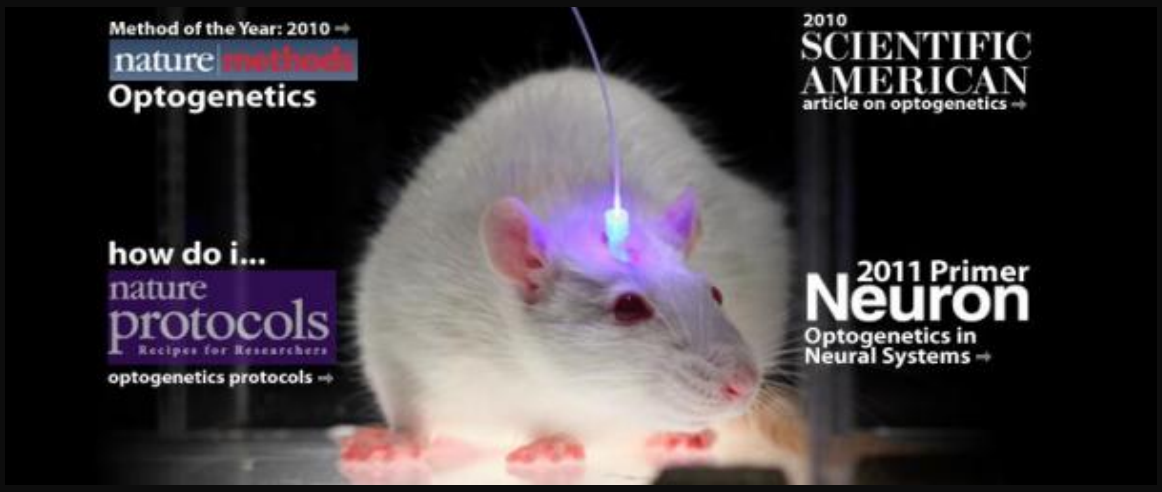

Optogenetics, Chemogenetics and Circuit Mapping of Brain Function

Central Virginia Chapter of the Society for Neuroscience
2015 Annual Symposium and Poster Session

March 20th, 2015

Virginia Commonwealth University

Kontos Medical Sciences Building
1217 E. Marshall St., Richmond, VA

CVCSN

Optogenetics, Chemogenetics and Circuit Mapping of Brain Function

Annual Symposium of the
Central Virginia Chapter of the Society for Neuroscience
Friday, March 20th, 2015

9:00 a.m.	**Registration in Kontos Medical Sciences Building (MSB) Atrium** Poster set up in Kontos MSB room 104 &105 Breakfast in Lower Atrium, Kontos MSB
9:30	**Symposium Opening Remarks** Raymond J. Colello, PhD, CVCSN President
9:40	***Novel viral based strategies for targeted neural circuit tracing*** **David C. Lyon, PhD** Associate Professor of Anatomy & Neurobiology/Cognitive Sciences University of California Irvine School of Medicine, Irvine CA
10:40	***Optical electrophysiology*** **Vincent A. Pieribone, PhD** Professor of Cellular and Molecular Physiology and Neurobiology, Yale University School of Medicine, New Haven CT
11:40	Lunch Break in Molecular Medicine Research Building rooms 1009 & 1011
12:30 p.m.	**CVCSN Symposium Poster Session**
2:15	**Data Blitz Oral Presentations**
3:15	Intermission
3:30	***From synapses to circuits: using light to understand brain wiring*** **Benjamin R. Arenkiel, PhD** Assistant Professor of Molecular and Human Genetics Baylor College of Medicine, Houston TX
4:30	***A neural basis for melanocortin-4 receptor regulated appetite*** **Michael Krashes, PhD** Chief, Section on Motivational Processes Underlying Appetite National Institute of Diabetes and Digestive and Kidney Diseases Bethesda MD
5:30	**Symposium Awards & Closing Remarks** Raymond J. Colello, PhD, CVCSN President

Data Blitz Oral Presentations

Annual Symposium of the
Central Virginia Chapter of the Society for Neuroscience
Friday, March 20th, 2015
2:15 to 3:15 pm

Novel neurofascin isoform: potential mediator of microglia-AIS interaction
Savannah D. BROOKINS
Anatomy and Neurobiology, Virginia Commonwealth University

***Noxious stimuli suppress nutrient sensation in neuron pair ASI in* Caenorhabditis elegans**
Kristen DAVIS
Biochemistry and Molecular Biology, Virginia Commonwealth University

Alpha4 nAChRs and septum ERK signaling regulate age-associated changes in anxiety-like behavior
Claire I. DIXON, PhD
Pharmacology and Toxicology, Virginia Commonwealth University

Pharmacological implications of $A_{2A}R$-D_2R heteromerization and the significance for Parkinson's disease
Candice HATCHER-SOLIS
Physiology and Biophysics, Virginia Commonwealth University

Environmental tobacco smoke exposure alters rat cerebellar development in behavior and synaptic transmission
Pretal MULDOON, PhD
Anatomy and Neurobiology, Virginia Commonwealth University

The role of K63-linked polyubiquitination in the activation of the type I interferon response by IRF1
Michael J. SURACE, PhD
Biochemistry and Molecular Biology, Virginia Commonwealth University

Monoacylglycerol lipase inhibitors produce opioid sparing effects in a murine model of neuropathic pain
Jenny L. WILKERSON, PhD
Pharmacology and Toxicology, Virginia Commonwealth University

Effects of HIV-1 Tat on oligodendrocyte viability: iGluR-mediated Ca^{2+} dysregulation and GSK3β activation
ShiPing ZOU
Anatomy and Neurobiology, Virginia Commonwealth University

Symposium Abstract/Poster Directory

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CVCSN 2015 Symposium Poster Abstracts

1 Undergraduate Award Finalist

Hypothalamic urocortin 3 is a novel neuroendocrine factor in regulating reproductive hormone secretion.

