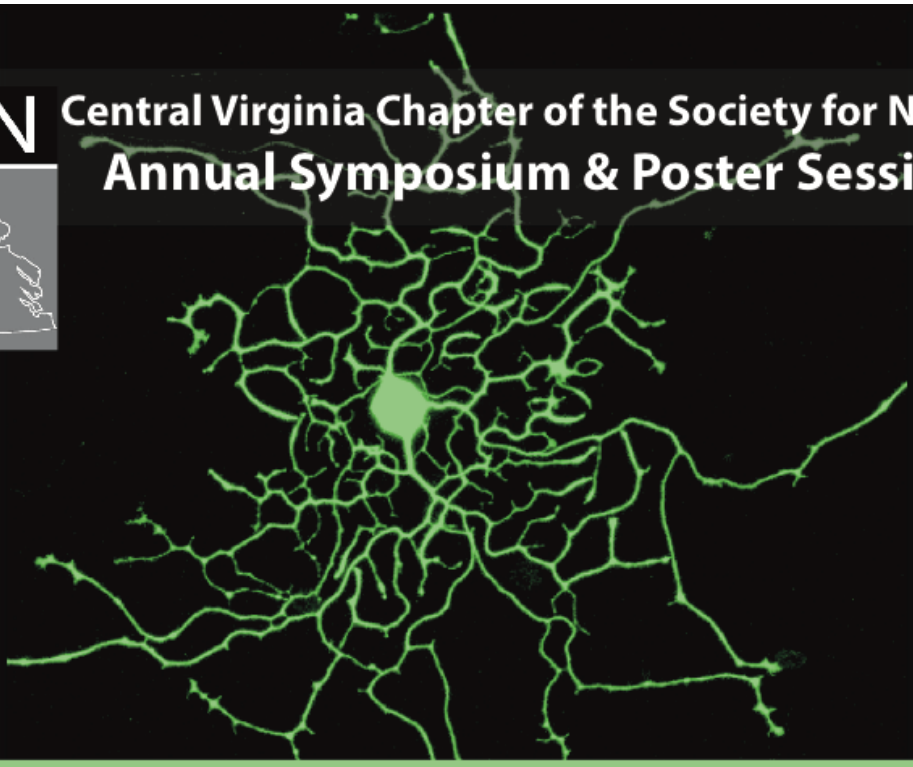
CVCSN

Central Virginia Chapter of the Society for Neuroscience

Annual Symposium & Poster Session 2017



NEURODEGENERATIVE DISEASES

From basic science to therapeutic interventions

March 26 - 27, 2017

Register: tinyurl.com/CVCSN2017

Virginia Tech Carilion Research Institute
2 Riverside Circle, Roanoke, VA 24016

GUEST SPEAKERS:

Kenneth Kosik, M.D., Ph.D.
University of California, Santa Barbara

Malú G. Tansey, Ph.D.
Emory University

John Bethea, Ph.D.
Drexel University

VIRGINIA STATE SPEAKERS:

Adam McQuiston, Ph.D., VCU
Linda Boland, Ph.D., UR
Michael Fox, Ph.D., VT
Michael McConnell, Ph.D., UVA
Michelle Olsen, Ph.D., VT
Scott Zeitlin, Ph.D., UVA
William Buchser, Ph.D., W&M

FOSTERING COLLABORATION AND COMMUNICATION AMONG VIRGINIA NEUROSCIENTISTS

Questions? Contact Dr. Michelle Theus, CVCSN Secretary Officer: CVCSNsymposium2017@gmail.com

VTC | Virginia Tech Carilion Research Institute

Central Virginia Chapter, Society for Neuroscience

Mission of the Society for Neuroscience

1. Advance the understanding of the brain and nervous system by bringing together scientists of diverse backgrounds, by facilitating the integration of research directed at all levels of biological organization, and by encouraging translational research and the application of new scientific knowledge to develop improved disease treatments and cures.
2. Provide professional development activities, information, and educational resources for neuroscientists at all stages of their careers, including undergraduates, graduates, and post doctoral fellows, and increase participation of scientists from a diversity of cultural and ethnic backgrounds.
3. Promote public information and general education about the nature of scientific discovery and the results and implications of the latest neuroscience research. Support active and continuing discussions on ethical issues relating to the conduct and outcomes of neuroscience research.
4. Inform legislators and other policy makers about new scientific knowledge and recent developments in neuroscience research and their implications for public policy, societal benefit, and continued scientific progress.

Virginia Tech Carilion Research Institute

The Virginia Tech Carilion Research Institute opened its doors on September 1, 2010, welcoming new faculty, fellows, students and staff from across the United States and throughout the world.

The institute provides state-of-the-art facilities for molecular medicine, imaging using lasers, high-power electron beams and magnetic resonance, high-capacity data handling, and human performance analysis.

The Roanoke-based institute is the hub for the worldwide hyperscanning network for interactive functional brain imaging. Connecting sites across the United States and throughout Europe and Asia, the network enables us to develop new insights into decision-making in healthy children and adults the effects of neuropsychiatric disorders on the decision-making process.

The Virginia Tech Carilion Research Institute houses 25 research teams, each led by a

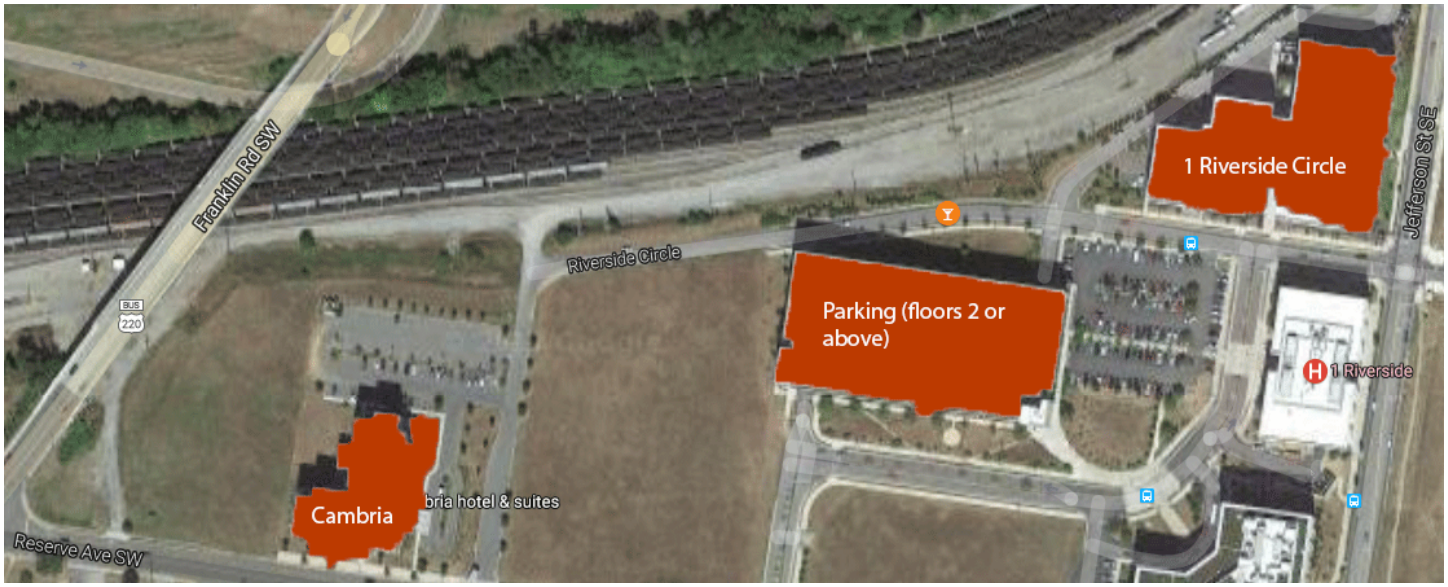
principal investigator who also holds a faculty appointment at Virginia Tech.

Our principal investigators—internationally recognized scientists—represent the departments of biological sciences, biomedical engineering and sciences, physics, and psychology at Virginia Tech. Their research teams are composed of postdoctoral fellows, graduate students, technicians and medical students from the Virginia Tech Carilion School of Medicine, as well as undergraduate students from Virginia Tech and other colleges.

These interdisciplinary research teams include investigators with expertise in biology, chemistry, computer science, economics, engineering, genetics, mathematics, physics and psychology. Working together, they seek to discover the fundamental processes of life and to solve the major health challenges facing the region, the nation, and the world. Our research teams work to address topics that include addiction and substance abuse, cancer, cerebral palsy, child neglect, developmental disabilities, epilepsy, heart disease, infectious disease, mental retardation, obesity and diabetes, Parkinson's disease, post-traumatic stress disorder, and traumatic brain injury.

Logistics

Parking: Please park on floors 2 or above in the parking garage across from 2 Riverside Circle, Roanoke, VA.



WiFi: Carilion Public WiFi

CVCSN 2017 Program

Sunday, March 26, 2017:

12:30 – 1:30 pm: Registration and poster setup

1:30 – 1:55 pm: Linda Boland, Ph.D., University of Richmond

A new model for studying excitability: Potential applications to assessing ion channel targets in neurodegenerative disease

1:55 – 2:20 pm: Michelle Olsen, Ph.D.; Virginia Tech

Dysfunction of astrocytic Kir4.1 in Rett syndrome

2:20 – 2:30 pm: Intermission

2:30 – 4 pm: Workshops for students on career opportunities and challenges

Session A: 2:30 – 3:15 pm: Undergrad students – summer and graduate programs

Session B: 2:30 – 3:15 pm: Grad students – choosing a mentor, tips for success in grad school

3:30 – 4 pm: Career challenges and options for scientists

4 – 5 pm: Poster presentations by undergraduate students

5 – 6 pm: Kenneth Kosik, M.D., Ph.D.; University of California Santa Barbara

Early Decisions in Neural Fate Determination

6:15 – 8 pm: Reception at the Cambria Suites

Monday, March 27, 2017:

8 – 8:50 am: Check in

8:50 – 9 am: Opening remarks

9 – 10 am: Malú G. Tansey, Ph.D.; Emory University

Role and Regulation of Inflammation and Immune Responses in Age-related Neurodegeneration

10 –10:10 am: Break

10:10 – 10:35 am: Michael Fox, Ph.D.; Virginia Tech

Mechanisms underlying visual circuit formation

10:35 – 11 am: Michael McConnell, Ph.D.; University of Virginia

Brain Somatic Mosaicism and Aged Human Neurons

11 – 11:20 am: William Buchser, Ph.D.; College of William & Mary

The Biology of the SARM1 Protein in Neurodegeneration and Cellular Adaptation

11:20 – 12:20 pm: Presentations by trainees

12:20 – 1:30 pm: Lunch Break

1-1:25 pm: Zeiss Microscopy

New trends on SEM and XRAY brain imaging

1:30 – 2:30 pm: John Bethea, Ph.D.; Drexel University

The role of Tumor Necrosis Factor (TNF) in CNS Injury and Repair

2:30 – 3:40 pm: Poster Session

3:40 – 4 pm: Symposium Awards

4 – 4:25 pm: Rory McQuiston, Ph.D.; Virginia Commonwealth University

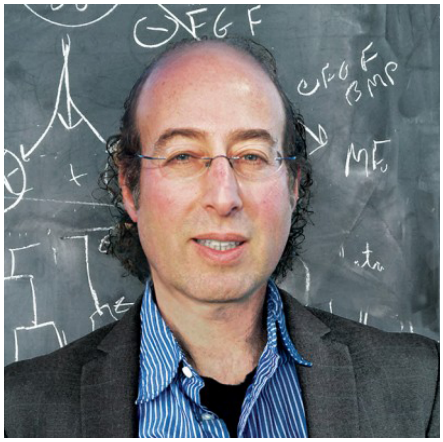
Physiological effects of pathogenic tau on medial entorhinal cortex inputs to dentate gyrus

4:25 – 4:50 pm: Scott Zeitlin, Ph.D.; University of Virginia

Modeling a gene therapy strategy for the treatment of Huntington's disease (HD) using Lac-regulatable HD knock-in mice

4:50 – 5:20 pm: Closing Remarks

Information on Guest Speakers



Early Decisions in Neural Fate Determination

Kenneth S. Kosik, M.D., Ph.D.
Harriman Professor of Neuroscience
University of California Santa Barbara

The earliest decisions concerning a neural fate begin while cells are still in a pluripotent state. Pluripotency has properties of a dynamic equilibrium in which individual cells transition stochastically between distinct metastable states that stably maintain the overall structure of the population. We found that single human embryonic stem cells (hESCs) have different and biased differentiation potentials toward either neuroectoderm or mesendoderm depending on the lengths of the G1 cell cycle phase even before the onset of differentiation. Therefore, single cell variation in G1 length establishes a probability distribution that determines the fate of the population. Environmental levels of WNT control the G1 length distribution curves, and thereby defines a continuum of pluripotent states.

Role and Regulation of Inflammation and Immune Responses in Age-related Neurodegeneration

Malú G. Tansey, Ph.D.
Associate Professor of Physiology
Emory University

The bidirectional communication between the immune system and the central nervous system are critical for brain health. The aging of the immune system and immune gene-environment interactions set the stage for age-related neurodegenerative disease. An example of how common genetic variation in genes that regulate antigen presentation synergizes with environmental exposures to determine risk for idiopathic PD will be presented. The role of LRRK2 in immune cells as a regulator of activation responses will also be presented as well as the extent to which LRRK2 levels are altered in subjects with iPD.

The role of Tumor Necrosis Factor (TNF) in CNS Injury and Repair

John R. Bethea, Ph.D.

Professor and Department Head; Department of Biology

Drexel University

Tumor necrosis factor (TNF) exists in two biologically active forms, soluble-TNF (solTNF) and transmembrane-TNF (tmTNF) that preferentially bind to TNFR1 and TNFR2, respectively, and elicit quite distinct biological responses. solTNF is recognized as a potent proinflammatory cytokine that contributes to the pathophysiology of numerous neurodegenerative and autoimmune disorders while tmTNF has recently been demonstrated to be neuroprotective and promote remyelination. In today's lecture I will: 1) discuss recent work from our laboratory demonstrating non-classical mechanisms though which solTNF/TNFR1 signaling is mediating pathophysiology in chronic spinal cord injury and 2) cell specific mechanism through which tmTNF/TNFR2 signaling is promoting remyelination in experimental autoimmune encephalomyelitis.

Information on Virginia State Speakers

A new model for studying excitability: Potential applications to assessing ion channel targets in neurodegenerative disease.

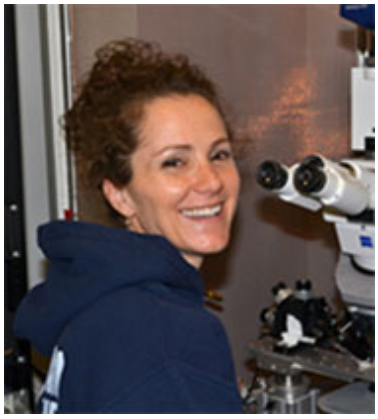
Linda M. Boland, Ph.D.

