic throughout life, and is one of the few brain regions to actively integrate new neurons into adulthood. The olfactory system encompasses several dedicated brain regions, such as the olfactory bulb (OB) and piriform cortex, which are connected by large, myelinated tracts. Precise control of conduction velocity and the synchronized firing of mitral cells, the myelinated projection neurons of the OB, are vital to olfactory sensory perception in higher brain regions. Plasticity in the OB has been widely studied using a model of sensory deprivation called unilateral naris occlusion, in which half of the bulb is deprived of sensory input. Dramatic, yet reversible, changes occur in the OB following unilateral naris occlusion, including downregulation of enzymes and a loss of cells. Despite the importance of myelin in the regulation of conduction velocity and the health of axons, plasticity of myelin has not been well studied in the olfactory system. Here, we use unilateral naris occlusion in adult animals to investigate myelin and axonal plasticity in response to sensory deprivation. Following 30 days of naris occlusion, we observed a significant shortening of mitral cell axon initial segments (AIS) in the occluded olfactory bulb. To investigate the electrophysiological consequences of these adaptations following naris occlusion, we are performing extracellular recordings of conduction velocity and axon refractoriness. Our data support an additional form of plasticity for the olfactory system in which axons and myelin adapt to changing olfactory inputs.

22. WIRELESS NEAR-FIELD OPTOGENETIC MANIPULATION OF THE PARAVENTRICULAR THALAMIC PROJECTION TO NUCLEUS ACCUMBENS IN HEROIN-SEEKING RATS

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Relapse to heroin seeking is often driven by the aversive mental states and physical dependence experienced during withdrawal. The paraventricular thalamus (PVT) projection to the nucleus accumbens (NAc) mediates aversion and opioid withdrawal symptoms after experimenter-administered chronic morphine (Zhu et al., 2016). We decided to test the hypothesis that optogenetic activation of this pathway might precipitate an aversive state capable of driving heroin seeking in a rat self-administration model. Using a wireless near-field optogenetic technology, and a combinatorial viral approach, we confirmed that photoactivation of PVT neurons projecting to the NAc generates a significant real-time place aversion during acute (24 h) withdrawal from heroin self-administration. At this same 24 h-withdrawal time point, we found that hyperalgesia is maximal compared to 12 h and 48 h. Finally, optogenetic activation of the PVT->NAc pathway during the first extinction session potentiated heroin seeking 24 h after the last heroin exposure but did not alter cued reinstatement of heroin seeking after extinction training (7d of withdrawal). These data confirm and expand previous studies showing that the PVT projection to the NAc is associated with aversion, and in our hands, optogenetic activation of this pathway was only capable of eliciting aversion during acute (24 h) heroin withdrawal. Furthermore, the same optogenetic stimulation pattern used to drive this aversive state is capable of driving heroin seeking under extinction conditions at this same time point. Future studies will explore different optogenetic stimulation patterns and test conditions to further delineate the role of PVT->NAc pathway in heroin seeking.

23. EXTENDING MULTIPHOTON MICROSCOPY TO DEEP BRAIN IMAGING IN FREELY MOVING MICE

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"Two-photon microscopy is used as a common technique for acquiring neuronal data from multiple neuronal layers in head-fixed mice. As the miniaturization of electronics and optics improve, it is becoming more feasible to use advanced microscopy techniques in head-mounted devices for the imaging of naturally-behaving mice. In this work, we show deep-brain imaging of the piriform cortex in a freely moving mouse using a two-photon fiber-coupled microscope (2P-FCM). A coherent imaging fiber-bundle is used for lateral imaging and an electrowetting tunable lens is used for rapid axial focusing with no mechanically moving parts. The 2P-FCM weighs only 2.5 g, with a lateral resolution of 1.5 microns and an axial resolution of 10 microns. We show imaging of a cylindrical volume of ~150 microns diameter by 150 microns depth. The 2P-FCM is coupled to a GRIN lens implanted in the anterior piriform cortex for the imaging of GCaMP6s-expressing neurons.

Behaviorally relevant neuronal activity is observed in multiple neuronal layers as female mice explore an environment with both their own bedding and unfamiliar male bedding. We show that the overall level of neuronal activity in the piriform cortex increases with decreasing distance to unfamiliar bedding. We could identify the responsiveness of specific neurons to each type of bedding. This set up can potentially be used for multi-plane single-neuron imaging of high-level olfactory processing as mice track odor plumes, which is currently not feasible under head fixed conditions.

24. MOLECULAR REGULATION OF THE EMBRYO-TO-ADULT TRANSITION (EAT) IN MOUSE TASTE BUD DEVELOPMENT

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Taste buds are collections of heterogeneous taste receptor cells (TRCs) that detect sweet, sour salt, bitter and umami. TRCs are continually replaced by proliferative keratin (K) 14 and 5+ progenitor cells situated adjacent to buds. In mouse embryos, taste bud precursors are first evident as small clusters of columnar epithelial cells or placodes on the tongue surface. Each placode comprises 10-20 cells that express both K8 and a secreted protein Sonic Hedgehog (SHH). SHH+ placode cells differentiate into TRCs in the first postnatal (P) week, but do not give rise to K14+ progenitors that support adult TRC renewal (Thirumangalathu et al., 2009 Development). This raised the questions of how and when TRC turnover commences. Using 3D image analysis, we find taste placode and taste bud cell number are static during embryonic development and within 1-2 days of birth, respectively; while EdU incorporation studies and K14 genetic lineage tracing reveal that the progenitor contribution to taste buds begins by P2, steadily increasing through P14 as taste bud cell number rapidly expands. During embryonic development, SHH represses taste fate, limiting the number and size of taste placodes (Hall et al. 2003 Dev Biol), while in adults SHH overexpression induces ectopic taste bud formation (Castillo et al. 2014 Development). Thus, we hypothesized the switch in SHH function coincides with the onset of TRC renewal. Using K14CreER to drive SHH overexpression at specific postnatal time points, we find that SHH drives ectopic taste cell formation starting at birth and this capacity ramps up over the first two postnatal weeks. Using RNAseq of P0 lingual epithelia, we have identified and are now exploring specific candidate transcription factors whose function may mediate this shift in the epithelial response to SHH.

25. MODULATION OF DOPAMINE D2-RECEPTORS SENSITIVITY FOLLOWING COCAINE EXPOSURE

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The dorsal (Dstr) and nucleus accumbens (NAc) receives dopamine input from separate midbrain neurons, are associated with different behaviors, and are modulated differently by drugs of abuse. How dopamine release is encoded by postsynaptic D2 receptors in each region is poorly understood. To measure D2 receptor activation, we virally overexpressed G protein-coupled inward rectifying potassium (GIRK2) channels in medium spiny neurons (MSNs). Using the resulting outward GIRK current, we found that the sensitivity of D2 receptors on MSNs was higher in the NAc than the Dstr (EC50: 2.45 vs 11.7 μM). The regional difference in D2 receptor sensitivity resulted from higher levels of Gαo expression in the NAc than Dstr. To examine how exposure to drugs of abuse might alter D2-receptor signaling across regions, we chronically treated animals with cocaine for 7 days (20mg/kg; ip). We found that cocaine reduced the sensitivity of D2-receptors and decreased the expression of Gαo selectively in the NAc. No change was observed in levels of Gαi and only a single day of cocaine treatment had no effect on D2-receptor expression or Gαo expression. Moreover, Gαo conditional KD mice have a higher cocaine induced behavior sensitization and cocaine induced place preference. These results suggest that Gαo is important for D2-receptors signaling and addiction related behaviors.

26. UTILIZING CRISPR TO PERTURB PHOTORECEPTOR/BIPOLAR ENHANCER MEDIATED CELL FATE DECISIONS IN THE MOUSE RETINA

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Two transcription factors, Blimp1 and Vsx2 influence rod photoreceptor and bipolar interneuron fate decisions late in retinal development. Normally these genes set rod to bipolar fates in a 10:1 ratio, but disruption of either factor individually changes these biologically critical ratios. Specifically, we are asking if these genes are in a mutually inhibitory network or merely working in parallel process to drive cell fate decisions. We are probing this gene regulatory network utilizing CRISPR/Cas9 to knock out both genes individually and simultaneously in vivo. We have also knocked out both genes' necessary cell type-specific enhancers individually and simultaneously. Results indicate that deletion of the cell type-specific enhancer for either Blimp1 or Vsx2 results in a fate-shift to rod biased or photoreceptor biased fates, respectively. Conversely, simultaneous deletion of both enhancers results in a dysregulated unbiased fate. Our findings suggest these genes work in a mutually inhibitory network regulated by these cell type-specific enhancers to set rod/bipolar cell ratios. This represents some of the first work to directly disrupt cell fate in a known gene regulatory network by simultaneous elimination of necessary enhancer regions with CRISPR/Cas9.

27. THE REGULATION OF TRANSCRIPTION FACTOR NFAT AND ITS GENE PRODUCTS IN RESPONSE TO NEURONAL ACTIVITY PATTERNS

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Synaptic plasticity, the process by which the strength of connections between neurons are modified, is thought to be the cellular and molecular mechanism responsible for learning and memory in the hippocampus. Long-term potentiation (LTP) and depression (LTD) at excitatory synapses require structural and molecular changes during activity-evoked synaptic strengthening or weakening. Gene transcription is necessary to support new protein synthesis that maintains synaptic strength during the late phases of LTP and LTD. The processes that link neuronal activity with changes in gene transcription (i.e. excitation-transcription (E-T) coupling) are not

completely understood. Here, I examine E-T coupling in the activation of genes regulated by the nuclear factor of activated T-cells (NFAT). During neuronal inactivity NFAT resides in its phosphorylated state in the cytosol. Neuronal depolarization causes Ca2+ influx that activates the phosphatase calcineurin to dephosphorylate NFAT. After dephosphorylation NFAT translocates to the nucleus where it acts as a transcription factor. Unpublished work from our group suggests NFAT translocation is dependent on Ca2+ influx at the soma, which is generated by the propagation of electrical signals from dendrites during synaptic stimulation. However, it is not known how different patterns of neuronal stimulation impact these signaling process to differentially regulate specific NFAT target genes. Here we investigate the regulation of NFAT and its gene products after neuronal stimulation.

28.REVERTING PRION DISEASE IN NEURONAL PROGENITOR CELLS AS A CELL REPLACEMENT THERAPY

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We propose the use of gene edited stem cells as therapy for neurodegenerative diseases. Olfactory neuronal progenitors (ONPs) can differentiate into neurons in adulthood. We hypothesize they will regenerate neurons that have been lost due to aggregation of disease-associated proteins. Mesenchymal stem cells (MSCs) can be derived from adipocytes of adult mice and further differentiated to neural stem cells (NSCs), which we will use as an alternative to ONPs. These therapies will be modeled by prion infected mice, which display the typical features of neurodegenerative disease. Prion protein (PrP) is highly expressed in neurons, and its misfolding results in disease. PrP knockout mice are resistant to prion diseases. CRISPR/Cas9 gene editing will be used to delete the prnp gene in WT ONPs. CRISPR/Cas9 will also be used to revert a mutation associated with the human prion disease Gerstmann–Sträussler–Scheinker syndrome (GSS), modeled by a single point mutation at codon 101 of mouse PrP. Alternatively, we plan to edit the prnp gene in M/NSCs to express a secretable, dominant-negative PrP. Single cell sorting will be used to produce homogenous populations of edited cells, which will be sequenced to identify relevant mutations. We will engraft gene edited ONPs using stereotactic injection and M/NSCs using nasal instillation into the brains of prion infected WT and GSS transgenic mice. We hypothesize that these cells will resist prion infection. ONPs will migrate throughout the brain to restore damaged neurons and M/NSCs will populate the olfactory bulb and secrete dominant negative PrP and anti-inflammatory cytokines.

29. TEMPORALLY PRECISE VNS ENHANCES MOTOR LEARNING AND PERFORMANCE OF A SKILLED FORELIMB REACH TASK

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Vagus nerve stimulation (VNS) paired with execution of a motor behavior leads to increased cortical plasticity and improved rehabilitation following stroke. However, the influence of temporally-paired VNS on motor behavior outcomes in a healthy animal has not been explored. We implement a chronic VNS mouse model to study the effects and underlying mechanism on motor learning in a healthy animal. Mice were implanted with a cuff electrode on the left cervical vagus nerve and were trained to perform a forelimb reach for a food pellet. VNS is applied in two timing conditions throughout a 14-day learning paradigm: 1) paired to successful reaches and 2) randomly throughout the behavior training session. VNS paired with reach success increases the rate of learning and overall proficiency with the largest effect in early days. Conversely, VNS applied randomly results in a learning deficit in early days. These results suggest that precise temporal delivery of VNS is necessary for augmented motor learning. We implement the machine-learning algorithm DeepLabCut for motion tracking to extract reach kinematics to dissect these learning differences. To explore the underlying mechanism of augmented motor learning, we perform opto-tagging to identify cholinergic and non-cholinergic neurons in the basal forebrain, an acetyl-choline dense neuromodulatory center, in an anesthetized mouse and recording firing activity during VNS. VNS can modulate the activity within basal forebrain by increasing the firing rate of individual neurons and altering local field potential dynamics. Using in-

vivo 2-photon microscopy of motor cortex, we demonstrate that VNS can modulate activity of layer 2/3 motor cortex neurons.

30. SYNAPTIC ULTRASTRUCTURE AT THE DROSOPHILA NEUROMUSCULAR JUNCTION

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The neuromuscular junction (NMJ) acts as a bridge between two important cell types in higher organisms: nerve and muscle. At the “active zone”, a specific presynaptic specialization of the NMJ, synaptic vesicles dock, prime, and ultimately fuse with the presynaptic membrane. Proteins required for each of these steps are highly concentrated and specifically organized at active zones. However, the ultrastructure of proteins at active zones remains unknown. My research aims to exploit the fast, cost effective, genetic system of Drosophila to discover functional relationships of active zone proteins and the molecular mechanisms mediating the vesicle cycle.

31. THE INNERVATION OF TASTE BUDS DIFFERS BETWEEN HUMANS, TREE SHREWS, AND MICE

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Gustatory nerve fibers innervate mammalian taste buds and express P2X receptors, reflecting the obligate purinergic nature of gustatory transmission. These fibers arise from the facial nerve to innervate the fungiform papillae and from the glossopharyngeal nerve to innervate the circumvallate and foliate papillae. Acetylated tubulin serves as a marker for all nerve fibers, while P2X3 labels gustatory fibers as well as subset of mucosal nerve fibers. We utilized immunohistochemistry with these two markers as well as electron microscopy to compare the patterns of innervation of fungiform and circumvallate papillae of mice, humans and tree shrews (Tupaia belangeri), a member of the Euarchontoglires superorder of mammals whose phylogenetic classification has produced controversy due to it sharing features of both primates and rodents. Although the general pattern of innervation is conserved across all three species, we find increased density of innervation by P2X3-immunoreactive nerve fibers in humans compared to mice and tree shrews. In addition, the nerve fibers in human taste buds appear thicker and more tortuous as compared to mice. Further ultrastructural analysis is necessary to determine whether the details of connectivity in humans differ from those in mice and tree shrews.

32.THERAPEUTIC TARGETING OF CTBP-MEDIATED NEUROINFLAMMATION IN TRAUMATIC BRAIN INJURY

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Traumatic brain injury (TBI) is a leading cause of death and disability in the United States. Mild TBI (mTBI or concussion) accounts for >70% of total TBI cases and is termed a “silent epidemic” because many patients may not be diagnosed until they begin to exhibit post-concussion symptoms. An estimated 15% of mTBI patients suffer prolonged disabling cognitive and neurobehavioral impairment. Human and animal studies indicate that neuroinflammation is an important contribution factor and a targetable aspect of the “secondary injury” phase after brain injury. Our preliminary studies have uncovered a novel and potent proinflammatory role of the transcriptional co-regulators CtBP1 and CtBP2 in TBI-triggered neuroinflammation. CtBP2 and CtBP-controlled target genes are induced in a time- and impact energy dose-dependent manner in the mouse CHIMERA (Closed-Head Impact Model of Engineered Rotational Acceleration) model that delivers diffuse axonal injury similar to mTBI in human. Moreover, we have developed two distinct CtBP-targeting compounds that are able to inhibit CtBP-mediated transactivation of proinflammatory genes. We tested two such compounds in the CHIMERA mice by intraperitoneal injection after mTBI. Both compounds can inhibit the induction of CtBP-controlled inflammatory genes in both the brain and the peripheral blood leukocytes and alleviate the neurobehavioral deficits elicited by

mTBI.

33. INDIVIDUAL DIFFERENCES IN SLEEP ARCHITECTURE DURING COMBINED SLEEP RESTRICTION AND CIRCADIAN MISALIGNMENT

Michael Kim[1], Kate E. Sprecher[1], Hannah K. Ritchie[1], Christopher M. Depner[1], Pieter C. Dorrestein[2], Monika Fleshner[3], Rob Knight[4], Christopher A. Lowry[5], Fred W. Turek[6], Martha H. Vitaterna[6], and Kenneth P. Wright Jr[1]

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Introduction: Sleep architecture has been shown to have trait-like consistency in sleep deprived individuals. However, sleep architecture during simultaneous sleep deprivation and circadian misalignment has not been widely reported. Methods: 20 adults completed a 39-day protocol that involved a two-week period of habitual 8 hours of sleep per night, followed by a four-day lab period. During the lab visit, participants were given an 8-hour sleep opportunity, followed by a 3-hour nighttime sleep opportunity, then two consecutive 3-hour daytime sleep periods. Participants then followed a 3-day recovery sleep period before repeating the home and laboratory procedures. Continuous polysomnography (EEG, EMG, and EOG) was utilized to assess sleep and wakefulness. Intra-class correlations coefficients (ICC) quantified individual differences in sleep architecture. Linear mixed effect models with subject as the random factor and visit and sleep period as fixed factors were utilized to assess the affects of time on sleep architecture. Results: During combined sleep restriction and circadian misalignment, N3 and REM were significantly increased ($p<0.001$). Individual consistency of N3 was moderate at baseline (ICC 0.45) and moderate to substantial under combined sleep restriction and circadian misalignment (ICC 0.47-0.70). Individual consistency of REM was fair at baseline (ICC 0.33) and slight to moderate under combined sleep restriction and circadian misalignment (ICC 0.19-0.50). Conclusion: Sleep architecture during combined sleep restriction and circadian misalignment showed consistent trait-like individual differences.

34. EXERCISE REVERSES HIGH-FAT DIET-INDUCED CHANGES IN PERINEURONAL NETS WITHIN THE PREFRONTAL CORTEX

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Overconsumption of foods high in fat contributes to the development of obesity and results in structural adaptations within the reward circuits of the brain. Our previous work showed that long-term exposure to a high-fat diet reduced the intensity of perineuronal nets (PNNs), specialized extracellular matrix structures that primarily surround fast-spiking interneurons, in both the prelimbic prefrontal cortex (PL-PFC) and the orbitofrontal cortex (OFC). However, whether the diet-induced changes in PNNs can be reversed is unknown. To address this question, we exposed Sprague-Dawley rats to a high-fat diet (60% by kilocalorie) in conjunction with exercise and assayed whether the presence and/or intensity of PNNs in the PFC changed with 28d of exercise. Rats were placed into one of four conditions: ad libitum chow with no exercise, ad libitum chow + exercise, ad libitum high-fat with no exercise, and ad libitum high-fat + exercise. Following the completion of exercise, we quantified PNN density and intensity in the PL-PFC, infralimbic prefrontal cortex (IL-PFC), and the OFC. Consistent with our previous work, we found that exposure to high-fat caused significant reduction in PNN intensity in both the PL-PFC and OFC (PL-PFC: Chow: 100.0±1.48; HF: 68.41±6.94; OFC: Chow: 100.0±2.57; HF-EX: 73.83±5.77; data are mean ± SEM). However, exercise combined with high-fat exposure showed a restoration of PNN intensity similar to the chow controls in the PL-PFC (PL-PFC: Chow: 100.0±1.48; HF+EX: 102.2±4.15) suggesting that

exercise may combat and/or rescue the effects of the high-fat diet in the PL-PFC. Surprisingly, exercise did not rescue the attenuation in PNN intensity in the OFC (OFC: Chow: 100.0±2.57; HF+EX: 76.37±3.89). Thus, we show for the first time that exercise can mitigate region specific dietary changes in PNN restructuring, which may help offset the maladaptive effects of obesity.

35. CONSERVED SITE ON NEUREXIN3-ALPHA MODULATES BALANCE BETWEEN EXCITATION AND INHIBITION

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Neurexins are a group of presynaptic cell adhesion molecules implicated in numerous neuropsychiatric disorders associated with imbalances in neuronal excitability. Neurexins derive from three genes that give rise to thousands of isoforms. Given the abstruse nature of several neurexin isoforms we functionally and morphologically further interrogate SS4 isoforms of Nrxn3a at hippocampal synapses, as SS4 has been shown to regulate ligand binding of neurexins as well as modulate plasticity in the hippocampus. Here, we probe the role of Nrxn3a in synaptic transmission using a mutated Nrxn3a (A687T) found in a heterozygous patient with acute intellectual disability and epileptic seizures. We show that this residue modulates ligand binding, and further reveal that Nrxn3a has profound effects on presynaptic morphology. Electrophysiology further brings to light Nrxn3α's involvement in excitatory transmission and long-term plasticity. These data exemplify the distinct role of Nrxn3α as a molecule necessary for the balance between excitation and inhibition at hippocampal synapses.