Associate Professor of Biology; Coordinator for Neuroscience

University of Richmond

Action potentials are the functional units of electrical signaling in excitable cells. A number of voltage-gated ion channels underlie the generation of action potentials and understanding how each of these proteins function and are regulated during electrical signaling is a subject of intense research. To improve our ability to study the molecular components of the action potential, we have converted non-excitable frog oocytes into electrically excitable cells. Oocyte action potentials replicate all of the key features of neuronal action potentials. In addition to its usefulness as an educational tool in inquiry-

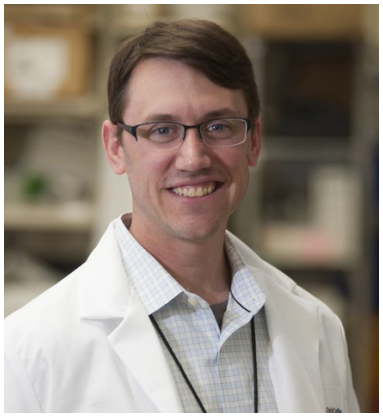
based learning, the new model has applications for understanding how action potentials are influenced by specific ion channel subunits, ion channel mutations, and pharmacological agents. Furthermore, neurodegenerative diseases sometimes reveal early functional changes in neuronal activity, prior to cell death. The seminar will propose how the oocyte action potential model may stimulate the development and validation of therapies directed at regulating cellular excitability in neurodegenerative disease.



Dysfunction of astrocytic Kir4.1 in Rett syndrome

Michelle Olsen, Ph.D.
Associate Professor; School of Neuroscience
Virginia Tech

Rett syndrome (RTT) is a neurodevelopmental disorder caused by loss-of-function mutations in the gene encoding methyl-CpG-binding protein 2 (MECP2). Symptoms of RTT include mental disability, autistic behavior, and seizures. In addition, severe respiratory dysfunction contributes significantly to poor quality of life and is associated with high mortality rate in this population. Evidence from RTT mouse models suggest that disordered breathing in RTT may result from disruption of central chemoreceptors (neurons that regulate breathing in response to CO2/H+), yet the cellular and molecular basis of MeCP2-dependent control of breathing remains largely unknown. Preliminary data provided indicate astrocytes in a brainstem region called the retrotrapezoid nucleus (RTN) sensing CO2/H+ by inhibition of inward rectifying K+ channels (Kir4.1). This channel is downregulated throughout the RTT brain including brainstem. Work underway tests the hypothesis that MeCP2 is required for expression of Kir4.1 in RTN astrocytes and loss of MeCP2 from astrocytes disrupts RTN chemoreceptor function and contributes to disordered breathing in RTT.



Novel mechanisms underlying visual circuit formation

Michael A. Fox, Ph.D.
Associate Professor; Director, Center for Developmental and Translational Neurobiology

Virginia Tech Carilion Research Institute

Developmental and age-related disorders that affect vision impart a major social and economic burden on the US economy. Many of these disorders specifically affect neurons that connect the retina (i.e. retinal ganglion cells [RGCs]) information to the brain. The most common of these disorders is glaucoma, a progressive neurodegenerative eye disorder that is the leading cause of irreversible blindness in the US. Currently, 3 million Americans are living with glaucoma, costing the US economy nearly 3 billion dollars each year. Glaucoma is not the only disorder that impacts RGCs. Currently there are no effective treatments for patients with glaucoma. In response to this unmet need, NEI has issued a goal of gaining new knowledge that will contribute to the development of regenerative therapies aimed at restoring connections between the retina and brain. To accomplish this goal, we need a better understanding of the mechanisms that drive the formation of these connections during normal development. Over the past 5 years, we investigated mechanisms that drive the growth of retinal axons into appropriate target regions of brain. During this work, we discovered that retinal synapses in the dorsal lateral geniculate nucleus (dLGN) are anatomically and functionally distinct from retinal synapses in all other retino-recipient regions. Because these synapses are crucial for transmitting visual information to cortex, understanding mechanisms of their formation is essential for restoring subcortical visual circuit function. With this in mind, a major goal of our laboratory is to identify target-derived cues responsible for the unique development of retinogeniculate synapses in dLGN.

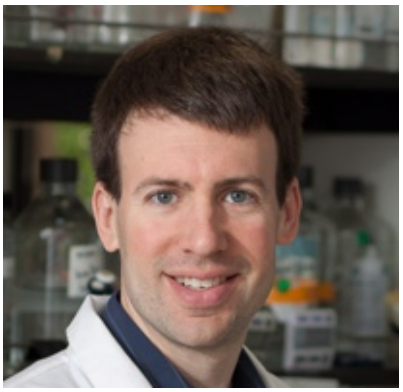
Brain Somatic Mosaicism and Aged Human Neurons

Michael J. McConnell, Ph.D.

Assistant Professor of Biochemistry and Molecular Genetics, and Neuroscience

University of Virginia School of Medicine

Every neuron has a different genome, this is called brain somatic mosaicism. Current estimates indicate that every neuronal genome contains ~1000 single nucleotide variants as well as at least one de novo retrotransposon insertion. In addition, between 10% and 40% of neurons contain a megabase (Mb)-scale de novo copy number variation (CNV) affecting >10 genes. My laboratory is focused on understanding the cause and consequence of mosaic CNVs in neuronal genomes, my talk at CVCSN will discuss recent data on aged human neurons.



The Biology of the SARM1 Protein in Neurodegeneration and Cellular Adaptation

William J. Buchser, Ph.D.
Visiting Assistant Professor
College of William & Mary

Rapid loss of axonal integrity in adult animals result from an active process that resembles axon-specific apoptosis—Wallerian degeneration. The molecules that execute this process are distinct from apoptosis, and are still mostly un-identified. Importantly, two of the key molecules which regulate the process are now known, and include a negative regulator NMNAT2 and the primary regulator, a neuro-inflammatory molecule called SARM1. Although SARM1 appears to be a TLR adaptor (based on the occurrence of a TIR domain similar to MyD88), it appears to have evolved in a distinct pattern. The inhibition of SARM1 in the setting of a variety of injuries is likely to rescue the otherwise damaged function of the nervous system. Specifically, SARM1 inhibition will be explored in the setting of noise-induced hearing loss and glaucomatous changes to the retina. Ultimately, a balance between homeostasis (where autophagy is a main player) and inflammation (controlled by SARM1 and other immunological pathways) will be needed to maintain axonal integrity in neurodegenerative disease and injury.

Physiological effects of pathogenic tau on medial entorhinal cortex inputs to dentate gyrus

Rory McQuiston, Ph.D.
Associate Professor, Department of Anatomy and Neurobiology
Virginia Commonwealth University

A hallmark of Alzheimer's disease (AD) is the formation of neurofibrillary tangles in the brains of AD patients. Neurofibrillary tangles arise from the formation of oligomers of hyperphosphorylated tau protein. Neurofibrillary tangle formation in the brain of AD patients occurs early in the entorhinal cortex and then spreads to synaptically connected regions of the brain. This seminar will describe our recent efforts to model

pathological tau formation at the initial stages of its spread and examine its impact on cellular and synaptic properties of downstream neurons in the dentate gyrus.

Modeling a gene therapy strategy for the treatment of Huntington's disease (HD) using Lac-regulatable HD knock-in mice

Scott O. Zeitlin, Ph.D.
Associate Professor of Neuroscience
University of Virginia School of Medicine

HD is an adult-onset autosomal dominant neurodegenerative disorder that is caused by the expansion of a polyglutamine stretch within the ubiquitously expressed Huntingtin (HTT) protein. In 2015, a Phase I clinical trial to test the safety of an anti-sense oligonucleotide (ASO) therapeutic strategy aimed at reducing the levels of HTT mRNA and protein was initiated by Ionis Pharmaceuticals and Roche. To determine when would be the best time to start such a therapy, and if there would be any deleterious consequences of terminating such a therapy in older patients, we are using *Lac*-regulatable HD knock-in model mice that permit repression (to levels typically attained using ASOs in animal models) or induction of mouse mutant *Huntingtin* (*Htt*) expression at different postnatal ages. We find that earlier repression of mutant *Htt* expression can delay the appearance of mutant Htt aggregation and ameliorate some behavioral phenotypes in older mice. However, induction of mutant *Htt* expression in old mice reduces whatever positive benefit that is obtained by repressing *Htt* expression earlier in life. The current status of this work, including preliminary RNAseq characterization of the mouse model cortical and striatal transcriptomes following either repression or induction of mutant *Htt* expression will be presented.

Sunday Poster Presenters

Undergraduate Students

Poster	Presenter	Institution
1	Matthew Adan	College of William and Mary
2	Afful Donatine	James Madison University
3	Cassidy Burke	University of Virginia
4	Thomas Caldwell	Washington and Lee University
5	Shiyu Chen	University of Virginia
6	Ian Colello	Virginia Tech
7	Shreyaska Dahal	Virginia Tech
8	Sanitra Desai	University of Richmond
9	David Hahn	University of Richmond
10	Christopher Handwerk	James Madison University
11	Sarah Izabel	Virginia Commonwealth University
12	Lucy Jin	University of Virginia
13	Melina Knabe	Washington and Lee University
14	Kathryn McDaniel	University of Virginia
15	Michael Mykins	Virginia Tech
16	Mary Pegelow	James Madison University
17	McKenzie Prillaman	University of Virginia
18	Lauren Ratcliffe	Roanoke College
19	Thomas Parks Remcho	University of Virginia
20	Kristin Sammons	James Madison University
21	Kathryn Sarfert	Washington and Lee University
22	Destin Shortell	University of Virginia
23	Jacqueline Sinnott	University of Richmond
24	Sauson Soldozy	University of Virginia
25	Austin Tapp	The College of William and Mary
26	Abigail Watterson	University of Richmond
27	Zachry Jennifer	Washington and Lee University

Undergraduate Students' Abstracts

Age-Specific Neurophysiological Adaptations to Endurance Training

Matthew Adan and Anna Seo
Department of Kinesiology, College of William and Mary

Endurance and resistance training are known recommendations for successfully treating and preventing chronic health conditions associated with age among adults and older individuals, including cardiovascular disease, stroke, Type II Diabetes, obesity, and arthritis. In order to enhance these methods of exercise training for this population, the possible adaptations and mechanisms of aged muscle after this kind of endurance training (i.e. aerobic style) must be described and understood, especially in juxtaposition with that of young muscle.

This study investigates the neurophysiological differences between young and aged rats after endurance exercise training. Functional analysis is used to measure force production of isolated soleus muscles from aged and young rats, after electrical stimulation to either the nerve or muscle. Parameters of interest include maximum force production, neuromuscular block, time to maximum force, and fatigability over the five-minute interval of stimulation.

This investigation found that muscle, when stimulated directly rather than stimulated via the nerve, is able to produce stronger contractions/greater amounts of force and that nerves experience fatigue in all parameters before muscles do. The percent decrease in force (fatigability) over the 5 minute interval was significantly greater when the nerve was stimulated versus when the muscle was directly stimulated, indicating that fatigue occurs more and faster during nerve stimulation.

Sensory Mechanisms Underlying the Escape Response to Looming Stimuli in Crickets

Donatine Afful, Aubrey Siebels, Ariel Childs, Alex Zeher, Corey Cleland
Department of Biology, James Maddison University

Animals respond to aversive stimuli with escape or withdrawal responses. In crickets, wind, which might normally be produced by an approaching predator, has been shown to evoke an escape response in which the cricket turns 180 degrees from the wind and then runs or jumps away. We have shown that crickets (Acheta domesticus) largely utilize the same turning strategy for looming stimuli, which provides both wind and visual sensory cues. However, there is lack of literature on the sensory modalities that underlie the escape. Our specific aim is to identify and compare the role of visual, cercal, filiform, and antennal sensory cues in the escape of the cricket from looming stimuli. Crickets (n=90) were stimulated with a 2.5" ball (1 m/s) projected at 45 degrees using an air cylinder and stopped 20 mm from the initial position of the cricket. Crickets were stimulated at 8 angles, in 45-degree increments around the body in random order. Above the platform was positioned a high-speed video camera (650 fps) and a LED ring

light. Prior to stimulation, a primary sense organ (eye, cerci or antenna) or a source of sensory information was ablated or removed to test necessity, or isolated to test sufficiency for the escape response. Crickets were blinded with lacquer nitrocellulose, or deprived of cercal or antenna information via lesions applied at the base of the appendage. A glass panel was placed between the animal and the stimulus to block wind cues to intact crickets. Crickets' compound eyes are only receptive to light below red wavelengths so 660nm red light was used to eliminate visual cues in intact crickets. Finally, a white ball against a white background was used to eliminate visual but retain wind cues. Results showed that both eyes and cerci, but neither filiform hairs not antennae, contribute to the escape response. The contributions of both eyes and cerci to the escape depend largely on the direction of incoming stimuli. The eyes mediate escape responses to anterior looming stimuli while the cerci mediates escape responses to posterior stimuli. In addition, our results showed that escape responses mediated by the cerci had a shorter latency but similar magnitude as compared to escape responses mediated by the eye. Taken together, our results suggest that crickets use both vision and wind to programs escape responses, and that the resulting movement differ in at least latency.

Epileptic circuits revealed using EpiPro, a novel synthetic activity modulated promoter

Cassidy Burke, Iuliia Vitko, Agnieszka Gawda, Ji Won Kim, Kathryn Brodie, Kyle Sullivan, Benjamin Walker, Matteo Ottolini, Deborah Perez-Reyes, Jaideep Kapur, Manoj Patel, and Edward Perez-Reyes
Department of Neuroscience, University of Virginia

Medial temporal lobe epilepsy (TLE) is a disease in which seizures originate in the hippocampus and can spread throughout the brain. To explore the epileptic circuit in a rat model of TLE, an activity modulated promoter (EpiPro) was designed to label active neurons in the hippocampus with GFP. To ensure preferential expression in epileptic neurons, EpiPro was developed using elements from genes that are upregulated after chronic stimulation and in human epilepsy, as well as highly expressed in the hippocampal circuit. In the dentate gyrus, epileptic animals were found to have significantly higher counts of GFP+ cells (expressing EpiPro) than naive animals. This finding provides in vivo evidence to support the hypothesis that dentate granule cells become hyperexcitable in TLE. Our data also validates the use of EpiPro to drive gene therapies to inhibit overactive neurons and prevent seizures.

Cycling Female Rats Are Resistant to Diet-Induced Obesity Cognitive Impairments

Thomas Caldwell and Uma Sarwadnya
Department of Neuroscience, Washington and Lee University

The obesity epidemic in the United States affects one out of three adults. Obesity is associated with an increased risk for numerous disorders, such as heart and liver disease. An often-overlooked consequence of obesity is decreased cognitive ability. Recent studies have shown that diet-induced obesity (DIO) alters

the performance of rats in behavioral tasks focused on cognitive functioning. However, these studies employed male rats, leaving many questions unanswered regarding how obesity influences the cognitive capacity of females, especially considering the variation in sex hormones. To investigate how DIO influences cycling female rats, we employed a high fat, high sugar (HFHS) diet and performed two behavioral tasks designed to test the cognitive ability of rats. The open field (OF) and novel object recognition (NOR) tasks were conducted, comparing the activity of female rats fed a control diet with those fed the HFHS diet. Vaginal cytology was performed to track the estrus cycle of the rats. Groups for statistical analysis were formed based on estrus cycle stage. The HFHS diet produced significantly greater body weights, hyperglycemia, and insulin insensitivity to effectively model DIO when compared to control rats. The tasks did not reveal any significant differences between the two diets, with both groups displaying similar activity in the OF and NOR assessments. There were no significant differences when estrus stage was considered. These negative results suggest that females are not as susceptible to cognitive impairment as males. This is possibly due to the presence of estrogen in female rats, which could act as a shield to the cognitive impairment male rats experience, prompting further investigation into this area of behavioral research.