36. A SPIKING NEURAL NETWORK MODEL MIMICKING THE OLFACTORY CORTEX FOR HANDWRITTEN DIGIT RECOGNITION

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The mammalian olfactory system uses odor-specified temporal codes to represent the quality and the quantity of different odors. In order to better understand this coding strategy, a biologically plausible spiking neural network(SNN) was built and evaluated. In this study, MNIST images of handwritten digits were used to mimic the two-dimensional representation of odor information by the glomeruli in the olfactory bulb. The images were used to train the SNN based on the spike-timing-dependent plasticity (STDP) unsupervised learning rule. The SNN model was implemented by Izhikevich neurons to represent both the pyramidal neurons and the GABAergic interneurons in the piriform cortex. The recognition accuracy of the SNN model was evaluated to gain insights for the temporal coding scheme in the olfactory system. The results suggested that the SNN model can effectively encode 2D neural representations with temporal codes and achieved discrimination accuracies close to animal behavioral performances in odor discrimination tasks.

37. TRAIT-LIKE INDIVIDUAL DIFFERENCES IN CARDIOVASCULAR REACTIVITY AND PAIN FOLLOWING SLEEP RESTRICTION AND CIRCADIAN MISALIGNMENT

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Objective: Determine whether trait-like individual differences exist for pain and cardiovascular reactivity to stress after sleep restriction and circadian misalignment (SRCM). Methods: 21 healthy adults aged 18-35y completed an 18-day protocol twice, consisting of 2 weeks 8h sleep/night at home, followed by a 4-day in-laboratory visit. This protocol was repeated after 3 days of ad libitum sleep. During the in-laboratory visit, participants slept 8h on night 1, 3h on night 2, and 3h on mornings 3 and 4 (total of 9h sleep over 73 hours). The cold pressor test (CPT) was performed the first morning after an 8-hour sleep opportunity and the fifth morning after 3 days of SRCM. Participants submerged their hand in ice water for 3 minutes. Systolic blood pressure (SBP) and heart rate (HR) were measured 5 minutes before (T-5), and at the 1st (T1) and 3rd (T2) min of the test. Pain was measured on a visual analog scale at T-5 and T2. Intra-class correlation coefficients (ICC) derived from linear mixed effects models were used to measure stability of individual differences. Results: SBP, HR, and pain ratings were significantly elevated in response to the CPT. HR, but not pain, was significantly elevated following SRCM compared to baseline conditions (p=0.035). ICCs following SRCM indicated moderate to almost perfectly stable individual differences in SBP, HR, and pain (0.4-0.84). Conclusions: Cardiovascular and pain responses to an acute stressor is consistent within individuals and varies between individuals in a trait-like manner after sleep restriction and circadian misalignment.

38. HIGH-RESOLUTION TOMOGRAPHY ANALYSIS OF DROSOPHILA VESICLE PRIMING

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Neuromuscular junctions (NMJs) are specialized contact sites, synapses, where nerve cells communicate with muscles to provide the efficient and rapid signaling required for movement. This requires transmitter-laden synaptic vesicles within nerve terminals to contact with the plasma membrane in a process called docking. Calcium influx into the terminal then triggers docked vesicles to fuse with the nerve terminal plasma membrane. Upon vesicle fusion, chemical transmitter is released into the synaptic cleft between the nerve terminal and muscle fiber, activating receptors on the muscle cells. Vesicles fuse at specialized sites called active zones, which are regulated by a variety of proteins collectively called the active zone material. Synaptotagmin is the primary calcium sensor that triggers vesicle fusion, while SNARE proteins mediate the fusion event. Despite the significance of active zone material and vesicle fusion at NMJs, little is known about the physical structures mediating key molecular mechanisms within this system. My project investigates vesicle priming by utilizing the unprecedented spatial resolution (2-3 nm) of electron tomography in the fruit fly (Drosophila melanogaster) model system. I measured area of contact between docked vesicles and plasma membranes as well as the membrane thicknesses. Larger contact areas correlate to how “primed” a vesicle is, or how likely it is to fuse upon a calcium signal. By analyzing the frequency distribution of these contact areas, we are better able to understand the physical and molecular mechanisms mediating vesicle fusion and thus neurotransmitter release.

39. DECODING OLFACTORY STIMULUS STRENGTH FROM OLFACTORY BULB LFP OSCILLATIONS

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The perception of odor concentration is vital for survival. Since increased concentration reduces firing latency for mitral cells in the olfactory bulb (OB), firing phase with respect to the low frequency oscillation (LFO: respiration, theta local field potential) may convey stimulus strength. This signal can be read out via single cell firing, through aggregate neuronal activity patterns in the local field potential (LFP), and through the interaction between these phenomena—through spike-field coherence. While the representation of stimulus strength in mitral cell firing has been studied in vitro, markedly less is known about how the network of principal cells in OB conveys this information.

Here, I implanted tetrodes into the glomerular layer of the dorsomedial OB of C57BL/6 mice. Single-unit activity and the LFP were recorded while the mice performed two variants of the behavioral go no-go task, discriminating between odor identity—and separately—odor concentration.

All mice learned both tasks within a few days of training. LFP power (all bands) increased for rewarded stimuli and decreased for unrewarded stimuli over task acquisition in both paradigms (identity and concentration). In the concentration task, LFP power correlated with the rewarded odors irrespective of concentration. Phase amplitude coupling (PAC), or the relationship between the oscillatory phase of the LFO and the power of a higher frequency gamma oscillation (from coherent mitral cell firing) was stronger for the identity task than the concentration task. Additional work using discriminant analysis will determine whether LFP power or PAC are able to decode which stimulus was presented in both the identity and concentration paradigms.

40. ALTERATIONS TO THE SOUND LOCALIZATION PATHWAY IN FRAGILE X SYNDROME

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Hypersensitivity to sound and impaired sound localization are some of the most common sensory symptoms described by people with autism. The sound localization pathway, a neural network within the auditory brainstem, enables us to not only localize the location of a sound source per se, but also to separate between multiple simultaneous auditory streams that enter our ears. Whenever we have a conversation in situations such as a crowded restaurant, a busy public place, or a room where background noises are present, our sound localization circuit helps us to parse this complex situation into multiple narrow spatial channels based on their location. Being unable to localize the source of a sound, and to focus on a conversation when distracting noises are present significantly impairs social interactions in autistic patients. Despite its importance, our understanding of how the sound localization circuit is impaired in autism is largely unknown. To explore alterations in the sound localization pathway in autism, we can use a mouse model (Fmr1 KO mice) for the most common genetic form of autism, Fragile X syndrome (FXS). We have shown that there are frequency-specific alterations to the auditory brainstem in FXS mice, we have continued this study by examining alterations to myelin fibers that innervate this area and contribute to the speed of sound processing. We have seen alterations in myelin in the brainstem of FXS consistent with possible alterations to sound localization ability. We have measured myelination using both Transmission Electron Microscopy (TEM) and Coherent Anti-Stokes Raman Scattering (CARS). Using these two techniques we can measure the diameter of myelinated axons as well as the thickness of the myelin in those areas. We have seen changes to the myelination in FXS in the fibers that innervate the medial nucleus of the trapezoid body (MNTB). Determining the cause of sound localization impairments in FXS will help determine future strategies for treatment of these impairments, and perhaps FXS in general.

41. A STEREOTAXIC PLATFORM FOR SMALL ANIMALS BASED ON 3D COMPUTER VISION AND ROBOTICS

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Neuroscience behavioral animal studies often require injecting DNA material or fluorescent dyes

into specific brain regions within the animal's skull. Currently, these types of injections or surgical procedures are done manually by skilled researchers using mechanically based stereotaxic platforms. However, alignment can be very time-consuming and prone to error due to the small size of brain targets. Here we propose to develop a next generation stereotaxic platform for small animals by combining a three-dimensional (3D) computer vision sub-system and a full six degree-of-freedom (6DOF) robotic platform to improve spatial accuracy and surgical speed. With this approach, a video projector projects a series of structured illumination patterns onto an animal skull. Two video cameras are then used to capture two-dimensional (2D) images of the skull and the captured 2D images are processed to reconstruct an accurate 3D skull profile based on geometrical triangulation. Using the reconstructed 3D skull profile, the skull can be guided and repositioned using a 6DOF robotic platform to precisely and accurately align a surgical tool with the intention of reaching a specific brain target. This new stereotaxic system may improve accuracy and speed of small-scale brain surgeries for neuroscience studies.

42. SYNAPSE REGULATION BY NFAT-DEPENDENT TRANSCRIPTION

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Activity-dependent synaptic alterations including synapse formation and elimination are supported by protein synthesis, often requiring transcription. Synaptic activity and Ca2+ signaling are coupled to transcription by a process broadly termed excitation-transcription (E-T) coupling—many forms of which operate downstream of L-type voltage-gated calcium channels (LTCCs). One form of E-T coupling that has been well characterized by our laboratory signals via activation of the nuclear factor of activated T-cells (NFAT) family of transcription factors. Specifically, LTCC Ca2+ influx activates Ca2+/calmodulin (Ca2+/CaM)-dependent protein phosphatase 2B/calcineurin (PP2B/CaN) that is localized to the channel by A-kinase anchoring protein 79/150 (AKAP79/150), and in turn CaN dephosphorylates NFAT to promote its nuclear translocation. Evidence suggests that dysregulation of this pathway may be involved in pathological alterations to synapse density in multiple nervous system disorders including Autism Spectrum Disorders, Alzheimer's Disease, and Schizophrenia. Here, we present recent unpublished data in support of this hypothesis suggesting that CaN-NFAT signaling regulates synapse density and excitatory-inhibitory (E/I) synaptic balance and that overactivation of this pathway may underlie the alterations to these properties seen in Alzheimer's disease.

43. SYNAPTIC REGULATION AND SLOW WAVE, SLEEP SPINDLE, AND THETA BURST EVENT ORDER IN SLOW WAVE SLEEP.

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Slow wave sleep (SWS) is critical for synaptic regulation and the process of memory consolidation. During SWS, neurons produce oscillatory activity in the form of events that include slow waves (SWs), sleep spindles (SPs) and theta bursts (TBs). The timing of SPs and TBs in relation to slow waves is hypothesized to allow efficient cross-frequency coupling and replay of hippocampal-mediated memory traces. Preliminary reports suggest de-synchronization of the SPs and TBs from SWs parallels age-associated memory decline and brain atrophy. Disrupted SWS, including loss of SWs and SPs, is associated with the development and progression of several neuropsychiatric conditions, including schizophrenia, depression, Alzheimer's disease, and Parkinson's disease. Here we sought to develop a novel analysis method to examine the timing of SPs and TBs with regard to SWs to better understand their relationships among healthy individuals and those with neurological illness. Using sleep electroencephalography (EEG) from our ongoing clinical trials research, we developed custom MATLAB scripts for automated identification of these electrographic sleep events. Here we demonstrate that time-frequency relationships between SWs, SPs and TBs are dependent on the temporal order of the events. We hypothesize

that the temporal order of slow waves and the time-frequency relationships of nearby SP and TB events can be used to identify unique SW subtypes. We anticipate that this method will provide additional understanding about time-frequency relationships in the context of normal physiology, as well as in aging and neurological illness.

44. IMAGING PIRIFORM CORTEX ACTIVITY DURING ODOR RECOGNITION TASKS

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Piriform cortex (PCTX) is the largest recipient of olfactory bulb projections, with recurrent circuits thought to serve an important function in olfaction in processes such as odor coding and pattern completion. Currently, the function of PCTX is not fully understood, but substantial progress has been made recording neural activity with elecrodes. Our group is developing a device to image neuronal activity in PCTX. The ventral location of PCTX, several mm deep within the brain, makes imaging PCTX challenging because of light scattering effects. This scattering makes it impossible to optically access PCTX through cranial windows. This lack of optical access prevents awake imaging of single-cell calcium dynamics and spatial localization of cells. Methods exist to increase optical imaging depth, but even with 3-photon excitation imaging beyond ~1 mm is difficult. To overcome this limitation, it is possible to image through implanted Gradient Index (GRIN) lenses: rod shaped glass lenses of small diameter. Here we present progress in our project showing high signal-to-noise ratio GCaMP6s recordings of single neuron PCTX activity recorded through a GRIN lens during odor recognition tasks in an awake-behaving mouse. In addition, we are incorporating transparent electrodes on the surface of the GRIN lens to create a GRINtrode to image neurons and record extracellular potentials simultaneously.

45. SUPER-RESOLUTION STIMULATED EMISSION DEPLETION (STED) MICROSCOPY AT CU ANSCHUTZ MEDICAL CAMPUS: 2-COLOR STED, PHOTO-ACTIVATION LIVE-CELL STED AND PROGRESS TOWARDS A MINIATURE FIBER COUPLED STED MICROSCOPE FOR BRAIN IMAGING IN AWAKE BEHAVING ANIMALS

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Super-resolution microscopy, including STimulated Emission Depletion (STED) Microscopy, brought about a paradigm-shift in fluorescence imaging through breaking the "diffraction limit" in optical microscopy. To take advantage of this capability we have built our own STED microscopes as shared instruments for the Advanced Light Microscopy core at the CU Anschutz Medical Campus. I will present the basics of STED microscopy and then delve into the specifics of the design and capabilities of the STED systems on campus, starting with the two-color STED. As an example of a neuroscience application of this microscope, I will present results from the Dell'Acqua group in which STED was part of the evidence used to establish the importance of AKAP signaling for the placement of Ca2+-permeable AMPA receptors in synapses for LTP. Since AKAP and its targets are organized at the sub-micron level, super-resolution was needed to resolve changes in structure and co-localization of nanodomains in neurons from an AKAP-mutated mouse as compared to wild type. I will discuss the design and capabilities of the live-cell photoactivation STED and present images and video of dendritic spines from this microscope. Finally, I will discuss our latest work on developing a miniature fiber-coupled STED microscope. The goal of this research is imaging sub-diffraction limited features such as synaptic spines or cilia,

inside the brains of awake-behaving mice.

46.DATA REDUCTION FOR REAL-TIME ENHANCED GROWING NEURAL GAS SPIKE SORTING WITH MULTIPLE RECORDING CHANNELS

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Neural data analysis algorithms that can rapidly and accurately determine brain states are need to be developed to allow the implementation of closed-loop neural feedback controls for neuroscience studies and neural disorder treatment. In the past, we developed an Enhanced Growing Neural Gas (EGNG) algorithm that can be used to rapidly sort streaming neural spikes in real-time with very limited computational resources, suitable for implementation using digital electronic technologies for system miniaturization. Further development of data reduction is needed to extend the EGNG algorithm to sort neural spikes recorded from a multichannel neural probe. Here, we propose to use two identification methods – peak intensity and area integration – to identify the strongest neural spike among the adjacent channels in order to reduce the size of the recorded data, before sending the neural spikes to the downstream EGNG algorithm for spike sorting. This modification may lead to capability enhancement for the EGNG algorithm to rapidly sort neural spikes recorded from a multi-channel electrode for future closed-loop neural control experiments and treatments.

47. ADVANCED LIGHT MICROSCOPY CORE - NANOSCOPY

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Nanoscopy and super-resolution imaging available at Advanced Light Microscopy Core facility on Anschutz Medical Campus

48. MYELIN LOSS DISRUPTS MOTOR CORTEX CIRCUIT FUNCTION

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Multiple sclerosis (MS) is an immune disorder that destroys oligodendrocytes, which are responsible for myelination, resulting in impaired motor behavior. Demyelination slows the propagation of action potentials and introduces complex changes to brain circuitry. In addition, demyelination drives cortical hyperexcitability in patients, as revealed through fMRI imaging, and single-cell electrophysiology in mouse brain slice. However, It is unknown how in vivo single-neuron activity is modulated by myelination levels, and how changes to neuronal activity alter motor behavior. In addition, motor rehabilitation in MS patients is a growing approach to speeding recovery of motor behavior, but the effect of rehabilitation on motor circuits is unknown. This work aims to understand the effects of demyelination on a skilled reach behavior and the corresponding neural activity in primary motor cortex (M1), along with potential benefits of rehabilitation therapy following demyelination. Using chronically implanted microelectrode arrays, we recorded in M1 of mice as they engaged in a skilled reaching behavior. Neural activity was recorded before, during, and after demyelination, achieved through dietary administration of a global demyelinating toxin (cuprizone). Demyelination impaired the performance of the reach and produced hyperexcitability in excitatory neurons involved in the reach behavior, but not in neurons uninvolved in the reach. In addition, modulation, or the degree to which the firing rate is increased during the reach, was reduced following demyelination. Fast-spiking, putative PV+ neurons also have increased firing rates following demyelination. Rehabilitation training after demyelination improved behavioral success and normalized firing activity during remyelination but did not affect modulation of movement-related neurons. This data suggests that altered myelination disrupts motor cortex circuit function, and that rehabilitation training can ameliorate some deficits.

49. THE EFFECTS OF ALTITUDE ON DEPRESSIVE-LIKE BEHAVIOR IN RAT MODELS

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Major depression is one of the most prevalent mental illnesses in the United States, with an estimated 16.1 million people suffering from a depressive episode every year. Colorado, Utah, Wyoming, and other states at high altitude report a higher prevalence of depression and suicide in both males and females, pointing to a possible link between low oxygen pressure at high altitude and increased prevalence of depression. Additionally, females report higher rates of depression when compared to males, indicating that there may be a sex difference in vulnerability to depression. Serotonin, a neurotransmitter in the brain that is responsible for maintaining mood balance and emotion regulation, has been implicated in depression and also responds to changes in oxygen. Thus, serotonin dysregulation may contribute to increased prevalence of depression at high altitude. However, little is known about the effects of acute vs chronic altitude exposure on depression and there are no previous studies that examine the effects of various acclimation durations to high altitude on behavioral changes. Furthermore, there is no established scientific protocol specifying appropriate acclimation periods for rodent testing at various altitude levels, despite the fact that decreased partial pressure of oxygen has the potential to introduce new physiologic variability in scientific research. In this study, we examined the effect of altitude and acclimation duration on the depressive-like behavior and hematology of both male and female rats in Boulder, CO (5,430ft) or San Diego, CA (633ft elevation). A clinical hematology and a sucrose preference test (SPT), which measures anhedonia (a depressive-like behavior) in rodent models, were performed in order to assess the effects of altitude and acclimation duration on rodent models. Here we show that the SPT revealed an effect of altitude on anhedonia in a sex dependent manner. These data show that exposure to altitude increased depressive-like behaviors in rodents during behavioral testing.

50. AUTISM-ASSOCIATED Δ-CATENIN G34S MUTATION PROMOTES GSK3B-MEDIATED PREMATURE DEGRADATION INDUCING NEURONAL DYSFUNCTION

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δ-catenin is a crucial component of a synaptic scaffolding complex, which regulates synaptic structure and function in neurons. Loss of δ-catenin function is strongly associated with severely affected autism spectrum disorder (ASD) in female-enriched multiple families. In particular, a G34S (Glycine 34 to Serine) mutation in the δ-catenin gene has been identified in ASD patients and suggested to exhibit loss-of-function. The G34S mutation is located in the amino terminal region of δ-catenin, where there are no known protein interaction domains and post-translational modifications. Notably, the Group-based Prediction System predicts that the G34S mutation is an additional target for GSK3β-mediated phosphorylation, which may result in protein degradation. Therefore, we hypothesize the G34S mutation accelerates δ-catenin degradation, resulting in loss of δ-catenin function in ASD. Indeed, we found significantly lower G34S δ-catenin levels compared to wild-type (WT) δ-catenin when expressed in cells lacking endogenous δ-catenin, which is rescued by genetic inhibition of GSK3β. By using Ca2+ imaging in cultured mouse hippocampal neurons, we further revealed overexpression of WT δ-catenin is able to significantly increase neuronal Ca2+ activity. Conversely, Ca2+ activity remains unaffected in G34S δ-catenin overexpression, which is reversed by pharmacological inhibition of GSK3β using lithium. This suggests the G34S mutation of δ-catenin provides an additional GSK3β-mediated phosphorylation site, inducing δ-catenin premature degradation, and resulting in loss-of-function effects on neuronal Ca2+ activity in ASD. Additionally,

inhibition of GSK3β activity is able to reverse G34S-induced loss of δ-catenin function. Thus, inhibition of GSK3β may be a potential therapeutic treatment for δ-catenin-associated ASD patients.

51. ALTERATIONS IN GLYCOSYLATION AFTER SPINAL CORD INJURY

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Glycosylation is a fundamental cellular process which occurs in endoplasmic reticulum (ER) and Golgi apparatus. Glycans can have a dramatic impact on the functionality of glycol-conjugates such as proteins or lipids and mediate many different biological interactions including cell migration, cellular signaling and synaptic interactions of neurons. Despite that glycosylation is one of the most dominant post-translational modifications of eukaryotic proteins, the process and consequences of glycosylation is often only superficially characterized. The nervous system is the most heavily glycosylated of all tissues yet the glycan repertoire within nervous system is never been established. We have studied the glycosylation state of the rat spinal cord in normal animals and in spinal cord of rats with a spinal cord injury (SCI). Spinal cord was dissected from uninjured animals, SCI 3 days post injury (DPI), SCI 14 DPI, sham surgery 3 DPI and sham 14 DPI. Spinal cord samples were homogenized, reduced and carboxymethylated, triptic digested, then N-linked and O-linked glycans are separated using PNGase F and reductive elimination. MALPDI-MS and MS/MS were performed to analyze glycan structures present in each sample. MS analysis shows a diverse and rich amount of glycan expression in all groups, with some polysaccharide structures differentially produced in SCI animals compared to uninjured controls and shams. These results reveal the rich diversity in glycan structure and abundance in spinal cord and that this is significantly altered after injury. Studies moving forward will characterize the functional importance of differentially produced glycans in SCI.