Injury-induced Supersensitivity in the Central Gustatory System

Shiyu Chen

Department of Psychology, University of Virginia

Gustatory system has unique plasticity comparing to other sensory systems, where taste bud regeneration and change of terminal field organization have been studied extensively. However, less studies focus on terminal field change after injury. This project will demonstrate if there is an underlying mechanism for injury memory in gustatory system and influence the terminal field volume after a second injury. In this project, all mice's glossopharyngeal nerve will be transected (IXX) first and then randomly divided into three groups. In control group, glossopharyngeal nerve (IX) will be labeled with cascade blue (CB) right after the transections. In one experiment group, glossopharyngeal nerve will be labeled with CB 60 days after IXX, with chorda tympani nerve and greater superficial petrosal nerve remaining intact. In the other experiment group, glossopharyngeal nerve will be labeled 60 days after IXX, while chorda tympani nerve and greater superficial petrosal nerve will be transected to induce secondary injury response.

Making American Football Safer: changing the NFL's policy on the type of helmets allowed on the playing field.

I.A. Collello, M. Grasso, O. Kemp, S. Muffly, K. OIU, A. Schloe, and R.J. CollelloI

Virginia Tech., Blacksburg, VA., and Anat. and Neurobio., Virginia Commonwealth Univ., Richmond, VA.

Helmet-to-helmet collisions are one of the primary means by which concussions occur in Football. With over 1,500,000 people playing football nationwide, helmet-to-helmet contact accounts for a significant proportion of the >100,000 concussions that occur yearly. To evaluate helmet safety, the STAR System was developed in 2011 that rated helmets on their ability to reduce g-forces experienced by the head across a range of impact forces measured on the field, with a 5-Star rating being the best available helmets and a 1-Star the worst. Nevertheless, litigation concerns made the NFL, in 2014, adopt the position of allowing players to wear any helmet provided it is, of a suitably protective nature and produced by a professional manufacturer. This led us to ask: 1.) what helmets do NFL players wear and, 2.) does the current NFL helmet policy make the game safer? To address this, we identified the specific helmets worn by 1000 NFL players on Weeks 13 and 1 of the 2015 and 2016 seasons, respectively. Using film footage from the 16 games played and helmet air-vent patterns to identify helmets, we found that players wore a wide range of Star-Rated Helmets. Using a helmet-to-helmet impactor system, we next determined the g-forces experienced by the head when helmets of varying Star rating collided. We found that, when two 5-Star helmets collided, the g-forces experienced by the head were roughly 25% less than that observed for two 3-Star helmets colliding and roughly 50% less than that observed for two 1-Star helmets colliding. Together, these results suggest that the NFL policy of allowing players to wear any helmet put players at increased risk of receiving a concussion during helmet-to-helmet collisions. Thus, the most straightforward way to reduce concussions in Football is to mandate that players only wear helmets that receive the highest safety rating.

The parasite, Toxoplasma gondii, causes degeneration of skeletal muscles and their synapses.

Shreyaska Dahal, Richard Jin, Elizabeth Wohlfert, Gregorio Valdez
Virginia Tech Carilion Research Institute

Toxoplasmosis is a parasitic infection caused by a protozoan known as Toxoplasma gondii. During the parasite's life cycle, it forms cysts in multiple regions of the body, including the brain and skeletal muscle. In normal humans, these infections result in flu-like symptoms, and can pose a much larger health risk to the immunocompromised. To understand the pathological changes caused by T. gondii, we recently began examining the effects of T. gondii on skeletal muscles in mice. We have found extensive skeletal muscle damage, myositis and motor deficits in infected mice. Additional analysis has revealed that the neuromuscular junction (NMJ), the synapse muscle fibers form with innervating motor axons, also degenerates in mice infected with T. gondii. These deleterious cellular changes persist long after the acute phase of infection, suggesting that T. gondii continues to perpetrate damages and/or impair repair mechanisms within skeletal muscles. These findings will help develop strategies aimed at mitigating the adverse effects of T. gondii to skeletal muscles, their NMJs and possibly brain synapses.

The Effect of SOD2 Antioxidant on Localization and Expression of ATG12 in a Drosophila Model of Machado- Joseph Disease

Sanitra Desai and John M. Warrick
Department of Biology, University of Richmond

Spinocerebellar ataxia 3, more commonly known as Machado-Joseph Disease, belongs to a family of dominantly inherited human diseases that induce neurodegeneration. This lethal disease of late onset is caused by the expansion of a normally occurring polyglutamine repeat region in the coding part of the Ataxin-3 (ATX3) protein (Maceil et al. 1995). We are studying the role antioxidants and autophagy play in MJD by looking at the localization of MJD proteins and proteins associated with autophagy. Autophagy has been suggested as a cellular defense against disease causing proteins. Antioxidants reduce oxidative stress, a disease component. Therefore, therapeutic strategies of stopping the degeneration caused by MJD may include removing aggregate protein through induced autophagy and eliminating oxidative stress, caused by reactive oxidative species (ROS), using antioxidants (Paulson 2012). However, it has been shown that levels of ROS influence autophagy. This research explores whether relationships between mutant polyglutamine, autophagy, antioxidants, and neurodegeneration exist in Drosophila melanogaster, or fruit fly, models of MJD. This will be done by comparing how Autophagy Related Protein 12 (ATG12) is localized under normal and diseased conditions and the effect an increased level of antioxidant Superoxide Dismutase (SOD2) has on that localization in the Drosophila melanogaster retina. We hypothesized the expression of ATX3-Q84 will decrease the expression and/or alter localization of ATG12, inhibiting autophagy and thus increasing neurodegeneration. Increasing the SOD2 level may further alter this expression and localization even more, as increased SOD2 has been shown to increase degeneration. Our preliminary data suggests that mutant ATX3 proteins are associated with ATG12; however, this association is reduced in the presence of up-regulated SOD2. Studying the effect of and increased level of SOD2 on localization and expression of ATG12 and ATX3 will help us understand MJD's pathology and identify potential mechanisms to develop an effective therapy or cure for this lethal disease.

The effect of ITPR mis-regulation on a Drosophila model of MJD

David Hahn and John M. Warrick
Department of Biology, University of Richmond

Spinocerebellar ataxia 3, more commonly known as Machado Joseph Disease (MJD), is a fatal, hereditary disease characterized by muscle control loss and neuronal degeneration. Inositol Trisphosphate Receptor (IP3R) expression is a crucial component of the calcium-mediated signaling mechanism in cells and has been linked to other similar neurodegenerative diseases. Thus we investigated the effects of IP3R on the progression of MJD. Using a Drosophila model for MJD, IP3R expression was first up-regulated and found to increase neurodegeneration in flies over the course of seven days as compared to control models. IP3R

expression was then down-regulated via RNAi which was found to decrease neurodegeneration after seven days. Based on these results, it has been concluded that IP3R is a significant factor of the overall MJD progression mechanism. Future experiments will be conducted to accurately describe the role of IP3R signaling in MJD pathology.

Dendritic Spine Pruning in Cerebral Cortex Before Eye Opening

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Developmental synaptic pruning in the cerebral cortex is a normal, experience-dependent process. Early in postnatal development, the cerebral cortex normally overproduces synapses, and then undergoes a period in which synapses are pruned away, leaving only synapses that are important for neural function. Dysregulation of synaptic pruning in excitatory neurons of the cerebral cortex may lead to neurological disorders such as schizophrenia and autism spectrum disorder. Previous results show that dysregulated synaptic pruning after eye opening in the mouse can lead to elevated excitatory synaptic density during developmental visual critical periods. To examine the role of experience in shaping cortical circuits early in life, here we label layer II/III visual and other cortical pyramidal neurons via in utero electroporation and examine their dendritic spines before eye opening, early in the process of synaptic pruning. Results offer insight into the time course and location of excitatory synaptic pruning in cerebral cortex.

Assessing Dendritic Identity and Protein Trafficking in Regenerated Dendrites in Adult Drosophila

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Neurons exchange information through synaptic signals or sensory inputs received by the dendrites, which may generate action potentials that can propagate along the axon. This exchange can be interrupted when axons or dendrites are damaged. Axonal regeneration has been widely studied, but dendritic regeneration has not received the same attention. For that reason we have focused this study on assessing injury recovery in Drosophila dendrites. The model we used has several desirable features: neurons that are easily imaged, a tractable genetic system, and the potential to build a framework for future studies. Previous research has assessed dendritic growth in response to acute injury using Drosophila larvae. We aim to evaluate such growth in adult animals. Drosophila larvae are still developing. We aim to understand how stable adult dendritic arbors respond to injury. When injuring the animals, we utilize lasers from a two-photon microscope to severe all dendrites at the first branch point by laser ablation, leaving a bald neuron. In assessing injury response, we image animals and perform immunohistochemistry staining. This staining assesses the injured neuron's maintenance of neuronal identity, ability to correctly traffic somatodendritic proteins and integrity of cytoskeletal structure. We stained these neurons using the

antibody against PPK-26, a neuronal type specific marker, to assess the identity of injured neurons. We also stain for PPK-26 in a non-permealized condition to attest that these proteins are located at the surface of the neuron, as in uninjured neurons. Finally, we test for stabilized microtubules, by staining for Futsch, the Drosophila MAP1B homolog. Preliminary results indicate the presence of PPK-26 in regenerated neurons suggesting that the dendrites present morphological similarities to those of fully functioning healthy neurons. We have also located presence of Futsch marker in regrown dendrites. Although certainty is only reached after quantitative analyses, we hope to find that these dendrites also contain microtubules in its regenerated expansions. Ultimately, we aspire to expand our knowledge of the cytoskeletal structure of regenerated dendrites and compare with findings of the larvae model, which have established that regrown dendrites maintain some but not all of the characteristics of uninjured dendrites.

Signaling in Early Proprioceptive Circuit Formation

Lucy Jin, Irene Cheng, Christopher Deppmann
University of Virginia

The proprioceptive nervous system provides information on the position and movement of body parts, namely limbs, in space. The ligand-receptor pair, Neurotrophin 3 (NT3)-TrkC represents the pro-construction signal required for proprioceptor survival and axon growth. In addition to pro-constructive signaling, pro-destructive signaling is required for fine-tuning and refinement of the proprioceptive system. Pro-destructive signaling that antagonizes NT3-TrkC may be mediated by the tumor necrosis factor receptor superfamily (TNFRSF) signaling, namely by p75 neurotrophic receptor (p75NTR) and TNFα-TNFR1/TNFR2. Here, we investigate the potential role of TNFRSF members in destructive signaling through immunohistochemical and behavioral analyses on knockout mice lacking TNFRSF members. Preliminary results show that the absence of p75NTR results in reduced proprioceptive cell survival and increased motor impairment. The absence of TNFα, though, rescues proprioceptor death induced by the absence of p75NTR. TNFR1 support motor function while TNFR2 impairs motor function. Contrary to our hypothesis that these proteins primarily play a role in destructive signaling, TNFRSF members appear to have roles in both constructive and destructive signaling.

Language translates to executive functions: investigating the bilingual advantage in inhibitory control

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Given that twenty-percent of the U.S. population speaks a language other than English at home, it is imperative to assess the effect of a second language on brain structure and function. The bilingual advantage hypothesis claims that command over two languages leads to enhanced non-linguistic cognition. The need of a bilingual to maintain both languages active simultaneously, inhibit one, and

flexibly switch between both may transfer to executive functions (EFs): a group of top-down control processes. The present study investigates the effect of mono- and bilingualism on inhibitory control and cognitive flexibility across age groups. Implementing an online format, mono- and bilinguals (ages 18-89, n = 334) of diverse language parities completed a language background and demographic questionnaire. EF performance was assessed using a Simon task, task-switching paradigm, and directed forgetting paradigm. In addition, Shipley-II vocabulary and block patterns tests served to assess crystallized and fluid intelligence. Results will (1) demonstrate at what age, if at all, the bilingual advantage becomes robust, (2) which linguistic experiences contribute advantageously to executive function, and (3) determine whether a directed forgetting paradigm can be implemented in bilingualism research. It is hypothesized that bilinguals will outperform monolinguals on all EF tasks and that this difference will be most pronounced in older ages. Finally, it is hypothesized that among bilinguals, age of active onset and amount of language switching will be most predictive of outcomes. Preliminary findings suggest that after controlling for various lifestyle factors, age is most predictive of task performance.

Trafficking and identity of the NGF-TrkA signaling endosome and its role in post-synaptic density formation

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Sympathetic neurons, responsible for the "fight or flight" response, have periods of growth and cell death during development that are regulated by the amount of available nerve growth factor (NGF). NGF is released by peripheral organs and functions as a signal that signals to growing neurons that they've reached their final destination. Upon the neurons reaching their final destination, NGF binds to its high affinity receptor, TrkA. TrkA and NGF internalize into an endosome which traffics retrogradely back to the soma to prevent apoptosis. While it is known that NGF-TrkA signaling endosomes (SEs) move retrogradely back to the soma, the Rab protein(s) associated with the SEs in the soma and beyond are unknown although multiple Rab proteins have been implicated. It has been shown that TrkA SEs are present in the dendrites of superior cervical ganglia (SCG) in mice and regulate post-synaptic density (PSD) formation. Additionally, if the MEK/MAPK signaling pathway is inhibited, TrkA SEs are not found in the dendrites and PSDs are not formed. Currently, we are investigating the pathway and identity of TrkA SEs to elucidate the route through which SEs arrive in the dendrites, the Rab protein(s) associated with the SEs at each point in that route, and the signaling pathways that are required for TrkA SEs to be present in the dendrites to regulate PSD formation.

Loss of FGFBP1 delays synapse development in the hippocampus

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Fibroblast Growth Factor Binding Protein 1 (FGFBP1) is a protein that functions to release FGF ligands from the extracellular matrix. In this manner, FGFBP1 increases the number of FGF ligands that bind and activate cognate receptors. Specifically, FGFBP1 binds to FGF7 and FGF22, which are important for the formation of inhibitory and excitatory synapses respectively. Our lab recently showed that FGFBP1 plays an important role in maintaining the structural integrity of neuromuscular junctions (NMJs), the synapse formed between motor neurons and skeletal muscles, during normal aging and progression of amyotrophic lateral sclerosis. Here we asked if FGFBP1 also affects brain synapses dependent on FGF7 and FGF22 for their proper development. We found that FGFBP1 expression decreases as synapses mature in the hippocampus. To explore the function of FGFBP1, we examined excitatory and inhibitory synapses in FGFBP1 knockout and control mice during development. Using immunostaining, we found that loss of FGFBP1 reduces the density of synaptic inputs positive for glutamatergic and GABAergic markers in the hippocampus during development. However, the density, and thus number, of synaptic inputs is unchanged in young adult animals. These results suggest that FGFBP1 plays an important function in the timely formation and maturation of neural circuits in the brain.

What is the nociceptive withdrawal response of unrestrained rats when noxious stimulation is delivered to the tail or feet?