52. CATHEPSIN K, A NOVEL MEDIATOR OF CHRONIC INFLAMMATORY PAIN

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Current therapeutic drugs for chronic pain temporarily relieve symptoms, have adverse effects (i.e. addiction and tolerance), and don't address the underlying pathology. Inhibition of cathepsin K activity, a cysteine protease, which has traditionally been studied in the context of osteoporosis has shown to reduce pain in the guinea pig model of osteoarthritis (McDougall et al., 2010). However, whether cathepsin K is a key mediator of inflammatory pain is unknown. Previously we showed that one-time inhibition of cathepsin K activity reduced the mechanical hypersensitivity induced by complete Freund's adjuvant (CFA) 1day post-injection (control=0.53g; cKi=1.23g). These pharmacological findings were corroborated in cathepsin K knockout mice (Cstk-/-). Cstk-/- mice showed a higher mechanical pain threshold than control mice after CFA injection (1d-post CFA: wt=0.75g; Cstk-/-=1.47g). Recently we have been able to reproduce the changes in pain threshold of Cstk-/- and control mice using SUDO method (1d-post CFA: wt=0.60g; Cstk-/-=1.63g). A similar reduction in mechanical hypersensitivity was seen after inhibition of Cathepsin K activity for 2 days after CFA injection (1d-post CFA: control=0.62g, cKi=1.15g; 2d-post CFA: control=0.71g, cKi=1.33g). We have also demonstrated that the mRNA levels from CFA-injected paws, dorsal root ganglia and spinal cords are elevated by 200%-600% 24h after CFA injection and cathepsin k protein expression is elevated in the paw of CFA-injected mouse compared to saline-injected mouse. From these results we hypothesize that cathepsin K is a key mediator in chronic inflammatory pain. The future goal for this work is to determine whether novel strategies to attenuate cathepsin K activity can be used to treat chronic pain.

53. NEUROMODULATION ENHANCES THE MOTOR CORTICAL CODING OF SKILLED MOTOR BEHAVIORS

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Motor cortex neurons are involved in movement preparation and execution: activating motor cortical neurons drives complex movement, while inhibiting these neurons stops the movement. Previous work from the lab

showed that stimulation protocols recruiting neuromodulatory pathways - vagus nerve stimulation (VNS) or basal forebrain (BF) stimulation - enhanced performance success rate on a dexterous reach task in mice. Understanding how motor cortical neurons' activity is modulated during stimulation-driven behavioral improvement is critical for optimizing neuromodulation technologies to enhance motor learning and rehabilitation.

Population neural dynamics were monitored in freely moving mice performing dexterous reach task with a miniscope to image neural calcium activity (GCaMP6) in layer II~III forelimb motor regions (M1). Preliminary data shows that individual neurons in M1 are selectively active in different phases of the task. Interestingly, population activity during reach preparation show greater number of active neurons and earlier activity before reach initiation in success trials than in failure trials, albeit the neural sequence is highly variable from trial to trial. We measured the neural responses in M1 during and after VNS or BF stimulation. Both stimulation protocols selectively enhance activity of a subset of M1 neurons, and BF seems to also suppress a different population of neurons. Future work will focus on how changes in M1 neural activity following phasic neuromodulation relate to behavioral alterations.

54. CUE-INDUCED REINSTATEMENT OF ORAL OXYCODONE-SEEKING BEHAVIOR IN MALE AND FEMALE MICE

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A significant portion of prescription opioid users self-administer orally rather than intravenously; however, most animal opioid research has been conducted using the intravenous route. These models have demonstrated that cues associated with intravenous use are sufficient to cause relapse. Our first objective was to determine whether oral drug-associated cues are sufficient to cause relapse. It is also clinically shown that preference to self-administer orally at least partially relates to the user's sex, leading to our second objective to determine whether sex differences exist in susceptibility to relapse. Mice orally self-administered escalating doses of oxycodone under postprandial and non-postprandial conditions and exhibited robust cue-induced reinstatement of extinguished drug-seeking behavior. An additional group of mice showed that oral self-administration under non-postprandial conditions is sufficient to support cued reinstatement. Because we discovered that female mice earned significantly more mg/kg oxycodone than male mice, subsequent gonadectomy studies were conducted to evaluate the effects of gonadal sex steroids on oral self-administration. Contrary to our initial hypothesis that these procedures would reverse the female to male imbalance of oxycodone oral self-administration, we found that ovariectomy prandial-dependently enhanced, while orchiectomy across dose and prandial-independently suppressed oxycodone self-administration. These studies establish that 1) oral drug cues are sufficient to cause reinstatement that is independent of prandial conditions, 2) earned oral oxycodone is larger in female mice compared with male mice, and 3) gonadectomy produces divergent effects on oral oxycodone self-administration between sexes.

55. THE PHOSPHATASE PTP-3 REGULATES TRANSPORT OF IONOTROPIC GLUTAMATE RECEPTORS (AMPARS) IN THE NEMATODE C. ELEGANS

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Normal cognition is dependent on AMPAR trafficking, which includes local synaptic trafficking and long-distance transport. Previous work has shown a critical role for the phosphatase leukocyte common antigen-related protein (LAR), in regulating the trafficking of AMPARs to synapses. LAR synaptic activity is mediated by a member of the LAR protein tyrosine phosphatase-interacting protein family, Liprin-α. These studies have shown that the localization of LAR to synaptic spines is dependent upon the degradation of Liprin-α by Ca2+/calmodulin-dependent protein kinase II (CaMKII). However, despite this knowledge we do not know how LAR regulates AMPAR transport. This study aims to identify the function of LAR in long-distance AMPAR transport. We have previously established an imaging platform enabling the tracking and manipulation of AMPAR transport in real-time in single neurons of intact transparent C. elegans animals. First, we measured synaptic GluA1 homologue, GLR-1 tagged with dual SEP::mCherry at proximal and distal synapses and found that GLR-1 numbers were decreased at proximal synapses, but increased at distal synapses in LAR loss-of-function mutants. Next, we

used time-lapse confocal imaging combined with photobleaching to track transport of single GLR-1 containing vesicles. LAR mutants display decreased transport of single vesicles. Lastly, we used fluorescence recovery after photobleaching (FRAP) and show that LAR mutants have decreased delivery of GLR-1 to synapses. Furthermore, LAR mutants exhibited a decrease in spontaneous reversals, a behavior dependent on functional synaptic GLR-1. Taken together, our results show a critical role for LAR in long-distance synaptic AMPAR transport.

56. DENDRITIC SPIKES EXPAND THE RANGE OF WELL-TOLERATED POPULATION NOISE STRUCTURES

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The brain operates surprisingly well despite the noisy nature of individual neurons. The central mechanism for noise mitigation in the nervous system is thought to involve averaging over multiple noise-corrupted inputs. Subsequently, there has been considerable interest recently to identify noise structures that can be integrated linearly in a way that preserves reliable signal encoding. By analyzing realistic synaptic integration in biophysically accurate neuronal models, I report a complementary de-noising approach that is mediated by focal dendritic spikes. Dendritic spikes might seem to be unlikely candidates for noise reduction due to their miniscule integration compartments and poor averaging abilities. Nonetheless, the extra thresholding step introduced by dendritic spike generation increases neuronal tolerance for a broad category of noise structures, some of which cannot be resolved well with averaging. This property of active dendrites compensates for compartment size constraints and expands the repertoire of conditions that can be processed by neuronal populations.

57. AKAP150 PALMITOYLATION REGULATES SYNAPTIC INCORPORATION OF CA2+-PERMEABLE AMPA RECEPTORS TO CONTROL LTP

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Ca2+-permeable AMPA-type glutamate receptors (CP-AMPARs) containing GluA1 but lacking GluA2 subunits contribute to multiple forms of synaptic plasticity, including long-term potentiation (LTP), but mechanisms regulating CP-AMPARs are poorly understood. A-kinase anchoring protein (AKAP) 150 scaffolds kinases and phosphatases to regulate GluA1 phosphorylation and trafficking, and trafficking of AKAP150 itself is modulated by palmitoylation on two Cys residues. Here, we developed a palmitoylation-deficient knock-in mouse to show that AKAP150 palmitoylation regulates CP-AMPAR incorporation at hippocampal synapses. Using biochemical, super-resolution imaging, and electrophysiological approaches, we found that palmitoylation promotes AKAP150 localization to recycling endosomes and the postsynaptic density (PSD) to limit CP-AMPAR basal synaptic incorporation. In addition, we found that AKAP150 palmitoylation is required for LTP induced by weaker stimulation that recruits CP-AMPARs to synapses but not stronger stimulation that recruits GluA2-containing AMPARs. Thus, AKAP150 palmitoylation controls its subcellular localization to maintain proper basal and activity-dependent regulation of synaptic AMPAR subunit composition.

58. IS CAMKII-A IMPORTANT FOR OLFACTORY LEARNING?

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Mutations to the α-isoform of calcium/calmodulin-dependent protein kinase II (CaMKII-α) are associated with an increased risk of developing a schizophrenia, a neuropsychiatric disorder characterized by impaired concentration, working memory, perception and social dysfunction. CaMKII-α is highly expressed in the brain including the hippocampus and prefrontal cortex two areas of the brain important for learning and memory. CaMKII-α is important for long term potentiation (synaptic plasticity) and memory. Heterozygous CaMKII-α knockout mice

(Het) have been described to show a schizophrenia-related phenotype including immature dentate gyrus (DG), hyperactivity, working memory deficits, and social withdrawal. Finally, CaMKII-α is expressed in the granule cells of the olfactory bulb. However, the role of CaMKII-α in olfactory learning is not well understood. To further investigate the role of CaMKII-α in schizophrenia, we used a go-no go olfactory discrimination task and an olfactory working memory task to assess cognitive learning deficits and awake behaving tetrode recording to measure neuronal oscillations in the hippocampus and prefrontal cortex. Mice learned to associate an odorant with a water reward in the go-no go task. Mice received double tetrode implants aimed at the CA1 region of the hippocampus and medial prefrontal cortex. All mice learned to differentiate between dissimilar odors. However, when the odor pair was similar and was reversed, Het mice took longer to learn the task. Furthermore, there was an increase in activity for the rewarded odorant in the prefrontal cortex and hippocampus. These observations suggest a key role of CAMKII in associative odorant learning.