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The nociceptive withdrawal response (NWR) is a protective response to a noxious stimulus. The response can vary due to many factors, including stimulus intensity, stimulus location and posture. In previous studies from our laboratory, rats were restrained in an acrylic tube during stimulation. It was observed that rats, when given a heat stimulus to the tail, moved their tails in the opposite direction of the stimulation site. It is unknown however, whether they concurrently exhibit postural body movements. The specific aim of this study was to examine postural changes in the body of rats evoked by a noxious heat stimulus to the tail or foot in an unrestrained, as opposed to retrained, setting. Sprague-Dawley rats (n=7) were first anesthetized with isofluorane and black marks were placed on the feet, tail, and body to target stimulation and track movement of the animal. Rats were then placed on an open glass table with a video camera (60 fps) positioned underneath to capture movement of the tail, feet and body. Localized stimuli were delivered via an infrared laser to one of five points on the tail or the plantar surface of one of the four feet. Using the recorded video and a tracking software (ProAnalyst), tail, foot, and body movement frames were recorded and changes in body angle, initial/final foot positions and number of steps were tracked. We found that when the tail or feet were stimulated, the stimulated body part was withdrawn between 1 and 3 seconds following stimulation nearly 100% of the time; however rats exhibited varying escape strategies depending on if the tail or feet were stimulated. When the tail was stimulated, rats tended to exhibit initial tail movement followed by a 180Â° rotation to face the stimulus. When the feet were

stimulated, there was far less turning and the rats tended to simply shift their body weight with minimal foot movement. Though these responses were relatively consistent among the rats, it is important to note that individual rats responded qualitatively differently. In summary, rats appear to use similar local withdrawal strategies, but different postural strategies. Consequently, testing rats while restrained may miss these associated and potentially functionally important postural strategies.

Tree Shrew: A Step Closer to Understanding Synaptic Circuity of Primate Taste Pathways

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While sensory thalamic nuclei display remarkable similarities in their inputs, intrinsic circuitry, synaptic organization, and neurotransmitters, whether organization through chemical sense pathways follows equivalent principles is unknown. Studying the gustatory pathway in rats, we recently revealed that synaptic circuitry in the gustatory thalamus (VPMpc) is considerably different compared to other mammalian thalamic nuclei (Holtz et al., 2015). The differences between the rat VPMpc and other sensory nuclei are: primary axons synapsing exclusively close to cell bodies, lack of both glomeruli and triadic arrangements, and dense core vesicles and CGRP label marking inputs from multiple origins. We argue that the unique properties of the rat gustatory thalamus are a reflection of pathway discrepancies between primates and rodents. The primate gustatory thalamus receives primary input directly from nucleus tractus solitarii (NTS). In rodents, the NTS instead projects to the parabrachial nucleus (PBN), then to the thalamus and amygdala. To test this, we examined the gustatory thalamus in the tree shrew, a close relative to primates. The tree shrew gustatory thalamus (VPMP) has well-differentiated cytoarchitectonic borders delineated by myelin and cytochrome oxidase. Unlike the rodent VPMpc, but like other thalamic nuclei, the tree shrew VPMP contains VGluT2 and lacks CGRP+ terminals. Electron microscopy reveals that VGluT2+ terminals engage in triads in VPMP, another hallmark of retinal inputs in the thalamus. The tree shrew VPMP also receives modulatory inputs, including cholinergic and GABAergic positive terminals. Our data confirm the hypothesis that synaptic organization of the tree shrew gustatory thalamus, unlike the rat VPMpc, carries common characteristics of sensory thalamic nuclei in mammals, providing evidence that synaptic organization principles in sensory thalamic nuclei are preserved across all senses. Our findings also highlight tree shrews as a necessarily preferred model over rodents for studying gustatory information flow to cortex.

The Effects of Alcohol on the cTMT for Ascending and Descending Limbs of BAC

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Department of Psychology, Roanoke College, Roanoke, VA

Blood alcohol concentrations (BACs) as low as 0.040 mg/ml generate cognitive and visual impairments such as planning, working memory, blurred vision, and difficulty finding objects in space. Neuropsychological tests, such as the trail making test (TMT), can assess the severity of impairment. This study aimed to investigate the effects of alcohol on executive functioning, visuomotor performance, and perceived self-impairment using a computerized trail making test (cTMT), which reduces practice effects. The influences of alcohol were tested over the variables of total time, median wait time, and version. Twenty-six participants (8 male, 18 female) with a minimum age of 21 years were recruited. Participants were randomly selected to be in the placebo group (n=7) or the experimental group (n=19). The experimental group performed the cTMT at four target points in relation to two drinks: baseline (0.000 mg/ml), ascending (0.060 mg/ml)—after first drink, peak (0.080 mg/ml)— after second drink, and descending (0.060 mg/ml). While participants who received alcohol tended to report themselves at a lower BAC than they actually were, there was no significant difference between perceived and actual BAC. Additionally, alcohol did not significantly affect performance on the cTMT. While there was a significant correlation of observed and reported BAC for the ascending and peak levels, there was not a significant correlation for the descending level. This indicates that people are worse at accurately assessing their BAC on the descending curve. Main effects of version on total time and median wait time reveal that Trail B took longer. Any level of impairment experienced at lower BACs was not detected by the cTMT. Nonetheless, the cTMT may identify impairments at higher BACs. Future studies could attempt to raise BAC levels to at least 0.100 mg/ml in order to examine more distinctive effects of alcohol on the specific cognitive tasks required by the cTMT.

p75NTR Involvement in Metabolic Control Pathways

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Obesity levels present an urgent public health challenge. The extensive sympathetic and sensory innervation of white adipose tissue (WAT) provides a target for the treatment of obesity. This innervation controls energy liberation from adipocytes. We have identified a putative protein, integral in the regulation of lipolytic events, the p75 neurotrophin receptor (p75NTR). The p75NTR is expressed in several tissue types and binds a wide variety of neurotrophins. A lack of p75NTR has been previously shown to reduce weight gain in response to a high fat diet by increasing the rate of energy liberation from fat stores. Here, we show that mice deficient in p75NTR lose less weight due to restricted caloric intake.
This development suggests p75NTR modulates energy homeostasis and does not solely upregulate lipolysis. We aim to understand the mechanism by which p75NTR maintains energetic homeostasis. To quantify the plasticity of p75NTR expression during dietary restriction we examined its levels in both sympathetic nerves and adipocytes, derived from WAT depots. To elucidate the cell-specific function of p75, we subjected two conditional knockouts of p75NTR, in sympathetic neurons and adipocytes, to caloric

restriction and analyzed their weight loss. The understanding of p75NTR involvement and, more broadly, the role of fat depot innervation allows for a more complete model for energetic homeostasis maintenance and suggests neuromodulation as an avenue for weight control.

Selective Stimulation of A-delta Nociceptors in Rat Hind Limb and the Resulting the Nociceptive Withdrawal Response

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Rats rapidly withdraw their hind limb in response to heat or other noxious stimulation, which is known as the Nociceptive Withdrawal Response (NWR). Two types of nociceptors may mediate the NWR: C-fibers and Aδ nociceptors. Among the differences between these two types of nociceptors, C-fibers have large receptive fields while Aδ nociceptors have much smaller receptive fields. Previous studies have shown that the direction of the NWR does not depend on stimulus location. However, these experiments used a method of heating that may have predominantly stimulated C-fibers. If C-fibers were stimulated, we might expect no dependence on stimulus location due to their larger receptive field compared to Aδ nociceptors. Therefore, it remains possible that Aδ nociceptors could mediate a response that is dependent upon stimulus location. The specific aim of our ongoing experiments is use preferential stimulation of Aδ ´ nociceptors using high intensity, short duration (100ms) pulses of heat to determine if the NWR depends in stimulus location. The length of the pulse was determined based on previous research showing that Aδ nociceptors are selectively activated with short, high intensity pulses of heat. Because Aδ fibers have small receptive fields, we hypothesize that the selective stimulus will result in a response that is dependent on stimulus location. Five small (1 mm) spots (three aligned rostral-caudal, three aligned lateral-medial) were blackened on the plantar surface of the left hind paw. These spots were stimulated in a randomized sequence and the initial and final positions of the paw were recorded with a camcorder (60 fps @ 1080p) placed underneath the rat. When stimulated, the rat picks up its paw and rapidly places it back down on the glass. The difference between the initial and final positions represents the NWR movement response vector. Unexpectedly, stimulus location still did not have an effect on the direction of the NWR. Rather, preliminary results (n=6) suggest that the direction of the response is determined only by the initial position of the paw, as observed in previous experiments where C-fibers were presumably stimulated. These results further substantiate our findings that the NWR is organized around initial posture rather than the details of the noxious stimulus.

Western-Style Diet Differentially Impacts Neuroanatomy and Memory Performance of Female and Male Rats

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A growing body of research demonstrates an association between diet-induced obesity and cognitive impairments; however, past studies have primarily utilized male subjects. Due to estrogens' effects on behavior and neuron structure, it is crucial to explore sex differences in the effects of obesogenic diets. The present study examined the effect of Western-style Diet (WSD) on memory and dendritic complexity of male (n = 18) and female (n = 38) Sprague-Dawley rats. All females were ovariectomized (OVX) and half were implanted with a slow-release 17 β-estradiol pellet (OVX+E). Following ten weeks of diet exposure, spatial and episodic memory were assessed using the Morris Water Maze (MWM) and Novel-Object Recognition (NOR) tasks, respectively. At termination, brains were removed and prepared with the Golgi-Cox method. Stained neurons in both the hippocampus and entorhinal cortex (EC) were imaged and digitally reconstructed. Results indicated significant differences in percent body fat and calorie consumption between hormonal and dietary conditions. Reduced NOR exploration ratios in WSD males and fewer MWM annulus crossings in WSD OVX+E females were also observed. OVX+E rats fed a WSD experienced significant decreases in the number of dendritic branches and terminal tips in the EC, as well as a decreased average dendritic length in the hippocampus compared to control-fed counterparts. Sholl analysis revealed that WSD reduced neuronal complexity in the EC of OVX+E rats and in the hippocampus of male and OVX rats, suggesting differential susceptibility to diets. These results demonstrate the importance of investigating sex differences, especially in obesity-related impairments.

The Role of ipRGCs in Light-Dependent Modulation of Learned Fear Responses

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The study of mechanisms by which environmental information travels to the brain and influences learned fear responses is necessary in order to better understand fear-related disorders. Previous research in mice using a tone-cued fear conditioning paradigm has established that light enhances learned fear responses. This effect is predominantly, if not exclusively, driven by rod and cone photoreceptors; however, the class of retinal ganglion cells conveying this photic information to central sites involved in fear responses remains unknown. Intrinsically photosensitive retinal ganglion cells (ipRGCs) have been shown to mediate light's effects on various behavioral, physiological, and higher-order cognitive processes. We hypothesized that light-dependent modulation of behavioral responses to learned fear is also mediated through ipRGCs. To investigate this, we tested mice with progressive loss of ipRGCs in either light or dark conditions, using a tone-cued fear conditioning paradigm. Mice lacking ipRGCs do not exhibit a statistically significant difference in freezing levels in dark or light conditions, despite possessing a full complement of rods and cones. In contrast, their wild type littermates' freezing levels are significantly higher when tested in light compared to dark. Thus, our data indicate ipRGCs are indeed required to convey photic information to sites in the brain that control fear learning. These findings have implications for the treatment of PTSD in

humans, as ambient lighting conditions during PTSD therapies may affect whether such treatments are ultimately successful.

Investigating the effect of AKAP on TREK-1 activation by arachidonic acid

Bridgette Heine, **Jacqueline Sinnott**, Linda M. Boland, Ph.D
Department of Biology, University of Richmond

TREK-1 is a two-pore domain (K2P) potassium channel that is regulated by a variety of stimuli including fatty acids, membrane stretch, pH, and phosphorylation. It plays key roles in the central nervous system, heart, blood vessels, kidneys, as well as other organs. Investigations aimed at characterizing regions of the TREK C-terminal domain have led to a greater understanding of the structure and function of this potassium ion channel as well as its regulation. Whiles studies have investigated the critical residues of the TREK C-terminus for these various stimuli, many studies fail to explain interactions between overlapping regions. One remaining gap exists between the relationship of a phosphorylation site that activates TREK by arachidonic acid (AA) and its overlap with the binding site of an A-Anchoring Kinase Anchor Protein (AKAP) that abolishes AA sensitivity of TREK. Using a combination of bioluminescence and electrophysiology assays, we aimed to study the connection between the critical S300 residue required for AA activation the binding region of AKAP binding region, both of which are found in the C-terminus. Bioluminescence assays of epitope-tagged TREK-1 C-terminal mutants suggest that the region between G308 and L332 is critical for channel expression in the plasma membrane. Whole oocyte recordings suggest that residues G294-G308 play a role in activation by arachidonic acid. In trying to explore the relationship between previously characterized critical regions, we are investigating the overlapping roles of AKAP scaffolding proteins and phosphorylation in activation of TREK by AA.

Mass-Univariate and Multivariate Approaches to Studying the Effects of Subconcussion on Functional Connectivity

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With an estimated 750,000 sports-related concussions (SRC) each year in the United States, concussion is increasingly recognized as a widespread issue. However, distinct from concussion is the entity of subconcussion. One review defined subconcussion as a "cranial" impact that does not result in a concussion on clinical grounds." The average college football player can experience over a thousand subconcussive hits a season, suggesting that further understanding subconcussion's effect on the brain is important for understanding the risks for players in high impact sports. Functional magnetic resonance imaging (fMRI) represents a very promising method for measuring the physiologic effects of

subconcussion.One study found a local functional connectivity measure, regional homogeneity (ReHo), to be disrupted in the days to weeks following a concussion, but found that global brain connectivity, weighted degree centrality (DC), was not changed in the same time frame. These metrics examine the connectivity of the entire cortex, and together provide complementary functional information that can help clarify subconcussion's effect on brain function.The present study uses measures of functional connectivity (ReHo, and DC) to investigate the effects of subconcussion from college football players in a single season. This study uses both mass-univariate and multivariate approaches to studying the effect of subconcussion on functional connectivity.

Effects of Medial Prefrontal Cortical Administration of the Orexin-2 Receptor Antagonist, TCS-OX2-29, on Attentional Performance in Rats

Austin Tapp

Department of Neuroscience, The College of William and Mary

Orexins are excitatory neuropeptides that come in two isoforms, Orexin A and Orexin B, and serve as ligands for the G-protein coupled orexin 1 and orexin 2 receptors (Ox1R and Ox2R, respectively). Changes in orexinergic transmission are thought to contribute to attentional processing. While several studies have examined the role of Ox1Rs in attention, less research has assessed the contribution of Ox2Rs. Moreover, several lines of evidence suggest that the right medial prefrontal cortex is particularly critical for visual attentional performance. Taking all of this into consideration, the goal of the present experiment was to test the effects Ox2R blockade, via administration of TCS-OX2-29, into the left or right medial prefrontal on visual attention. The results suggest that low dose administration of TCS-OX2-29 into the right, but not into the left, medial prefrontal cortex enhanced attentional performance. We speculate that relatively mild antagonism of Ox2Rs may have increased the sensitivity of these receptors to subsequent orexin transmission, thereby enhancing attentional performance. Ongoing projects in our laboratory are assessing whether these effects are observed when TCS-OX2-29 is infused into other brain regions known to be critical for attentional performance.