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59. MUTATIONS IN THE SYNAPTOTAGMIN C2A DOMAIN OF DROSOPHILA MELANOGASTER

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Synaptotagmin is a vesicular transmembrane protein localized to the pre-synaptic region of neurons. While there is some variation in synaptotagmin across species, overall function and composition are conserved. This is useful when observing synaptotagmin in Drosophila melanogaster, the common fruit fly. As a result of the conservation of synaptotagmin, researchers are able to extrapolate information gathered from research performed on fruit flies to human neurophysiology. Fruit flies are an ideal model organism for genetic research, due to their short generation time and well-documented genetic information. Previous research performed in the Reist lab indicates synaptotagmin as the calcium sensor in fruit flies. Current research focuses on changes to the amino acid composition of the calcium binding pocket in the C2A domain of synaptotagmin. In the Reist lab genetic research is performed by breeding fruit flies to achieve the desired genotypes. This allows for discovery of the effect of predetermined mutations. The current research project utilizes a crossing scheme that isolates a mutation in the C2A domain where one or more aspartic acids (negatively charged) are mutated to asparagines (uncharged). The future goal of this project is to determine the effect of mutating varying numbers of aspartic acids to asparagines. We have hypothesized based on previous research performed in the Reist lab and based on the chemical properties of the amino acids that this mutation will result in decreased frequency of vesicle fusion events than in synaptotagmin with normal amino acid composition.

60. CAMKII NITROSYLATION REGULATES SYNAPTIC LOCALIZATION

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Hippocampal long-term potentiation (LTP) is a form of synaptic plasticity underlying learning, memory, and cognition. Further, it is well established that LTP requires the Ca2+/calmodulin (CaM)-dependent protein kinase II (CaMKII), its Ca2+-independent autonomous activity, and its movement to excitatory synapses. Autonomous CaMKII can be generated by protein modifications such as autophosphorylation of residue T286 or simultaneous S-nitrosylation of residues C280 and C289. Autonomous CaMKII activity generated by T286 autophosphorylation is well known to play a key role in LTP, and, by extension, learning, memory and cognition. However, the role of CaMKII S-nitrosylation in LTP remains unknown. Recently, a study has suggested that CaMKII S-nitrosylation may play a role in age-related cognitive decline. My preliminary imaging data suggest that S-nitrosylation of wild-type (WT) CaMKII, induced by the nitric oxide donor, DEA-nonoate, is sufficient to induce CaMKII translocation to excitatory synapses under basal conditions and that this effect is abolished in non-nitrosylable CaMKII mutants (CaMKIIΔSNO, C280/289V; CaMKIIC280V; CaMKIIC289V). The results of this project elucidate the

physiological role of CaMKII S-nitrosylation in regulating synaptic targeting and plasticity and may contribute to a better understanding age-related cognitive decline.

61. OLFACTORY ACTIVITY SELECTIVELY REGULATES THE NEUROGENESIS OF A SUBSET OF OLFACTORY SENSORY NEURONS

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In mammals, olfactory sensory neurons (OSNs) are born throughout life, ostensibly for the sole purpose of replacing damaged OSNs. Prior to maturation, each OSN progenitor chooses to express, out of hundreds of possibilities, a single odorant receptor (OR) gene. OR choice is thought to be a stochastic process, wherein each OR is chosen with a fixed probability that determines the fraction of newborn OSNs that express each OR. Here we show that manipulating olfactory experience by blocking olfactory stimulation on one side of the nose dramatically reduces the neurogenesis of OSNs that express a specific subset of ORs. These findings suggest that odor-stimulated mature OSNs may send signals to stem cells to promote the production of new OSNs that express particular ORs. Thus, the OR identities of newborn OSNs are determined in part by olfactory experience. We suggest that this form of plasticity may play a role in olfactory learning.

62.REGENERATION OF COMPLEX PERIPHERAL NERVE DEFECTS USING ALLOGRAFTS TREATED WITH LOCALIZED IMMUNOSUPPRESSIVE THERAPY

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Peripheral nerve regeneration after segmental defects occurs naturally, but is often sub-optimal. The most effective current clinical option is a sensory autograft of a freshly removed nerve, with degradable biomaterial conduits and decellularized grafts of lesser efficacy. Allografting of freshly isolated live nerves is as or more effective than autografts, but is not widely practiced due to the serious risks of systemic immune suppression. Our group has been developing methods to localize the immune response surrounding only the graft in an effort to reduce risks and enable use of this highly effective strategy to regenerate peripheral nerves. Data shows that localized immune suppression achieved with drug and cell based strategies allow for full regeneration equivalent or superior to mixed nerve autografts in critical sized defects in the rat model. This strategy has also proven effective for regenerating critical sized defects that encompass bifurcations and complex nerve structures. Allografts are uniquely positioned to address this critical aspect of peripheral nerve injury.

63. PHOSPHORYLATION OF S845 REGULATES CLATHRIN-MEDIATED ENDOCYTOSIS OF GLUA1

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Introduction: During scaling-up, AMPARs accumulate at synapses, restoring synaptic strength. In most scaling-up protocols, GluA2-lacking AMPA receptors accumulate selectively at the synapse, despite the fact that the great majority of AMPARs are GluA1/2 heteromers. Although it has been shown that GluA1 S845 phosphorylation reduces endocytosis, the molecular basis for regulation of GluA1 endocytosis is not well understood. Objective: To study how GluA1 S845 phosphorylation regulates clathrin-mediated endocytosis during neuron activity. Methods: Cortical neurons were obtained from E18 Sprague-Dawley rat embryos and cultured as mixed cultures for immunofluorescence and co-immunoprecipitation, after 48h with TTX of chemical LTP induction. Membrane fraction of Sprague-Dawley rat whole brain was used for the GST pull-down assay.

Unpaired two-tailed Student's t-tests were used in single comparisons. For multiple comparisons, we used one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test to determine statistical significance. P value < 0.05 was considered statistically significant. CEUA / 005/2012. Results: Previous results from the group have shown that 48h TTX treatment increases GluA1 S845 phosphorylation (Kim and Ziff, 2014). 48h TTX treatment reduces GluA1 endocytosis (CTL=1; TTX=0.63±0.02) and increases surface expression (CTL=1; TTX=1.17±0.04). Blockage of dynamin with 25 μM Dynole increases binding of clathrin adaptor AP2 to GluA1 (CTL=1; DYN=1.46±0.13). 48h TTX treatment reduces B-Adaptin binding to GluA1 (CTL=1; TTX=0.43±0.11). Induction of chemical LTP increases phosphorylation of GluA1 S845 (CTL=1; cLTP=5.55±0.84) and decreases B-Adaptin binding to GluA1 (CTL=1; cLTP=0.24±0.08). GST pull-down assay show less binding of GluA1 to the S845D phosphomutant (GLUA1=1; S845D=0.31±0.06). Conclusion: TTX treatment for 48 hours, a well stablished scaling up protocol, diminishes GluA1 endocytosis rate and the binding of β-Adaptin, a subunit of the AP2 adaptor, to GluA1. GluA1 S845 phosphorylation decreases the binding of AP2 to the CTD of this AMPA receptor subunit, unveiling a mechanism of phosphorylation-regulated clathrin-mediated endocytosis of GluA1. References: Kim S, Ziff EB (2014) Calcineurin Mediates Synaptic Scaling Via Synaptic Trafficking of Ca2+-Permeable AMPA Receptors. PLoS Biol 12(7): e1001900. doi:10.1371/journal.pbio.1001900

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64. *IN VIVO* CELL TRACKING AND CLEARED TISSUE IMAGING WITH EXTENDED FIELD OF VIEW SELECTIVE PLANE ILLUMINATION MICROSCOPY

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Single Plane Illumination Microscopy (SPIM), a type of light sheet fluorescence microscopy (LSFM), is continuously evolving to new demands for rapid and high-resolution volume imaging of biological samples. The OpenSPIM framework [1] was introduced as an open access blueprint to aid wide adoption of light sheet methods. Because of the inherent trade off in field of view (FOV) and resolution when generating the exciting light sheet, the original OpenSPIM has a limited FOV and modest axial resolution. Building on OpenSPIM and existing work on scalable light sheets [2], we introduce an open source Extended Field of View Single Plane Illumination Microscope (eFOV-SPIM). eFOV-SPIM has an adjustable field-of-view that enables both high-resolution imaging of in vivo dynamics and large cleared tissue samples on the same platform. eFOV-SPIM requires minor modification to the OpenSPIM excitation pathway combined with dedicated in vivo and cleared tissue sample chambers and sample mounts. Here we present the optical characterization of eFOV-SPIM system as well as experimental demonstration of single-cell tracking in Danio rerio and near-isotropic imaging of optical cleared tissue.

65. ELUCIDATING THE MECHANISMS OF HEAD AND NECK RADIOTHERAPY-INDUCED TASTE DISRUPTION IN A MOUSE MODEL OF FRACTIONATED IRRADIATION

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Head and neck cancer patients receiving conventional fractionated radiotherapy (daily treatment for up to 7 weeks) suffer from taste dysfunction and xerostomia that persist months to years following treatment and can lead to weight loss and poor clinical outcomes. To understand the cellular and molecular mechanisms underlying functional taste loss, we established a fractionated irradiation mouse model, where the head and neck of mice is exposed to 4 Gy daily for 5 consecutive days. Here, we focus on the circumvallate taste papilla (CVP) and the

Von Ebner's minor salivary glands (VEG) that are attached to CVP and have been proposed to regulate taste cell turnover via secreted factors. In controls, taste cell progenitors located outside taste buds proliferate and give rise to post-mitotic cells that enter taste buds and differentiate into Type I, II or III taste cells (glial-like, sweet/bitter/umami receptors or sour/high salt detectors, respectively). Upon fractionated irradiation, disruption of taste cell homeostasis included both death of differentiated cells and interruption of the supply of new taste cells coincident with reduced proliferation and depletion of the progenitor population. Interestingly, we also found increased cell death and reduced proliferation in the VEG, indicating that renewal kinetics of both regenerating epithelia are affected by radiation injury. Specifically, we found proliferation, as assessed by Ki67 expression, is reduced in both the CVP and VEG, and the total number of progenitors is reduced in the CVP. Expression of markers for all three taste cell types and the number of Type II cells are also diminished. Irradiation is further associated with increased cell death in both taste buds and the VEG. Because Wnt/β-catenin is required for taste progenitor survival and proliferation, we posited that Wnt signaling levels would correspond with radiation-induced changes in proliferation. Expression of β-catenin (Ctnnb1) and LEF1 was reduced concomitantly with the reduction in Ki67 in response to irradiation, suggesting that Wnt/β-catenin signaling is important to maintain proliferation. However, upregulation of Wnt signaling after irradiation lagged behind upregulation of Ki67 suggesting that, upon radiation-induced injury, other signaling pathways control the resumption in proliferation. Further, recovery of Wnt pathway gene expression occurred prior to the recovery of all three taste cell type markers, consistent with the demonstrated role for Wnt signaling in taste cell differentiation.