The effect of misregulation of HAT Tip60 on a Drosophila model of MJD

Abigail Watterson and John M. Warrick

Department of Biology, University of Richmond

Machado-Joseph Disease (MJD) is a spinocerebellar ataxia caused by a polyglutamine repeat expansion in the Ataxin-3 protein that leads to motor neuron degeneration. The mechanisms by which the toxic ATX3 protein causes degeneration are not yet known. In MJD, the Ataxin-3 protein misfolds, leading to the formation of aggregates. Histone acetyltransferases (HATs) may get trapped in the aggregates, therefore preventing proper regulation of histone acetylation and gene transcription. As a result, neurons may become dysfunctional and die due to transcriptional dysregulation. The histone acetyltransferase Tip60

functions in neuronal gene control and apoptosis, and elevated levels have been found to rescue axonal transport defects, characterized by locomotive phenotypes, in a Drosophila melanogaster model of Alzheimer's disease (Johnson et. al). However, HAT Tip60 has not yet been studied in MJD. This research investigates the effect of up-regulating and down-regulating HAT Tip60 in a MJD Drosophila melanogaster model to determine how levels of Tip60 expression affect the progression of the disease in hopes that this research will eventually lead to an effective target for MJD and other neurodegenerative disease treatments.

HighSugar, HighFat Diet Increased Estradiol Surge during Proestrus and Induced Polycystic Ovaries in Cycling Female Rats

Jennifer E. Zachry, Veronika V. Pogrebna , Jackson A. Roberts, Katrina M. Volk and Natalia Toporikova, PhD

Washington and Lee University

A High-Sugar, High-Fat (HSHF) diet causes numerous metabolic abnormalities. However, it is unknown whether diet can disrupt pre-ovulatory hormone levels, leading to reproductive complications. We hypothesize that a HSHF diet alters the E2 surge during proestrus, which is important for ovulation, causing cycle irregularities. Furthermore, a HSHF diet may disrupt folliculogenesis by inducing cyst formation and reducing corpora lutea. Female Sprague Dawley rats (age 23 days) were separated into diet groups. Controls (n=14) received standard chow and water ad libitum and HSHF rats (n=16) received 60% calories from fat, 30% sucrose solution and water ad libitum. Rats were weighed every other day and cycles tracked daily using vaginal cytology. After three weeks, HSHF rats weighed significantly more than controls (p<0.01833). After 14 weeks, there were significantly more irregular and non-cycling HSHF rats compared to controls. All rats underwent jugular cannulation the morning of proestrus. Blood samples were taken at ZT11 and ZT13 during the pre-ovulatory proestrus surge and ZT1 and ZT3 of estrus for baseline. Serum LH and FSH (Multiplex assay) and E2 and progesterone (ELISA) were measured. Terminal blood samples were taken on diestrus-2 and basal testosterone levels were measured (ELISA). Results showed a significant increase in E2 in HSHF group during the proestrus surge (6.2±0.57 pg/mL control vs. 14.8±3.26 pg/mL HSHF ; p=0.0104). Term testosterone levels in HSHF diet group, indicating hyperandrogenism (26.42±1.77 ng/mL control vs. 32.85±2.21 HSHF ; p = 0.031 fixed, frozen, sectioned, and stained (H&E). Follicular counts showed significant increases in cyst number and decreases in number of corpora lutea, indicating polycystic ovaries in HSH rats. Our results demonstrate that HSHF diet causes higher E2 surge during the preÂ¬ovulatory proestrus stage, and induces acyclicity and polycystic ovaries. We conclude a HSHF diet significantly impacts the reproductive system.

Monday Poster Presenters

Research Associates

Poster	Presenter	Institution
1	Mandakh Bekhbat	Virginia Commonwealth University
2	Margot Bjoring	University of Virginia
3	Jessica Boni	Virginia Tech
4	Justin Brown	James Madison University
5	Gabriela Carrillo	Virginia Tech
6	Lata Chaunsali	Virginia Tech
7	Kareem Clark	Virginia Commonwealth University
8	Brian Connor	Virginia Tech
9	Aaron Corbin	University of Richmond
10	Quentin Fischer	Virginia Tech
11	Mark Gabriele	James Madison University
12	Rockelle Guthrie	Virginia Commonwealth University
13	Amanda Hazy	Virginia Tech
14	Benjamin Heithoff	Virginia Tech
15	Leanne Holt	Virginia Tech
16	Molly Hyer	Virginia Commonwealth University
17	Uri Kahanovitch	Virginia Tech
18	Djanenkhodja Kalikolov	Virginia Tech
19	Alicia Kerr	Virginia Tech
20	Courtney Knill	Virginia Tech
21	Yeonwoo Lebovitz	Virginia Tech
22	Eden Maness	William & Mary
23	Nicholas Maxwell	Virginia Tech
24	Nadine Michel	University of Virginia
25	William Mills III	Virginia Tech
26	Aboozar Monavarfeshani	Virginia Tech
27	Benjamin Okyere	Virginia Tech
28	Oleksii Shandra	Virginia Tech
29	Jianmin Su	Virginia Tech
30	Elizabeth Sugg	Virginia Tech
31	Natalia Sutherland	Virginia Tech
32	Thomas Taetzsch	Virginia Tech
33	Bhanu Tewari	Virginia Tech
34	Alex Winemiller	Virginia Tech
35	Jianping Wu	Virginia Tech

Research Associates' Abstracts

Chronic Adolescent Stress Leads to Sex-Specific Neuroimmune Alterations and Transcriptomic Remodeling in the Rat Hippocampus

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Chronic stress adversely impacts health through disrupting the precisely coordinated regulation of neuroendocrine systems and behavior. Males and females respond differently to physiological and psychosocial stressors, and exhibit distinct neuroinflammatory and behavioral outcomes. We hypothesized that when experienced during the developmental period of adolescence, chronic stress would cause an exaggerated pro-inflammatory response upon a secondary immune challenge in adulthood. Furthermore, we hypothesized that males and females with a history of chronic adolescent stress (CAS) would respond differently to this immune probe. Male and female adolescent rats underwent a mixed-modality CAS paradigm whereas a subset of littermates received no stress. On post-natal day 94, all rats received a single, systemic injection of either a low dose of lipopolysaccharide (LPS), a potent immune stimulant, or vehicle. Total RNA from the hippocampus was used to perform RNA-Seq (Illumina HiSeq). The expression of key CAS-responsive neuroimmune genes identified from the RNA-Seq were next verified via targeted qPCR experiments. Gene set enrichment analysis (GSEA) revealed that LPS treatment led to a significant enrichment of more than 500 gene sets including immunologic signature sets and canonical pathways in all groups regardless of sex and experience of stress. Among all animals that received LPS simulation, male, but not female, rats that underwent chronic adolescent stress (CAS) displayed enhanced enrichment of the NF-kappa-B signaling pathway compared to their non-stressed counterparts. Assessment of plasma cytokine concentration and NF-kappa-Bâ€™s DNA-binding activity in the spleen suggested that the central effects of CAS on the NF-kappa-B pathway are independent of peripheral inflammation. Our results indicate that chronic stress experienced during the adolescent period leads to long-lasting changes to the hippocampal genomic profile, and profoundly alters the genomic response to an immune challenge encountered in adulthood.

Receptive field estimation for simulated phasic and tonic neurons in the auditory system of songbirds

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Birdsong is a complex vocalization that bears important similarities to human speech. Critical to recognizing speech or birdsong is the ability to discriminate between similar sequences of sound that may

carry different meanings. Although birds lack the cortex of mammals, their auditory system has a hierarchical organization that resembles mammalian primary auditory cortex (A1). The caudal mesopallium (CM) is a secondary auditory area in the auditory system of songbirds that may be analogous to layer 2/3 of mammalian A1. Many neurons in this area respond selectively to the songs of particular conspecifics, indicating CM as a potential site for song discrimination. Here, we are particularly interested in two populations of neurons in CM distinguished by their tonic or phasic firing responses to electrical current stimulation. These two types of response patterns transform a signal in different ways and may contribute to the ability of CM to discriminate between songs by providing complementary information about their identities. Using biophysical models of tonic and phasic neurons, we simulate the responses of neurons in these populations to an auditory stimulus and use the simulated responses to estimate the neurons' receptive fields. This technique allows us to explore systematic differences in the receptive fields of phasic and tonic neurons in CM and how these differences contribute to the identification of a complex stimulus.

The role of astrocytic Kir4.1 in the development of epilepsy

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Kir4.1, a glial specific, inwardly rectifying potassium channel contributes significantly to astrocyte membrane properties and extracellular K+ homeostasis. Reduced protein expression and channel function is observed in nearly every CNS pathology and astrocyte specific rescue reduces neuronal hyperexcitability and dysfunction, leading to the notion that Kir4.1 may represent a novel therapeutic target. Using a pilocarpine model of status epilepticus and spontaneous recurrent seizures in adult male rats we observed a 50% reduction in hippocampal Kir4.1 expression 24 hours post status epilepticus that was associated with hippocampal extravasation of Evans blue dye, used as an indicator of blood brain barrier breakdown. Reduced Kir4.1 protein levels were maintained through the latent period, preceded the onset of spontaneous recurrent seizures and were maintained in epileptic animals. Work underway is aimed at addressing 1) identifying the spatio-temporal extent of Kir4.1 reduction during the latency period of epileptogenesis 2) determine if the sustained reduction in Kir4.1 expression is due to changes in DNA methylation 3) can rescuing Kir4.1 expression in astrocytes of the CA1 region of the hippocampus reduce neuronal excitability?

The Extracellular Matrix Protein F-spondin is Required for the Maintenance of Circadian Rhythms

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Over 20 classes of retinal ganglion cells exist in the mammalian retina, each with unique functions, morphologies and projection patterns. In previous studies aimed at elucidating how different classes of

RGC axons target different retino-recipient nuclei, we identified Reelin (an extracelluar matrix protein) as being important in directing the targeting of M1 intrinsically photosensitive RGCs (ipRGCs) to the ventral lateral geniculate nucleus (vLGN) and the intergeniculate leaflet (IGL). In mice lacking Reelin, axons from M1 ipRGCs were misrouted into inappropriate regions of the mouse thalamus. However, this specific class of ipRGCs, which encodes for non-image forming responses to light that are necessary for circadian photoentrainment, target other regions of the brain where Reelin is not expressed, such as the suprachiasmatic nucleus (SCN). In the current study we sought to understand what unique cues M1 ipRGCs use to target the SCN. Using a bio-informatic approach, we identified three candidate targeting cues enriched in the developing SCN: F-spondin (also called Spon1), Slit1, and ALCAM. Using targeted mouse mutants we tested the necessity of each cue for retino-hypothalamic targeting and for establishing normal circardian rhytyms. All three cues appeared largely dispensable for retinohypothalamic targeting. Moreover, Slit1 and ALCAM were not required for normal photoentrainment or the maintenance of circadian rhythms. While F-spondin-deficient mice (spon1 -/-) were able to photoentrain in normal light:dark conditions, they displayed dramatic arrhythmic behavior and an absence of free-running behavior in total darkness. Behavioral analyses suggest that these mutants lack intrinsic circadian behavior and that their ability to photoentrain in normal light:dark conditions is the result of light-derived masking. These results demonstrate the strong influence of light-derived signals in regulating innate circadian behaviors and reveal a novel role for F-spondin in maintaining circadian rhythms.

Alteration in peritumoral hyperexcitability in a pediatric glioma model

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Pediatric glioblastoma is a rare group of aggressive primary brain tumors and one of the leading causes of cancer-related deaths in children that remains incurable despite current therapies. Pediatric glioma is phenotypically and molecularly distinct from adult gliomas but they are often accompanied by seizures as a comorbidity. Previous studies have shown that GABAergic disinhibition in peritumoral neurons as a mechanism involved in glioma-associated seizures in an adult glioma model. In these neurons, the plasmalemmal expression of KCC2- a potassium-chloride transporter, which establishes the low Cl_ concentration required for GABA receptor-mediated inhibition, was significantly decreased. The expression of KCC2 is developmentally regulated which causes GABA to be depolarizing early in development. It is unknown if and how gliomas affect the expression and function of KCC2 early in development. To determine this, we developed a pediatric glioma mouse model where postnatal day 2-3 pups are intracranially injected with pediatric patient-derived glioma cells. Our electrophysiological recordings revealed that peritumoral layer 2/3 pyramidal neurons are more depolarized and have a lower threshold for firing action potentials compared to neurons from sham-operated animals. Moreover, peritumoral neurons displayed pronounced spontaneously occurring epileptiform activity compared to adult peritumoral neurons and sham controls. We also found a decrease in the expression of KCC2 in pediatric

peritumoral neurons. Although KCC2 expression was also decreased in pediatric peritumoral neurons, their enhanced hyperexcitability suggest that other mechanisms might be involved in the pediatric brain. Further studies will determine whether tumor-induced changes in NKCC1 expression and altered GABAergic synaptic transmission contributes to the peritumoral hyperexcitability observed in the pediatric glioma model.

Alteration of Axon Initial Segment Stability and Function in In Vivo and In Vitro Inflammatory Models

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Axonal pathology is a key contributor to long-term disability in multiple sclerosis (MS), an inflammatory demyelinating disease of the central nervous system (CNS), but the mechanisms that underlie axonal pathology have not been fully clarified. While most axonal pathologies characterized in MS result as a direct consequence of myelin loss, we have recently identified a primary axonal insult in mice that develop experimental autoimmune encephalomyelitis (EAE), a mouse model commonly used to mimic the pathogenesis of MS. This primary insult is the disruption of the axon initial segment (AIS), a subdomain of the axon that acts as the trigger zone for action potential generation, identified through decreased immunolabeling of crucial AIS proteins. Although we have shown structural alterations in the AIS, the functional consequences as well as the underlying mechanism of this disruption remain unclear. Here we present preliminary evidence, through electrophysiological recordings, of altered neuronal function in neurons that have lost AIS protein clustering in EAE. These findings highlight the important contribution that this disruption may have on the neurological deficits associated with MS. Additionally, our previous work implicated oxidative stress as a potential mediator of this axonal insult, as treatment with a free radical scavenger attenuated AIS loss. In order to further investigate the role of oxidative stress in modulating AIS stability, we have employed an in vitro model in which primary cortical neurons are exposed to SIN-1, a spontaneous reactive oxygen and nitrogen species generator. Through this approach, we have preliminary evidence that oxidative stress is capable of AIS disruption in a dose-dependent manner and potentially acts through induction of calcium influx through L-type voltage gated calcium channels resulting in calpain activation.

The Effect of Blood Plasma on Astrocytic Kir4.1 and Glt-1 Expression

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Astrocytes are glial cells within the Central Nervous System (CNS) that function to maintain potassium and glutamate homeostasis, mediate cerebral blood flow and maintain the blood brain barrier (BBB), among other tasks. In the forebrain, potassium and glutamate homeostasis is maintained, in part, by an inwardly

rectifying K+ channel, Kir4.1, and the glutamate transporter Glt-1. Our Preliminary data show Kir4.1 and Glt-1 down-regulation in areas with microbleeds in a mouse model of mild TBI as early as 30 minutes after the injury. Yet, the molecular mechanisms mediating the pathologic changes in the expression of Glt-1 and Kir4.1 are unresolved. This research project seeks to determine if damage to the BBB following mild TBI results in the loss of astrocytic Kir4.1 and Glt-1 expression. We hypothesize that downregulation of Kir4.1 and Glt-1 in astrocytes is caused by a blood plasma factor entering the CNS in areas with damage to the BBB. Mouse astrocytes were isolated: 1. traditionally by growth of post-natal neural tissue in FBS-containing media followed by passaging of the cells into FBS-containing or serum-free media (Sato + HB-EGF). 2. by magnetic cell sorting (MACS) of dissociated brain tissue followed by direct plating in FBS-based or Sato + HB-EGF media. Kir4.1 and Glt-1 expression was assessed using immunocytochemistry (ICC). Plasma Exposure does not down-regulate Kir4.1 or Glt-1 in FBS media when astrocytes were grown in FBS-containing medium initially. These observations contradict the in-vivo finding that breakdown of the blood brain barrier in mild TBI results in down-regulation of Kir4.1 and Glt-1. In Sato, a serum free medium, Kir4.1 expression appears down-regulated by 24-hour plasma exposure following ACSA-2 sorting, yet Glt-1 expression seems relatively constant under these conditions. Cell densities and Kir4.1 expression are both decreased in Sato as compared to FBS. In conclusion, our primary astrocytes cultured exclusively in a chemically defined medium responded to plasma exposure with down-regulation of Kir4.1 while those cultured in a serum-based media did not. Glt-1 expression levels are unchanged or even slightly increased in both media conditions after exposure to whole blood plasma.

A method to create excitability in Xenopus oocytes

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Action potentials (APs) are signals only found in muscle and nerve cells. Therefore, to study APs one has to use muscle tissue and brain tissue, which has its own limitations. It is challenging to obtain a large quantity of tissue to get an accurate representation of data. Additionally obtaining data from tissues require the use of drugs, which can have unknown effects and or damages on other channels and or tissue, to block out unwanted channel activity. In this research, we have developed a method to create excitability within Xenopus oocytes (frog eggs) by injecting various concentrations of RNA encoding for vertebrate Na+ and K+ channels. Expression of the channel proteins occurs over several days and currents and membrane potentials are studied by modifications of the two-electrode voltage clamp recording technique. We have obtained results that not only establish the basic criteria needed for oocytes to evoke APs, but also show that oocytes evoke APs similar to naturally observed excitable cells. Our goal is to use this method as an efficient alternative technique for studying the effects of different ion channels, specific channel mutations, and channel-specific drugs on action potentials.

Stimulus regularity differentially influences synaptic plasticity outcome in control rats vs rats with mild traumatic brain injury

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Typical protocols for inducing synaptic plasticity use stimulus patterns with constant interstimulus intervals (ISIs), while neurons in vivo receive synaptic input with irregular ISIs. Studying more physiologically relevant ISI patterns is important for understanding the role ISI regularity may play in synaptic plasticity induction. Here we evaluate how ISI regularity influences synaptic plasticity induction in the visual cortex of 10-12 week-old normal rats and rats which received a mild traumatic brain injury (mTBI) 2-3 weeks prior. In acute slices, we stimulated layer 4 and made whole-cell patch recordings of evoked postsynaptic potentials (PSPs) from 122 layer 2/3 pyramidal cells. Conditioning stimulation consisted of 900 pulses, at a mean frequency of 1Hz, with 1 of 3 different ISI patterns defined by a coefficient of variation (CV): regular (CV=0), slightly-irregular (CV=0.2), or highly-irregular (CV=1). PSP amplitudes evoked by 0.1Hz stimulation were measured before and after conditioning, and the post-/pre-conditioning ratio used to assess plasticity. Plasticity outcome distributions differed significantly between mTBI and control rats for CV=1 (1.21±0.15 vs 0.85±0.07; P=0.03, t-test), but not CV=0 (0.88±0.07 vs 0.87±0.06; P=0.94, t-test) or CV=0.2 conditioning (0.73±0.06 vs 0.75±0.05; P=0.85, t-test). For CV=1 conditioning, this reflected an increase in neurons expressing LTP (38% vs 16%), and a decrease in neurons expressing LTD (47% vs 31%) for mTBI vs control rats. We also compared PSP half-width, rise time, decay time, and latency before and after CV=1 conditioning. In mTBI rats, half-width and decay time increased only for cells expressing LTP ($P < 0.05$, t-tests). In contrast, for control rats half-width and latency decreased only for cells expressing LTD ($P < 0.05$, t-tests). Finally, preconditioning somatic calcium levels were significantly increased in mTBI vs control rats (91.2±5.5 nM vs 63.2±3.2 nM; $P < 0.001$, t-test). Our results suggest ISI regularity modifies the efficacy of synaptic plasticity induction and that mTBI can alter this interaction.

Complementary Modular-Extramodular Patterning in the Lateral Cortex of the Inferior Colliculus in Developing Mice

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The inferior colliculus is a major relay hub situated in the midbrain, and is subdivided into a central nucleus and surrounding dorsal and lateral cortices. Recent studies show that the lateral cortex of the inferior colliculus (LCIC) is multimodal, receiving inputs from auditory, somatosensory, and visual sources. The precise organization of patterned inputs to the LCIC and their development has yet to be fully established. Mounting evidence suggests a modular framework with surrounding extramodular zones that provide an anatomical substrate for input-output arrays. Previously, we identified acetylcholinesterase

(AChE), cytochrome oxidase (CO), glutamic acid decarboxylase (GAD), nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d), and parvalbumin (PV) as discrete markers of LCIC layer 2 modular fields. The present study builds upon these findings and establishes calretinin (CR) as a complementary extramodular marker. CR-specific labeling was observed in LCIC zones surrounding presumptive layer 2 modules at all ages, yet became increasingly more distinct at later developmental stages. This finding somewhat contrasts previous results in developing rat in which LCIC CR patterns were more evident prior to hearing onset (Lohmann and Friauf, 1996). NADPH-d and CR double-labeling experiments confirm a complementary modular-extramodular LCIC substrate that is established during the early postnatal period. Ongoing studies aim to determine the alignment of similarly organized Eph-ephrin guidance expression patterns in relation to these markers and developing multimodal projection patterns.

The Effects of Adolescent Stress on Gene ExpresThe Effects of Adolescent Stress on Gene Expression in the Adult Hippocampus sion in the Adult Hippocampus

Rockelle S. Guthrie, Sydney A. Rowson, Mandakh Bekhbat, Gretchen N. Neigh
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In recent years, the focus has shifted from the study of mainly neurons and glia to include analysis of the vascular endothelium. One of the pathways by which atrophy can occur is through lack of sufficient supply of nutrients to tissues. The blood vessels within the brain are responsible for supplying brain tissue with the nutrients necessary for healthy cell survival and proliferation. Given that depression is more prevalent in those suffering from cardiovascular illnesses, characterization of the state of microvessels within the hippocampus—a region involved in regulating the stress response, learning, and memory—is a valid avenue of research in the elucidation of the physiology of depression. The purpose of this study is to determine if chronic adolescent stress (CAS) causes persistent changes in brain vasculature, as measured by expression of genes indicative of vascular health. To test this hypothesis, rats were subjected to CAS followed by assessment of vascular outcomes in adulthood in both males and females. Assessment of VEGFa demonstrated a sex by adolescent stress interaction, such that adult males with a history of chronic adolescent stress, had reduced expression of VEGFa as compared to adult males without stress exposure. Analysis of gene expression is ongoing and includes additional factors indicative of vascular health that have been highlighted in the literature as being associated with hippocampal atrophy seen in animal models of chronic stress. A better understanding of the influence of early life environment on cerebrovascular health will aid in the development of novel treatment methods.

Interrogating the Role of Peripheral-Derived Hematopoietic Cells in Tissue Homeostasis Following Brain Trauma

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Traumatic Brain Injury (TBI) is a leading cause of acquired central nervous system injury worldwide. The immune system is a critical factor in TBI progression, and targeting aspects of this response has been identified as a promising therapeutic approach for minimizing functional deficits and long-term disability. Brain trauma elicits peripheral-derived mononuclear cell (PDMC) migration, infiltration and polarization leading to alterations in the microenvironmental cytokine-chemokine profile which can affect vascular, neuronal and glia cell health. Preliminary work from our lab has identified the Eph/ephrin pathway as a novel regulator of the hematopoietic response to TBI. Transgenic mice lacking Eph signaling in endothelial and hematopoietic compartments display smaller lesion volumes, improved motor function and altered cortical gene expression following the murine controlled cortical impact injury (CCI) model of TBI. Using flow cytometry we find Eph expression on numerous cell types extracted from the bone marrow, including monocytes and endothelial progenitor cells. To directly address the role of PDMCs and Eph signaling in tissue homeostasis following CCI injury, we performed adoptive transfers (AT) using freshly isolated wildtype or Eph knockout (KO) eGFP-positive bone marrow injected into irradiated wildtype mice followed by CCI injury at 30 days post-AT. Analysis of the density, identity and spatial distribution of the cells as well as lesion volume, neuronal cell death and glial activation using non-biased StereoInvestigator Stereology is under way. Initial findings, however, indicate that wildtype mice reconstituted with bone marrow derived from Eph KO mice display smaller lesion volumes compared to those with wildtype cells at 3 days post-CCI injury. Interestingly, ex vivo isolation of M1 and M2 monocyte/macrophages from Eph KO mice display decreased levels of TNF compared to wildtype cells. Based on these preliminary findings, we hypothesize that Eph signaling contributes to cortical tissue damage by mediating PDMC activities such as migration, cell polarization and/or cytokine-chemokine expression. Findings from this study will advance our basic understanding of the mechanisms underlying the peripheral-derived inflammatory response to brain trauma.

GFAP is protective in repeated mild traumatic brain injury/concussion.

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Approximately 3.8 million people in the US sustain a traumatic brain injury (TBI) every year, with a single TBI incurred every 5 seconds. Most TBIs are mild/concussive and patients might not pre-sent symp-toms. After severe TBI, astro-cytes form pro-tective glial scars, while astro-cytes in milder TBI cases might adjust their gene and pro-tein ex-pression more subtly. Both cases cause reactive astro-glio-sis and feature astro-cyte upre-gulation of the in-ter-mediate filament GFAP. The reliability of GFAP upre-gulation in all contexts of neuro-logical dis-ease or trauma has led to the de-velop-ment of several GFAP knock-out mice to elucidate GFAP func-tion. How-ever, moderate/severe brain injury para-digms show lack of GFAP alone as incon-se-quential to injury size or re-cov-ery likely due to com-pen-sation by other in-ter-mediate filaments such as vimentin. Yet, the role of GFAP in mild (repetitive) TBI (mTBI) has not been elucidated.

We modeled mTBI using an impact acceleration model with a metal plate for skull pro-tection. A weight drop of 100g from 50-60cm height induced diffuse injury with no visible lesions, even after three repetitions. We stained for GFAP to assess changes in astro-cyte immuno-histochemistry after repetitive mTBI. Increased GFAP mRNA ex-pression was ob-served in astro-cytes of male wild-type mice after mTBI com-pared to shams. In contrast to the scar lesions caused by moderate/severe TBI, we ob-served upre-gulation of GFAP pro-tein occasionally near large blood vessels and small groups of astro-cytes scattered through-out the cortex indicative of diffuse astrogliosis. GFAP deletion sig-nifi-cantly pro-longed righting reflex re-cov-ery times, indicative of loss of consciousness, after repeated injury when com-pared to wildtype control mice. GFAP knock-out mice incurred more acute vascular damage than wildtype controls even after single mTBI. Vimentin appears to com-pen-sate neither in the mTBI para-digm nor in uninjured GFAP knock-out mice. Surprisingly, we also found sig-nifi-cantly in-creased levels of glu-tam-ate trans-porter trans-cripts in uninjured GFAP knock-out mice, sug-gesting an unanti-cipated role for GFAP regu-lating astro-cyte homeo-static func-tion. In summary, we pro-pose that GFAP has pre-viously undocumented roles in pro-tection of the brain from mild TBI/concussion.

Astrocytes are a primary target for neuronal BDNF: implications on the regulation of astrocyte morphological complexity

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Mature astrocytes are one of the most morphologically complex cells in the central nervous system. This complexity is associated with several of the most well characterized functions of this cell type, including neurotransmitter reuptake, K+ homeostasis, and blood-brain barrier maintenance. While we know the developmental time window when astrocyte morphological maturation and refinement occurs, we know little else governing this process. Brain derived neurotrophic factor (BDNF) is a critical growth factor secreted largely by neurons and involved in the development and maturation of neurons, including neuronal growth and synapse refinement. Preliminary data demonstrates that astrocytes express high levels of the BDNF receptor TrkB when compared to neurons. In particular, the truncated version of TrkB, TrkB.T1, is the predominate receptor expressed in astrocytes. TrkB.T1 expression is highest in astrocytes during the critical period of astrocyte morphological refinement and maturation, a developmental time window which also coincides with the highest neuronal BDNF expression levels. Loss of BDNF expression is a hallmark of neurodevelopment disorder Rett Syndrome, and recent publications indicate that astrocytes have a significantly reduced morphological complexity and are dysfunctional in this disease. These findings have led us to hypothesize that BDNF/TrkB.T1 signaling is an important mediator of astrocyte morphological maturation and that reduced neuronal BDNF expression contributes to aberrant astrocyte morphology in Rett Syndrome.

Chronic adolescent stress alters learning and memory in adult female rats

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Chronic adolescent stress (CAS) has been implicated in increased incidence of cognitive and emotional deficits in adulthood. Sexually dimorphic effects of CAS are evident in the expression and extent of these deficits. Specifically, males show context-dependent deficits in learning and memory while females show no changes in similar tasks. These sexually dimorphic effects suggest that reproductive hormones contribute to CAS-induced alterations to neural function. Learning and memory are mediated by the hippocampus – a brain region that is rich in hormone receptors, providing a site of action for hormones to directly alter behavior. Interestingly, CAS-induced impairments in learning and memory in women are only evident following reproductive senescence suggesting that the presence of female reproductive hormones, such as estrogen, may provide a buffer against the negative effects of CAS on learning and memory. We tested this hypothesis in female Wistar rats exposed to CAS or control conditions and then assessed for learning and memory performance in adulthood using the Barnes Maze task. In adulthood, while memory was unaltered, CAS females showed a slight deficit in spatial learning with increased errors and an attenuated reduction in latency to locate the goal box over repeated trials. Following baseline testing, females were ovariectomized (OVX) to remove gonadal hormones. Analysis of the absence of gonadal hormones on Barnes Maze performance is ongoing. However, we expect that, while both control and CAS females will show deficits in learning and memory following OVX, CAS females will show exacerbated deficits. These findings may suggest that hormones associated with reproduction may provide a buffer against CAS-induced deficits in learning and memory in females. The work from this study is particularly relevant for women experiencing deficits in hippocampal function following menopause and has implications for hormone replacement therapy to alleviate the negative symptoms associated with reproductive senescence in humans.