66. BURSTING ACTIVITY IN PARKINSONIAN BASAL GANGLIA NEURONS: A COMPARISON OF ANALYSIS ALGORITHMS

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Neuronal activity can be organized into various motifs that are relevant to cognition and behavior. One such motif is a 'burst': a discrete period of multiple action potentials in rapid succession (e.g., 3 or more action potentials in <25 ms). Bursting activity occurs in a diversity of neuronal populations and brain regions as well as in circuits in vitro and is altered in various disease states. Parkinson's disease (PD) is one such condition in which bursting activity seems to be impacted, specifically in the basal ganglia, but the exact relationship between neuronal bursting patterns and basal ganglia (BG) dysregulation remains poorly understood. Moreover, there is no universal definition of a burst, and as such the many different analysis methods that extract bursts from electrophysiological data must be rigorously compared before they can be confidently applied in future research. To address both issues, we have produced a computational pipeline that runs spike trains through a battery of well-validated burst analysis algorithms and compares the results. We have applied this pipeline to intracranial electrophysiological data obtained from the subthalamic nucleus (STN) of human Parkinsonian patients undergoing deep brain stimulation electrode placement. We have found that a spike train from a single STN neuron in these patients can contain up to several hundred bursts during recording epochs of up to 500 seconds. Furthermore, we found substantial variation between burst profiles obtained from each analysis method, potentially indicating differences in their capacity to characterize human BG neuronal characteristics. Future extensions of this work include selecting the optimal burst method for differentiating between distinct subtypes of PD (i.e., akinetic vs tremor dominant) and identifying optimal therapeutic stimulation targets.

67. DISTINCT ROLES OF GLUA2-LACKING AMPA RECEPTOR EXPRESSION IN DOPAMINE D1 OR D2 RECEPTOR NEURONS IN ANIMAL BEHAVIOR

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Dopaminergic signaling in the central nervous system regulates several aspects of animal behavior. In the dopaminergic circuits, there are two classes of neurons that can be differentiated by their expression of dopamine receptors, D1 or D2 receptors (D1Rs or D2Rs). Notably, Ca2+-permeable GluA2-lacking glutamate AMPA receptors (CP-AMPARs) are important for gating synaptic plasticity and gene expression in neurons, and their expression particularly in the striatum affects various forms of animal behavior. However, differential effects of GluA2-lacking

AMPARs in D1R or D2R neurons on animal behavior have not been addressed. Here, we employed the Cre-Lox recombination system to remove GluA2 selectively in D1R or D2R neurons to express CP-AMPARs and carried out multiple behavior assays. First, the open-field assay revealed that D2R GluA2 knockout (KO) mice showed hypoactivity, while GluA2 KO in D1R neurons had no effect on locomotor activity. We also revealed that D1R GluA2 KO mice showed delayed learning in the accelerating rotarod test compared with control animals, whereas D2R GluA2 KO animals exhibited complete loss of motor learning. In the sociability test, GluA2-lacking AMPAR expression in D1R neurons induced hypersociability, whereas D2R GluA2 KO mice elicited loss of sociability. Both D1R and D2R GluA2 KO mice consumed less food compared with control animals, while D1R GluA2 KO animals showed significantly more weight gain. Finally, D1R GluA2 KO induced anti-depressant effects, while GluA2-lacking AMPAR expression in D2R neurons promoted depression-like behavior. Taken together, GluA2-lacking CP-AMPAR expression in D1R and D2R neurons differentially affects animal behavior.

68. ENGINEERING A PHOTOACTIVATABLE BOTULINUM NEUROTOXIN FOR LIGHT-DEPENDENT SYNAPSE SILENCING

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Conditionally silencing populations of neurons has been a powerful approach for linking circuit activity to behavior. Robust optogenetic approaches have been developed for short-term disruption of neuron firing, but there remains a major gap in the optogenetic toolkit for long-term disruption of neurotransmission. Clostridium neurotoxins, which block neurotransmitter release by proteolyzing vesicle fusion proteins, have been widely utilized for long-term disruption of neurotransmission but their current utility is limited by lack of spatial and temporal control. Here we engineered botulinum toxin B so that it can be activated with blue light for rapid, local and persistent synapse silencing. We demonstrate its utility for blocking excitatory neurotransmitter release, but we expect this approach to be broadly applicable to diverse forms of regulated secretion ranging from neuromodulators to neuropeptides and hormones.

69. BETA-AMYLOID INDUCES HYPEREXCITABILITY VIA SELECTIVE NICOTINIC ACETYLCHOLINE RECEPTOR-MEDIATED DISINHIBITION IN ALZHEIMER'S DISEASE

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Beta-amyloid (Aβ) peptide accumulation has long been implicated in the pathogenesis of Alzheimer's disease (AD). New lines of evidence show there is hippocampal network hyperexcitability in the early stages of the disease which leads to increased epileptiform activity and eventually cognitive decline. Given this, we analyzed intracellular Ca2+ dynamics in response to Aβ using a genetically encoded Ca2+ indicator, GCaMP, in cultured mouse hippocampal neurons. We found that acute application of 250nM soluble Aβ42 oligomers increased Ca2+ activity in hippocampal neurons in parallel with a significant decrease in activity in Aβ42-treated interneurons. This finding suggests Aβ42 affects GABAergic interneurons, reducing their inhibitory inputs to pyramidal cells to induce pyramidal cell hyperexcitation. Of importance, a potential target of Aβ42 on interneurons is the nicotinic acetylcholine receptor (nAChR). Three major subtypes of nAChRs (α7, α4β2, and α3β4) have been reported in the human hippocampus. Notably, simultaneous pharmacological inhibition of both α7 and α4β2 nAChRs mimicked the Aβ42 effects on both hippocampal pyramidal neurons and inhibitory interneurons. However, inhibition of all three subtypes showed the opposite effect. These lines of evidence suggest Aβ42 works selectively on α7 and α4β2 nAChR subtypes. In addition, simultaneous activation of α7 and α4β2 nAChRs was able to rescue Aβ42-induced neuronal hyperexcitation. Given that neuronal hyperexcitability in the pre-symptomatic stages

of AD may play an important role in the disease progression, knowledge of Aβ42's interaction with GABAergic inhibitory neurons via selective nAChR inhibition may yield potential therapeutic targets for patients with AD.

70. DIFFERENTIAL PARKINSONIAN DEFICITS AND BASAL GANGLIA OUTPUT IN STIMULUS-GUIDED AND INTERNALLY-SPECIFIED MOVEMENTS

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Parkinson's disease (PD) is a progressive neurodegenerative disorder caused by loss of dopaminergic neurons (DANs) in the basal ganglia (BG). The link between loss of DANs and onset of PD motor symptoms is well substantiated; however, the degree of impairment depends on the context of the movement (e.g., whether it is habitual). Although well documented in the clinical literature, the underlying neural mechanism leading to some movements being more impaired than others is not well understood. To investigate, we record neural activity from BG output nuclei of hemi-PD and control mice trained on a two-alternative forced choice task. We elicited unilateral DAN loss via 6-hydroxydopamine infusion into the left substantia nigra pars compacta. To acquire neural recordings, we implanted drivable tetrodes into a principal output nucleus of the BG, the substantia nigra pars reticulata (SNr), ipsilateral to DAN loss. Control mice were similarly implanted. We compared behavior and neural activity between two conditions requiring otherwise-equal orienting movements: stimulus-guided or internally-specified. Under the stimulus-guided condition, the direction of movement (left vs. right) was selected based on the identity of the stimulus. Under the internally-specified condition, the direction of movement was selected based on recent history of movements (left vs. right) and whether they were rewarded. Blocks of stimulus-guided and internally-specified trials were interleaved within the behavioral session, allowing for within-session comparisons of behavior and neural activity between the two conditions. We hypothesize that the BG differently process internally-specified and stimulus-guided movements.

71. BEHAVIORALLY-RELEVANT NEURAL ACTIVITY MODULATES OLIGODENDROCYTE PROLIFERATION AND DIFFERENTIATION

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Oligodendrocyte precursor cells (OPCs) persist in the CNS throughout life and make up 3-5% of all cells in the grey matter and 7-9% in the white matter. OPCs migrate continuously, extend ramified processes with dynamic filopodia, and respond to neuronal activity through specialized synapses. OPCs proliferate through asymmetric cell division and differentiate into mature oligodendrocytes to myelinate axons and modulate action potential firing in active circuits. However, the precise links between neuronal signaling and the intracellular processes that regulate cell division, differentiation, and circuit integration remain unknown. As the differentiation process is characterized by extensive cytoskeletal reorganization, one possible target of neuronal activity is the OPC actin cytoskeleton. To study the effects of neural activity on OPC cytoskeletal dynamics, we used anesthesia to reduce cortical neuronal firing by ~50% and acquired high-resolution in-vivo 4D timeseries images of NG2-mEGFP-positive OPCs in motor cortex. We analyzed branching and filopodial growth to assess differences in cytoskeletal dynamics in the awake, behaving vs. anesthetized states. Next we leveraged a longitudinal in-vivo imaging approach to study the effects of learning a forelimb reach task on oligodendrocyte behavior. In this study, mice were trained every day for 7d to reach through a small window, grasp, and retrieve a small food pellet. We performed in-vivo two-photon imaging in either NG2-mEGFP or Olig2-tdtomato mice every 2-3d for one week prior to training, during training, and for three weeks following training, to assess the effects of forelimb reach-driven neuronal activity on oligodendrocyte proliferation, differentiation, and circuit integration. Understanding the mechanisms through which behaviorally-relevant neuronal activity modulates the oligodendrocyte life cycle may improve rehabilitating therapies for demyelinating disorders like multiple sclerosis.

72. DEFINING THE POTENCY AND WNT-RESPONSIVENESS OF SOX2-POSITIVE TASTE EPITHELIAL CELLS USING LINGUAL ORGANOID CULTURE

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Taste perception is accomplished when stimuli are transduced into electrochemical signals by taste receptor cells (TRCs) within taste buds. TRCs regenerate throughout life with taste perception being tightly conserved despite frequent cell turnover. Loss of this tight regulation—often a side effect of anti-cancer therapy—underlies perceptive dysgeusia (altered taste) or ageusia (loss of taste) (Ovesen et al., 1991 Clin Nutr). Recent work has uncovered the importance of the SRY-box transcription factor, SOX2, as a regulator of cell renewal. We know: (1) SOX2 is required for the renewal of TRCs (Castillo-Azofeifa et al., 2018 Dev); and (2) all types of TRCs are derived from epithelial cells expressing SOX2 (Ohmoto et al., 2017 Chem Senses). Despite the multipotent nature of SOX2-expressing cells, there is no description of their potency using organoid culture technology. Intriguingly, SOX2 expression levels are highly variable in taste epithelium, and the degree to which SOX2 level correlates with multipotency is unknown. We used fluorescence-activated cell sorting of CVP epithelium from SOX2-GFP mice to collect high and low GFP+, as well as GFPneg, populations and assessed each group's capacity to generate TRC-containing lingual organoids. We find cells with either high or low SOX2-GFP expression robustly generate organoids, while GFPneg epithelial cells do not. Further, we find organoids derived from differentially fluorescent SOX2-GFP populations have distinct proliferation kinetics, morphologies, and transcriptional profiles. We are now testing how these very same properties are influenced when organoids are exposed to varied levels of Wnt protein as well as Wnt pathway activators/inhibitors.