Disrupted Kir4.1 expression and function in a murine model of Rett syndrome

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Rett Syndrome (RTT) is an X-linked neurodevelopmental disorder typified by apparently normal development until 6-18 months of age, when motor and communicative skills regress and hand stereotypies, autonomic dysfunction, and seizures present. Over 95% of all reported RTT is caused by spontaneous mutations in a single gene, methyl-CpG-binding protein 2 (MeCP2). Nearly all RTT research has focused on neuronal dysfunction in RTT, however, it was recently demonstrated that restoration of MeCP2 function selectively to astrocytes reversed several deficits in a murine model of RTT, suggesting astrocytes contribute to the RTT phenotype. The mechanism of this rescue is unknown. Here we

demonstrate that Kir4.1, a glia-specific inward-rectifying potassium channel that mediates several critical astrocyte membrane properties is disrupted in MeCP2 deficient mice. Astrocytes from MeCP2-deficient mice express significantly less Kir4.1 mRNA and protein, which corresponds with a 50% reduction in Ba2+-sensitive Kir4.1 mediated currents. Indeed, Kir4.1 protein and mRNA expression is significantly reduced well before symptom onset. ChIP analysis revealed a direct molecular interaction between MeCP2 and the Kir4.1 gene promoter in WT animals, an interaction that is lost in MeCP2 deficient mice. These are the first data implicating a direct molecular target of MeCP2 in astrocytes and provide novel mechanistic insight explaining how astrocytic dysfunction may contribute to RTT. Future work will concentrate on elucidating the role of MeCP2 loss in the brainstem in the breathing dysfunction phenomenon in RTT.

Role of 10 Hz highly-irregular conditioning stimulation on the induction of synaptic plasticity in visual cortex after mild traumatic brain injury (mTBI)

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Deep brain stimulation (DBS) is an effective treatment for a variety of disorders. However, the most effective therapeutic parameters, including specific combinations of stimulation frequency and temporal pattern are not well delineated. We investigated the role of highly irregular 10 Hz stimulation on the induction of synaptic plasticity in visual cortex of control (normal) rats, sham operated rats, and rats with mTBI. Whole-cell recordings were made from layer 2/3 pyramidal cells in 10-12 week-old rats, with sham or mTBI treatments performed 2-3 weeks prior. Highly irregular conditioning stimulation consisted of an interstimulus interval distribution coefficient of variation of 1.0, with either a continuous train of 900 pulses at 10 Hz for 90 sec, or a discontinuous train of 9 bursts of 100 pulses each at 10 Hz with a pause between bursts over a total of 900 sec. Postsynaptic potential (PSP) responses were evoked at 0.1 Hz for 10 min pre- and 20 min post-conditioning. For continuous conditioning, controls showed a net LTD (PSP amplitude post-/pre- conditioning = 0.86±0.04; n=19, while individual cells either underwent statistically significant LTD, no significant change (NC) or LTP (0% LTP, 42% NC, 58% LTD); shams, exhibited no net change (0.96±0.04; n=20; 15% LTP, 55% NC, 30%LTD), but mTBI rats showed a net LTP (1.26±0.11; n=20; 50% LTP, 30% NC, 20% LTD). For discontinuous conditioning, controls showed net LTD (0.63±0.07; n=19; 5% LTP, 5% NC, 90% LTD), and shams showed net LTD (0.72±0.07; n=14; 7% LTP, 14%NC, 79%LTD), but mTBI rats showed net LTP (1.17±0.12; n=18; 50% LTP, 22% NC, 28% LTD). Thus, highly irregular stimulation shifts a net LTD in cells from control rats to a net LTP in cells from mTBI rats, and this should be considered in selecting therapeutic parameters for DBS to potentially rebalance weights of altered synaptic circuits after mTBI.

Novel roles for CASK in visual system development and in Optic Nerve Hypoplasia

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Optic Nerve Hypoplasia (ONH) is currently the leading cause of childhood blindness and its prevalence has been rising steadily over the past decade. Despite its prevalence we know little about the genetic, molecular or cellular mechanisms underlying ONH. A previous study has described ONH in a cohort of patients with mutation in the X-linked gene CASK. CASK is a MAGUK protein with well-established roles in pre synaptic function. Here we report that genetic perturbation of CASK in mice results in the development of ONH perinatally, defective connectivity between the retina and the brain, and a loss of retinal ganglion cells (RGCs), the projections neurons of the retina. It remains unclear, however, why these defects arise in CASK mutants or in human patients with ONH and whether the loss of RGCs and retina-brain connectivity in ONH is the result of failed formation of these cell types or their neonatal degeneration. For these reasons, we are using the CASK-model of ONH to gain fundamental knowledge of the cellular and molecular mechanisms underlying this disease. Although CASK is considered a synaptic protein, preliminary data show its expression in retina is highest during retinogenesis. We are therefore exploring whether retinogenesis requires CASK. In addition to the loss of RGCs we have observed in the postnatal retina, we have discovered that CASK mutants displayed shifts in other retinal cell populations as well, including horizontal cells and amacrine cells. These results suggest that the loss of RGCs and retinogeniculate connectivity in CASK mutants may result, at least in part, from the genesis and specification of retinal cell-types in patients with ONH.

Cell-type specific expression of Collagen XXV, and unconventional transmembrane collagen, in mouse brain

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Collagens are triple helical extracellular matrix molecules with well-studied roles outside of the nervous system. More recently it has become evident that collagens are expressed throughout the nervous system and have bio-active roles the development and maintenance of neural circuits. For example, a subclass of collagens are transmembrane proteins that are generated by both motor neurons and muscle, and play important roles in the development and function of neuromuscular circuits. To date we know very little regarding the distribution or function of these transmembrane collagens in brain. Collagen XXV is one such transmembrane collagen, and here we aimed to determine its expression pattern in the developing brain. We found Collagen XXV is generated by cells in both the telencephalon and dorsal lateral geniculate nucleus (dLGN) throughout mouse development. Not only is Collagen XXV expressed in these specific regions, it is generated by different cell types in these regions. In the telencephalon it is expressed by subsets of interneurons, whereas in dLGN it is expressed largely by excitatory neurons. The cell-type specific expression of Collagen XXV suggests individualized roles in the tightly regulated process of neural

circuit formation within different brain regions and forms a basis for investigation of the specific functions of these transmembrane proteins during mammalian brain development.

The role of maternal gut microbiome in perinatal neurodevelopment: Implications for neurodevelopmental disorders

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Epidemiological studies on neurodevelopmental disorders, such as autism spectrum disorders and schizophrenia, highlight antibiotic usage and disruptions to the maternal immune system during pregnancy as major correlates of subsequent diagnoses in children. These results support additional findings that antibiotic use in pregnancy can cause perturbations to the developing neuroimmune system, and that defective microglial cells impair functional brain connectivity and induce aberrant social behaviors in animal models. Importantly, antibiotics create dysbiosis in the gut microbial environment ("microbiome") that can significantly affect the production of key circulating factors, such as short-chain fatty acids (SCFAs), which are critically involved in neuroimmune regulation. As such, we hypothesized that the maternal gut microbiome plays a necessary and sufficient role in proper neurodevelopment due to regulation of the fetal neuroimmune system. Through an antibiotics-based approach, mice were used as animal models of broad gut bacterial depletion during pregnancy. Preliminary data using immunohistochemistry showed neonatal pups born to bacteria-depleted (BD) dams possessed irregular microvasculature, neurogenesis, as well as premature morphology and increased numbers of resident microglia in comparison to conventionally-raised (CONV) controls. In contrast, the persistence of a single species of gut bacterium in a second set of pregnant BD dams (RES) resulted in microglial characteristics more comparable to CONV than BD pup brains. RES pups also performed similarly to CONV pups under behavioral assays, such as the 3-chamber social test. These results suggest that specific strains of bacteria and/or their metabolites may be necessary for proper microglial development and also excludes drug-specific adverse effects. Compared to RES and CONV dams, BD dams possessed atypical metabolite and inflammatory cytokine profiles. In particular, BD dams showed decreased levels of key SCFAs, which are common metabolites released from the microbiome that serve multiple roles in glial metabolism, synaptic function, and cerebrovascularization. These data indicate maternally-derived gut bacteria play a vital role in proper neuroimmune development in utero.

Effects of N-desmethylclozapine on attentional performance following loss of basal forebrain corticopetal cholinergic inputs

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Corticopetal cholinergic neurons play a vital role in attentional processing, and dysregulation of this system contributes to central nervous system disorders whose main attributes include an inability to focus for an extended period of time, such as Alzheimer's disease (AD). The cholinergic muscarinic-1 (M1) receptor is known to be necessary for normal attentional processing. The goal of the present experiment is to evaluate whether N-desmethylclozapine (NDMC), which acts on allosteric M1 receptor sites, can reverse attentional deficits following loss of cortical cholinergic inputs. After training in an attention-demanding task requiring differentiation between signal trials (500, 100, and 25ms illumination of a central panel light) and non-signal trials (no light illumination), Sprague Dawley rats received intrabasalis infusions of either saline or the cholinergic neurotoxin 192 IgG-saporin. The effects of intracerebroventricular infusions of NDMC were tested after post-surgical performance stabilized. In general, NDMC impaired attentional performance, particularly for lesioned animals. These findings suggest that NDMC may reduce the binding of acetylcholine by way of orthosteric competition or that the actions of NDMC at other receptor sites disrupts any beneficial effects of NDMC at the M1 receptor

Degeneration of spinal cord synapses precede atrophy of α-motor neurons in aging rhesus monkeys and mice

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The impact of aging on α-motor neurons and their connections in the spinal cord remains largely unexplored despite the central function these cells play in relaying voluntary motor commands. Here, we examined α-motor neurons and their synaptic inputs in the spinal cord of young and old rhesus monkeys and mice. Using light microscopy, we found that α-motor neurons resist age-related atrophy despite containing large amounts of lipofuscin in their cytosol. Further indicating that α-motor neurons remain viable in old animals, levels of genes primarily expressed in motor neurons are unchanged in the spinal cord of old compared to young mice. Next, we examined excitatory synaptic inputs critical for the function of α-motor neurons in rhesus monkeys and mice. In aged monkeys, excitatory synaptic inputs positive for vesicular acetylcholine transporter (VAChT) are decreased in the ventral horn and specifically on the soma and dendrites of α-motor neurons. In aging mice, these VAChT inputs were unchanged in the ventral horn, but inputs positive for vesicular glutamate transporter (VGluT1) were decreased. Corroborating these findings, proteins associated with these excitatory synaptic inputs are altered in the spinal cord of aged mice. Thus, neural circuits responsible for conveying and modulating somatic motor function degenerate prior to atrophy of α-motor neurons in aged rhesus monkeys and mice.

DNA double strand breaks in human induced pluripotent stem cell-derived neurogenesis

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Somatic mosaicism is a common consequence of normal development. DNA repair is simply not perfect, and each cell's genome incurs continuous DNA damage as a consequence of transcription, replication, and other cell biological stresses. Although somatic mosaicism has been reported in many tissues, it is particularly noteworthy in the brain for two reasons. First, by contrast to other organs with regular cell replacement, the vast majority of an individual's neurons are with that individual for life. And second, neural circuits give rise directly to behavioral phenotypes. Brain somatic mosaicism, now revealed and tractable due to advances in single cell 'omic approaches, has emerged as an intriguing and unexplored aspect of neuronal diversity. Neuronal copy number variations (CNVs), like most CNVs, are likely brought about by DNA repair mechanisms acting on transcription- or replication-induced DNA damage. In previous work, we showed that large CNVs and aneuploidy were prevalent in human induced pluripotent stem cell (hiPSC)-derived neurons, but CNVs were infrequent in hiPSC-derived neural progenitor cells (NPCs). Accumulating evidence suggests that DNA damage repair proteins are essential for neurogenesis to occur. We have quantified DNA damage by examining DSB (double strand break) formation in hiPSC-derived NPCs and neurons in proliferative and differentiating conditions, and in response to various perturbations (e.g., topoisomerase inhibition, replication stress). During neurogenesis, neurons show a unique increase in DNA damage compared to other cell types. Ongoing experiments aim to identify the role of specific DNA damage proteins in neurogenesis.

Impairments of the Gliovascular Unit in Alzheimer's Disease

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Amyloid beta (Aβ) deposits are a pathological hallmark of Alzheimer disease and the deposition of Aβ on cerebral vasculature has been thought to impair cerebral blood flow and the integrity of the blood-brain barrier (BBB). Furthermore, the gliovascular unit, composed of glial, neural, and vascular cells, assures sufficient blood supply to active brain regions by modulating vessel diameter and contributing to the maintenance of the BBB. While the importance of the gliovascularunit and its ability to regulate regional blood flow based on neuronal activity is now well recognized, surprisingly little is known about this interface in diseases such as AD. Here, we studied how the gliovascular unit is affected in a mouse model of Alzheimer disease, using a combination of ex vivo and in vivo imaging approaches. Using the dye methoxy-XO4 to specifically label vascular amyloid, we elicited vessel responses ex vivo using either pharmacological stimuli or calcium uncaging, as well as in vivo using multi-photon imaging in conjunction with label-free optical activation of vascular smooth muscle cells. These imaging studies identified vascular

amyloid deposits physically separate astrocytic end-feet from endothelial vessel walls, and only where vascular amyloid is present, does stimulation of astrocytes or vascular smooth muscle cells via ex vivo Ca2+uncaging or in vivo optical activation produce poor vascular responses. Furthermore, the administration of vascular dyes identifies breakdown of the BBB in areas with substantial amyloid burden. We conclude, therefore, that vascular amyloid significantly interferes with the function of the gliovascular unit and the integrity of the BBB, likely leading to decreased vascular regulation and reduced cerebral blood flow. As impaired cognitive function, the clinical hallmark of Alzheimer disease, results from inappropriate blood flow to critical brain regions, our results demonstrate a mechanism that could account, in part, for the pathology seen in Alzheimer disease. Current studies are aimed at elucidating which constituents of the BBB are lost as a result of vascular amyloidosis, and how amyloid accumulates along the vasculature.

LRRTM1 contributes to the assembly of complex retinogeniculate synapses in mouse visual thalamus

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Retinogeniculate (RG) synapses are critical for regulating the flow of visual information from retina to primary visual cortex (V1). We recently discovered that RG synapses in the mouse dorsal lateral geniculate nucleus (dLGN) differ anatomically and physiologically from retinal synapses in all other retino-recipient nuclei. Not only are retinal terminals significantly large and strong in dLGN, but they can be classified into two distinct morphologies: simple RG synapses that contain a single retinal terminal and complex RG synapses that contain numerous retinal terminals that converge onto the same region of postsynaptic dendrite. In the present study we aimed to identify target-derived factors that could drive the unique differentiation of retinal terminals in dLGN. RNAseq analysis identified Leucine-Rich Repeat Transmembrane Neuronal 1 (LRRTM1) as a developmentally regulated synaptic organizer enriched in dLGN, but not other retino-recipient nuclei, during the developmental maturation of RG synapses. To test the role of LRRTM1 in RG synapse formation, we assessed retinal terminals in targeted mutant mice lacking LRRTM1 (lrrtm1-/-). Anterograde labeling of retinal terminals by intraocular injection of fluorophore-conjugated Cholera Toxin B (CTB) and immunostaining for Vesicular Glutamate Transporter 2 (VGluT2) revealed smaller retinal terminal structures in the absence of LRRTM1. These results suggest that each retinal terminal was smaller in mutants or that complex RG synapses were absent. To answer this question we used serial block face scanning electron microscopy (SBFSEM). Ultrastructural analysis revealed a significant reduction in the number of complex RG synapses in lrrtm1-/- dLGN accompanied with an increase in the size of individual retinal terminals in dLGN of lrrtm1-/- mice. Moreover, preliminary data suggest a change in visual behavior of lrrtm1-/- mice which suggest, for the first time, complex RG synapses have functional significance.