73. REGULATION OF DAPK1 LOCALIZATION AND FUNCTION BY SYNAPTIC PLASTICITY

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Long-term depression (LTD) and potentiation (LTP) are opposing synaptic processes that underlie learning and memory. Normal LTP requires the synaptic translocation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) via its interaction with the NMDA-receptor subunit GluN2B, whereas LTD requires that this translocation be suppressed. Our recently published findings demonstrate this suppression is mediated by death-associated protein kinase 1 (DAPK1), by inhibiting CaMKII binding to GluN2B. LTD induces the activation of DAPK1, which promotes both synaptic retention and binding to the GluN2B C-terminus at a region overlapping the established CaMKII binding site. Indeed, DAPK1 and CaMKII competitively bind GluN2B, presenting a likely mechanism by which DAPK1 regulates CaMKII synaptic translocation. LTP disperses DAPK1 from synapses, presumably allowing CaMKII to bind GluN2B. However, it is currently unknown what mechanisms regulate DAPK1 synaptic targeting. Interestingly, I have discovered that the neuronal cytoskeleton, composed primarily of filamentous actin (F-actin), is required for the basal synaptic targeting of DAPK1, and its dynamics are involved in the dispersal of DAPK1 during LTP. Further, disruption of the cytoskeleton no longer disperses DAPK1 after treated with a chemical LTD stimulus, indicating GluN2B binding may mediate synaptic retention of DAPK1 during LTD.

74. A CLOSED-LOOP, AUTOMATED REACHING TASK TO STUDY MOTOR LEARNING IN MICE

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Phasic activation of neuromodulatory systems enhances motor learning. Food-deprived mice will reach through a narrow slit to grab 3-mm round food pellets positioned on top of a diameter-matched post. During a 20-minute session, only a fraction of the attempts to obtain food is successful. Sessions are repeated daily, and the

number of successful attempts to obtain food per session increases as mice learn this coordinated motor task. Optogenetic stimulation of basal forebrain cholinergic neurons following successful reaches modulates activity of neurons in motor cortex and increases reach success. While the reaching task is an effective means to study learning in the motor cortex, throughput is low because food pellets must be manually placed on the post for the duration of each session. Additionally, the manual triggering of optogentic stimulation can be subject to human error and variability. To increase throughput and reduce stimulus variability, we have developed a system which automatically places the pellet on the post, monitors the mouse behavior in three dimensions, and triggers optogenetic stimulation in a paradigm that is closed-loop and unbiased. Using this system, up to five sessions can be run concurrently, increasing throughput by a factor of five.

75. TYPE I TASTE CELLS: BOTH FORM AND FUNCTION SUGGEST A ROLE IN TASTE SIGNAL TRANSMISSION AND/OR MODULATION

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Type I taste cells are described as the glial-like support cells of the mammalian taste bud, and remain the least well-understood of the three main taste cell types. Like many CNS glia, Type I cells wrap around neighboring cells, and likely degrade excess neurotransmitter. To ask whether Type I cells might also participate in either modulation or direct transmission of taste information to afferent nerves, we examined aspects of Type I morphology and physiology. Using Serial Blockface Scanning Electron Microscopy, we observed possibly specialized contact points between Type I cells and nerve fibers in the mouse circumvallate taste bud. These nerve fibers often closely approach Type I cell nuclei. In 3D reconstructions of segmented cells, nerve fibers are often “nested” in the invaginations of Type I cell nuclei. Type I cells are also well poised to participate in cell-cell communication within the bud. They are commonly interposed between Type II and Type III cells, and perhaps facilitate signaling between the two. If so, Type I cells should respond to neurotransmitters released by taste cells. To ask whether Type I cells might respond to ATP released by Type II cells, we imaged calcium fluctuations in Type I cells marked by Gad65-Cre-driven tdTomato fluorescence. Fluorescent cells responded to ATP with a calcium increase, and did not show voltage-gated calcium influx consistent with Type I cells. These data indicate a more expansive role for Type I cells in the transduction and integration of signals in the taste bud than has been previously considered.

76. IMPACT OF ENVIRONMENTAL CONTAMINANTS ON THE DOPAMINE SYSTEM ASSESSED WITH FSCV AND IMMUNOASSAYS

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Environmental contaminants frequently encountered through occupational and deliberate exposures are a major public health concern. A 2011 study from the National Institute for Drug Addiction revealed that an estimate ofestimated 21.7 million people aged 12 or older used inhalants containing high concentrations of volatile organic compounds. Chronic use of these compounds degrades white matter resulting in dementia-like cognitive impairment. Inhalants have been reported to alter the striatal dopamine system, similar to other drugs of abuse. However, the exact mechanism by which inhalants impact the dopamine system is still obscure. Furthermore, noise contamination has also been reported to elicit neuronal changes in the central auditory pathways. These changes may underlie noise induced pathologies including tinnitus. While knowledge of dopamine’s effects in the auditory pathways is emerging, not much is known about how. Our lab seeks to combine both electroanalytical and molecular-based tools to elucidate how inhalants and noise affect the dopamine system. Characterizing the effects of these contaminants on already complex neuronal networks compels elegant methods of inquiry regarding changes in neurotransmitters and neuron physiology. Fast scan cyclic voltammetry (FSCV) can evaluate neurochemical release and uptake using carbon fiber microelectrodes for minimally invasive, real time measurements in the brain. To assess cell physiology, protein identification and quantification assays rely on the interaction of proteins and antibodies. Flow cytometry relies on antibody interactions to analyze the presence

and relative expression of receptors in brain tissue. Thus, combining FSCV with flow cytometry allows a more comprehensive examination of the impact of environmental contaminants on the dopamine system.

77. INSUFFICIENT SLEEP ALTERS AFTER-DINNER CONSUMPTION OF HIGH-CARBOHYDRATE SNACKS

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Introduction: Understanding mechanisms underlying the relationship between insufficient sleep and weight gain has importance for public health. Previous findings showed, although total energy expenditure is higher during insufficient sleep, food intake especially after-dinner snacks, increases beyond this energy expenditure, resulting in positive energy balance and weight gain. We examined the number of times subjects selected high-fat and high-carbohydrate snacks, and total calories consumed from after-dinner snacks during experimental sleep restriction.

Methods: Sixteen healthy subjects (8F 22±5yrs) completed a randomized cross-over inpatient protocol including 3-baseline days (9h sleep opportunity per night), followed by 5-days of insufficient (5h sleep opportunity) and 5-days of adequate (9h sleep opportunity). Various items were offered ad-libitum as after-dinner snacks and were weighed pre-and post-consumption. Analysis used mixed-model ANOVA with subject as a random factor, and condition and condition order as fixed factors.

Results: Subjects consumed more calories ($p=0.017$) as ad-libitum after-dinner snacks in the 5h sleep condition. There was no difference in the choice of snacks selected between conditions ($p=0.24$), but the number of times subjects selected ($p<0.001$) and total calories consumed from high-carbohydrate snacks ($p=0.004$) was higher during insufficient sleep. Conversely, the consumption, the number of times subjects selected, and total calories consumed from high-fat snacks were similar between conditions (all $p>0.23$).

Conclusions: Selection of high-carbohydrate after-dinner snacks promotes the positive energy imbalance observed during insufficient sleep.

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78. INVESTIGATING NEURAL CIRCUITRY OF ORIENTING BEHAVIORS: ROLE OF CEREBELLAR INPUT TO THE SUPERIOR COLLICULUS

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The neural mechanisms of orienting behaviors are fundamental to directing and redirecting attention. Precise orientation of attention enables meaningful interaction and engagement with the environment and is a crucial process underlying virtually all types of behavior (i.e. motor, sensory, cognitive, social). The cerebellotectal pathway, a major neural circuit between the cerebellum (Cb) and the superior colliculus (SC), is known to be important for proper execution of orienting behaviors. However, the neural mechanisms of this pathway and their role in modulating behavior is unknown. To investigate the role of this pathway in orienting behaviors, we developed an optogenetically inducible cerebellotectal mouse model by injecting adenovirus containing fluorescently labeled channelrhodopsin into deep cerebellar nuclei (CbN). We confirmed the anatomy of the pathway by identifying fluorescently labeled axonal projections from CbN that terminated within motor layers of the SC. Using whole cell neural recordings in the SC in head-fixed anesthetized animals, we were able to

validate our model by successfully driving changes in SC activity in response to CbN stimulation. Presently, we are designing a head-fixed spatial orientation task to assess the role of the cerebellotectal pathway in orienting behaviors.

79. GENERATION OF TAU-YFP CELL LINES FOR MODELING ALZHEIMER'S DISEASE

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The incidence of Alzheimer's disease is on steady rise due to the growing aging population around the world. The disease is pathologically defined by extracellular β-amyloid plaques and intracellular neurofibril tangles. Recent studies have suggested that aggregated tau, but not Aβ aggregation, is the key driving force that initiates the neuropathology and its progression. Therefore, it is important to develop treatments targeting tau protein metabolism involving phosphorylation, aggregation, clearance, and its neuronal toxicity. To model tauopathy in vitro, we have created neuronal N27A cell lines which stably express human P301L mutant tau gene (Mut-tau-YFP) and wild type tau gene (WT-tau-YFP). These cells can be monitored in live culture as they express YFP gene. We have found that the constitutive expression of mutant tau gene led to protein aggregation and formation of single inclusion body in every cell, mimicking neural fibril tangle occurred in tauopathy. Immunostaining and Western blotting assay confirmed that tau is hyperphosphorylated in Mut-tau-YFP cell line. Interestingly, the Mut-tau-YFP cell line can directly secret tau protein into culture medium. In addition, we found that Mut-tau-YFP cells are more vulnerable to oxidative stress than WT-tau-YFP cells. In summary, we have generated tau-YFP cell lines which recapitulate pathological features of tauopathy. These cell lines could provide a useful in vitro model for studying the molecular mechanisms of tau aggregation and neuronal toxicity, and for screening drugs that can prevent protein aggregation and protect neurons for Alzheimer's disease.