MECHANISMS REGULATING COLLATERAL REMODELING AFTER ISCHEMIC STROKE

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Leptomeningeal anastomoses or collaterals play a critical role in regulating vascular re-perfusion following obstruction, and their ability to remodel is a determinant of the severity of injury after ischemia. However, the mechanisms regulating their development and injury-induced remodeling remains under investigation. Our previous findings show that Eph receptor is a novel negative regulator of collateral development and remodeling after hindlimb ischemia. To implicate Eph in collateral remodeling after ischemic stroke, we induced stroke using the permanent middle cerebral occlusion model (pMCAo). Presently, we demonstrate that Eph expression is reduced on remodeling collaterals in the ipsilateral side compared to the contralateral side in CD1 mice at 1 and 4 days post pMCAO. To elucidate the role of Eph in collateral remodeling, we used LoxP/Cre to delete Eph expression in endothelial cells (EC). Interestingly, EC-specific loss of Eph, Ephf/f/Tie2::Cre (KO), resulted in increased collateral remodeling compared to wild type (Ephf/f) mice at 4 and 14 days post-pMCAO. This correlated with significant improvement in blood reperfusion and sensorimotor recovery in the KO mice compared to wild type. These enhancements may be due to several factors; ECs isolated from KO mice display a 3-fold increase in proliferation, enhanced migration, tube formation and elevated protein levels of phospho(p)-Akt compared to WT ECs. Also, there are altered expression patterns for genes that regulate cell proliferation, vascular development, extracellular matrix and immune-mediate responses, namely increased levels of MCP-1, MMP2 and angiopoietin-1. We further elucidated the cross-talk between Eph and the Ang-1/Tie2 pathways in KO ECs. Inhibition of the Tie2 receptor signaling using soluble Tie2-Fc reduced p-Akt protein expression, and consequently proliferation and tube formation of KO ECs compared to wild type. These findings suggest Eph limits arteriogenesis by regulating EC-specific Tie2 receptor and downstream factors necessary for vascular remodeling. Targeting Eph pathways may represent an attractive new target for therapeutic intervention aimed at tissue protection and functional restoration following stroke.

Astrocyte cell death contributes to spontaneous seizure development in a new mouse model of post-traumatic epilepsy

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Traumatic brain injury (TBI) is the most common cause of acquired epilepsy. Despite our awareness of the initiating events, prevention of post-traumatic epilepsy (PTE) with antiepileptic drugs has been unsuccessful. To date, nearly all research has focused on neurons and treatments almost exclusively address neuronal dysfunction. Yet, the field has identified other potential underlying causes that individually decrease seizure threshold, including astrogliosis and vascular dysfunction. These

mechanisms are actively induced by TBI. It is unclear if these phenomena contribute to the development of PTE, or if they vary in different injury paradigms and patient subpopulations. In patients, the risk for developing PTE is highest in severe injury, yet still significantly increased even after mild TBI. Currently, the field is limited to two PTE models with confirmed spontaneous seizures induced by moderate to severe TBI. In these models PTE incidence is low and heterogeneity from animal to animal has interfered with the emergence of a concise picture detailing disease mechanisms and PTE predictors. We developed a model of PTE induced by repetitive mild TBI. Repetitive mild TBI was induced (100 g weight, 50cm height, 3 impacts, inter-injury interval of 45 min) in a small cohort of mice. After the final righting time was assessed, mice were fitted with electrodes for electroencephalographic (EEG) recordings. EEG monitoring was started 4 days after injury. We found that all recorded animals had at least one seizure within 6 weeks post injury. The first seizure was captured on day 21 post injury. Seizures recurred in a subset of mice and were accompanied by behavioral abnormalities including freezing, facial automatisms, tail extension, rearing and falling. Recurrent electroclinical and behavioral seizures in the settings of repetitive diffuse mild TBI provides proof-of-concept evidence that our mouse model of mild TBI is a promising animal model for studying the pathogenesis of PTE and will help in finding new potential therapeutic targets. Evidence points to astrocyte cell death as potential originator or contributor of neuronal hyperexcitability in the model.

Slit2 contributes to class-specific targeting in the developing hippocampus

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In all areas of the developing brain, axons from different neuronal types, and even from different classes of these neuronal types, use specific sets of molecular cues to guide them to appropriate target neurons. At present, the identities of these essential targeting cues are poorly understood. For example, in the developing hippocampus, different classes of excitatory and inhibitory axons innervate unique hippocampal regions or layers. Some class-specific targeting cues have been identified in the developing hippocampus, such as reelin, an extracellular matrix protein necessary for layer-specific entorhino-hippocampal connections. Recently we identified a unique class of axons that project specifically to neurons in the CA2 region of the hippocampus. In an effort to identify what drives class-specific wiring of these CA2 projecting axons, we identified a member of the Slit family of secreted proteins as potential class-specific hippocampal targeting cues. In the developing brain, slit family members function as a repellent for for many types and classes of axons. Here we found that slit2 is strongly expressed by many neurons in the CA3 region of hippocampus but not in CA1 or CA2 regions. Expression levels of slit2 are strong at birth, as axons begin to innervate the hippocampus, but decline postnatally as these axons are assembled into circuits and as these circuits mature. To test whether slit2 is an essential cue for CA2 projecting axons we assessed circuit formation in slit2-deficient mutant mice. In these mutants lacking slit2

CA2-projecting excitatory axons were either lost completely or were mistargeted to adjacent regions of the hippocampus. Axons and synapses from other excitatory neurons or from local inhibitory interneurons appeared unaffected by the loss of slit2. Taken together these results suggest a novel role for slit2 in regulating class-specific targeting of axons to the CA2 region of hippocampus.

Roles for Collagen XIX in Peripheral Circuit Formation and Function

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Collagen XIX is a non-fibril forming collagen that is present in the extracellular matrix (ECM) of central and peripheral nervous system tissues. In the brain, recent studies have shown that interneurons generate this non-fibril forming collagen and its presence is necessary for the formation of a select population of inhibitory synapses (Su et al. 2010, 2016). Moreover, a small C-terminal fragment of Collagen XIX (which is endogenously relased by proteolytic cleavage) is sufficient to trigger synapse formation (Su et al. 2016). Within the PNS, several developmental studies have indicated collagen XIX is a critical ECM cue for esophageal muscle patterning and for motor axon growth (Sumiyoshi, 2004) (Hilario, 2010). Moreover expression studies have suggested that Collagen XIX (and other non-fibril forming collagens) may be present at the neuromuscular junction, a specialized synapse between motor neurons and skeletal muscles. For this reason, we explored synaptic and extrasynaptic expression of col19a1 mRNA (the gene that encodes Collagen XIX) in mouse muscle. Typically, genes expressed at higher levels by synaptic myonuclei play a role in function or maintenance of the synapse. Digital droplet PCR (ddPCR) showed significantly higher col19a1 expression in the synaptic versus extrasynaptic muscle tissue, demonstrating differential collagen XIX expression by synaptic myonuclei. This suggests that collagen XIX may contribute to the formation or maintenance of peripheral synapses, as it does it the brain. We further assessed NMJ morphology in col19a1 mutant mice with whole mount immunohistochemistry. These studies are currently ongoing, and have yet to reveal gross morphological abnormalities in mutants.

A primary neuronal culture system to study ALS: cultured sensory neurons are affected by ALS-causing mutations.

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We previously showed that nerve endings of proprioceptive sensory neurons (PSNs) degenerate in mice expressing mutant genes known to cause Amyotrophic Lateral Sclerosis (ALS), SOD1G39A and TDP43A315T. In this study, we asked if PSN nerve endings degenerate due to intrinsic or extrinsic changes caused by SOD1G39A and TDP43A315T expression. To distinguish between these two possibilities, we examined PSNs expressing SOD1G39A and TDP43A315T in culture. We found that

expression of SOD1G39A and TDP43A315T slows the rate of PSN neurite extension compared to control PSN. However, the soma of SOD1G39A and TDP43A315T PSN were indistinguishable to control PSN. These findings suggested that expression of SOD1G39A and TDP43A315T impairs the growth and maintenance of neurites. To test this hypothesis, we examined PSN neurites in the presence of vincristine, a microtubule inhibitor that progressively causes neurite degeneration when applied at low concentrations. Compared to control PSN, the presence of vincristine caused the neurites of PSNs expressing SOD1G39A to rapidly unravel. Along with these morphologic observations, we found changes in the expression and distribution of stress-related molecular markers in PSNs expressing SOD1G39A and TDP43A315T. Altogether, the conclusions from this study suggest that ALS-afflicted sensory neurons are intrinsically affected by the disease and could potentially serve as a model system for designing and testing novel therapeutics for ALS.

Loss of miR-133b accelerates myofiber degeneration in a mouse model for Duchenne muscular dystrophy

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The muscle and synaptically enriched microRNA, miR-133b, has been implicated in the biogenesis and maturation of muscle fibers. While mir-133b is increased in Duchenne muscular dystrophy (DMD), it remains unknown whether the induction of miR-133b is required to mitigate muscle fiber in this disease. To assess the role of miR-133b in DMD-affected skeletal muscles, we genetically ablated miR-133b from the mdx mouse model for DMD. We have found that loss of miR-133b exacerbates muscle degeneration in the tibialis anterior (TA) muscle. In the absence of miR-133b, the TA becomes populated with muscle fibers exhibiting a rather small cross-sectional area (CSA) and containing centralized myonuclei. Loss of miR-133b also increases the numbers of mono-nucleated cells in the interstitial space between muscle fibers. Our data suggest that the presence and upregulation of miR-133b in muscular dystrophy serves to slow the degeneration of muscle fibers.

Epileptogenic glioma causes perineuronal net degradation and differentially alters the spike frequency of excitatory and inhibitory neurons

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Perineuronal nets (PNNs) are extracellular matrix assemblies, which surround fast spiking parvalbumin (PV) expressing GABAergic interneurons in the cerebral cortex. The lattice like architecture of PNNs consists of chondroitin sulfate proteoglycans (CSPGs), tenascin-R, hyaluronan and link proteins. Due to high density of negatively charged CSPGs, PNNs can act as a selective barrier for cations and form temporary buffering

sites for the local cations during fast spiking activity. Although PNNs are extensively studied for their role in inhibition of synaptic plasticity, recent studies suggest a broad spectrum of PNN functions in physiology and pathology. Alteration in extracellular matrix and PNNs has been implicated in wide range of nervous system disorders including epilepsy. We examined the integrity of PNNs and the physiological attributes of PNN expressing PV neurons in a glioma-associated seizure model. Intracranial injections of patient-derived glioma in mouse brain induces seizures due to copious amount of glioma-released glutamate in the extracellular space. Gliomas are also know to release proteases, which degrades the extracellular matrix proteins to facilitate tumor invasion. Our results show a progressive degradation of PNNs in brain areas near the tumor border, the peritumoral cortex. Peritumoral cortex exhibited significant reduction in the number of neurons and PNNs and accompanied by deformed PV interneurons surrounded by fragmented PNNs. Peritumoral PV neurons associated with fragmented PNNs showed altered electrophysiological properties including significantly lower spike frequency compared to sham controls. On the other hand, excitatory neurons in peritumoral cortex exhibited higher spike frequency than the sham controls. Enzymatic degradation of PNNs with Chondroitinase ABC in sham controls also decreased the spike frequency of PV interneurons indicating the role of PNNs in determining the high spike frequency of PV neurons. Our data suggest that PNNs are a crucial determinant of PV interneurons physiological function and glioma-induced degradation of PNNs can disturb the excitation-inhibition balance by reducing spike frequency.

Mild TBI induces neurovascular unit dysfunction due to loss of perivascular astrocytes and vascular smooth muscle cells/pericytes

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Almost four million people in the United States incur a traumatic brain injury (TBI) every year. Up to 80% of these injuries are classified as mild or concussive. Yet, even single mild TBI can cause long-term cognitive impairments for patients and enhance the risk of patients to de-velop- neuro-degenerative dis-eases such as Alz-heimer Disease and Chronic Traumatic Encephalo-pathy. In the absence of obvious primary brain damage mecha-nisms leading to slowly pro-gressing neuro-degene-ration have remained elusive. Continuous cerebral blood flow (CBF) is essential for neu-ronal health and func-tion and even mild TBI patients can pre-sent with re-duction in CBF. If players involved in blood flow regu-lation such as astro-cytes, vascular smooth muscle cells (VSMC) or pericytes, are impaired by mild TBI and how this affects their role in neuro-vascular coupling is unknown. Here, we used an impact-acceleration weight-drop mouse model (100g, 50cm height) to simulate mild TBI in trans-genic mice that ex-press GFP or dsRed in astro-cytes and VSMC/pericytes respectively. Using immuno-histochemistry against astro-cyte markers including Glt-1, S100b, GFAP and Kir4.1 as well as the use of the astro-cyte-spe-ci-fic reporter mouse line Aldh1l1-eGFP revealed that astro-cytes frequently dis-appear surrounding larger penetrating

vessels. In vivo 2-photon imaging allows imaging of animals at baseline and acutely and chronically after induction of mild TBI. Using this appro-ach we found that some pericytes are missing from blood vessels im-me-di-ately after single mild TBI. Label-free cell-spe-ci-fic laser stimulation of astro-cytes or pericytes in vivo induces vaso-constriction or dilation of neighboring vessels in uninjured animals. Pre-liminary data sug-gest that this vascular re-sponse is re-duced after mild TBI, po-tentially as a result of damage to perivascular astro-cytes and VSMC/pericytes during injury. In conclusion, we pre-sent evidence that even single mild TBI affects astro-cyte and VSMC/pericytes survival and sug-gest that loss of these important neuro-vascular unit players dysregulates CBF modulation.

Regular and irregular (Poisson) conditioning trains induced synaptic plasticity between individual neurons in layer 4 of mouse visual cortex

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Primary visual cortex Layer 4 neurons receive synaptic input from their neighboring neurons, such that local synaptic integration of early visual information processing has been hypothesized to act as an amplifier of thalamocortical inputs. To better understand the plasticity of synaptic connections between L4 neurons and the effect of different temporal patterns of synaptic input on plasticity, we performed dual and triple whole cell patch clamp recording from L4-L4 pairs or triplets in visual cortical slices. Two different 15min 10Hz conditioning protocols with either Poisson (CV=1.0) or regular(CV=0.0) were used to induced long term plasticity. A total of 43 pairs of synaptically coupled neurons were recorded. The baseline synaptic strength, failure rate and paired pulse ratio (PPR) were measured and compared before and after the conditioning. Eighteen pairs underwent the Poisson protocol, the net synaptic strength and PPR decreased, while the failure rate increased. 13 underwent LTD, 4 remained unchanged , and 1 displayed LTP. Another 25 pairs of connection underwent the regular protocol, the net synaptic strength and PPR decreased and the failure rate increased. 24 pairs of connections exhibited LTD and one pair exhibited LTP. In summary, 1)Both conditioning protocols induced long term depression in the sample of recorded synaptically connected pairs and it related to the failure rate increase; 2)The long term plasticity outcome profiles between regular and irregular conditioning are distinct: Poisson conditioning shifts the plasticity outcome toward potentiation while regular conditioning towards depression; 3)In neocortical microcircuits of L4 in V1, both types of conditioning can induce divergent or convergent synaptic connected pairs to undergo different plasticity outcomes.