Alyssa BRUNAL[1], Christine van Hover[2], Chien Li[2]

Department of Psychology, Virginia Polytechnic Institute and State University, Blacksburg VA[1] and Department of Neuroscience and Pharmacology, University of Virginia Health System, Charlottesville, VA[2]

Corticotopin-releasing factor (CRF) family peptides play a critical role in coordinating the hypothalamo-pituitary-adrenal axis (HPA) activity and integrates endocrine, autonomic, and behavioral responses to stress. The function of the peptide family is mediated by two G-protein coupled receptors, namely type 1 and 2 CRF receptors (CRF_1 and CRF_2). The two receptors exhibit distinct binding characteristics to members of the CRF family: CRF binds CRF_1 with high affinity, while Urocortin 1 (Ucn 1) binds both receptors with equal high affinity. On the other hand, Ucn 2 and 3 are selective ligands for CRF_2, with minimal binding to CRF_1. In the pituitary, CRF_1 is expressed mainly in the corticotropes to mediate ACTH secretion, while CRF_2 is localized predominately in gonadotropes. In the present study, we provided preliminary data to suggest a novel neuroendocrine pathway involving Ucn 3 and CRF_2 in regulating reproductive hormone secretion. In the central nervous system, neurons expressing Ucn 3 are found in the hypothalamus and medial amygdala. Ucn 3 nerve fibers innervate a number of brain areas including the ventromedial hypothalamus (VMH), the lateral septum, and the median eminence (ME), particularly the external layer of the ME. In the hypothalamus, Ucn 3-positive neurons are found clustered near the rostral perifornical hypothalamic area (rPFH) and then extended rostrally into the anterior part of the pavricellular part of the paraventricular nucleus of hypothalamus (PVHap). Our previous study has shown that Ucn 3 cells in the rPFH project mainly to the lateral septum, while cells in the PVHap appear to target the VMH. To further delineate the efferent projections of Ucn 3 cells in the PVHap, a conditional viral tracing was performed. An adeno-associated viral vector expressing a Cre recominase (Cre)-regulated channelrhodopsin-mCherry reporter was injected into the PVHap area of transgenic mice that express Cre in Ucn 3-positive cells (Ucn 3-cre). Three weeks after viral injection, mice were then perfused and brains were processed to detect mCherry. We observed abundant mCherry-positive fibers in the VMH, confirming that Ucn 3 cells in the PVHap project to this nucleus. Abundant mCherry-positive fibers and terminals were also found in the external zone of the ME. This result raises the possibility that Ucn 3 may reach the pituitary to regulate gonadotrope function. To test this hypothesis, LH secretion from LβT2 cells, a mouse clonal gonadotrope cell line, was examined. We found that basal LH secretion in LβT2 cells were significantly reduced by Ucn 3 (10 nM) treatment. Importantly, GnRH-induced LH secretion was greatly attenuated in cells pretreated with Ucn 3. Together, our results indicate that hypothalamic Ucn 3 through CRF_2 in the pituitary may constitute a novel neuroendocrine pathway to regulate gonadotropin secretion.

2 Undergraduate Award Finalist

Chemogenetic approaches to probe the role of astrocytes in the motivation for ethanol

POLAND, R.S, Bull, C., Freitas, K.C.C., Zou, S., Syed, W.A., *Urban, D.J., Minter, S.C., Shelton, K.L., Hauser, K.F., Negus, S.S., Knapp, P.E., and Bowers, M.S.

Virginia Commonwealth University School of Medicine, Richmond VA and *University of North Carolina, Chapel Hill NC

Alcoholism is associated with adaptations that occur within discrete brain structures. The main focus of the literature is neuron-mediated events and neuronal plasticity, but it is now understood that astrocytes can significantly influence neurotransmission in many regions including the corticolimbic projection. We studied corticolimbic astrocyte adaptations to various schedules of ethanol (20% v/v) self-administration by measuring astrocyte density in subregions of the prefrontal cortex (PFC) and the nucleus accumbens. These regions are centrally involved in the reinforcing properties of commonly abused substances. This involvement is thought to be the underlying cause of the abuse potential of these substances. In response, we studied astrocytic adaptations in rats exposed to one of three commonly used ethanol self-administration paradigms. Those paradigms were continuous two-bottle ethanol access (CEA), intermittent two-bottle ethanol access (IEA), and operant ethanol access (OEA). Astrocyte number and packing density was determined using unbiased stereological measures of cells expressing the astrocyte marker, glial fibrillary acidic protein (GFAP). GFAP is a commonly used marker of astrocyte plasticity as well as an indicator of pharmacological insult or injury. The number of GFAP-positive astrocytes increased in the prelimbic and anterior cingulate, but not in the infralimbic or orbitofrontal prefrontal cortex subregions after IEA. After abstinence, the CEA cohort showed a reduction in astrocyte number in the prelimbic and orbitofrontal cortices. A reduction was also observed in the orbitofrontal cortex of the OEA cohort. We also found that the density of astrocytes expressing GFAP increased in the core, but not shell regions of the nucleus accumbens. There were no changes in region volume or number of cells $GFAP^-$ cells. Interestingly, no change was observed in the number of astrocytes expressing a second astrocyte marker aldehyde dehydrogenase 1L1 (ALDH1L1) in either the shell or core. This suggests that only a subset of the astrocyte population responds to ethanol self-administration in these brain regions. Because the nucleus accumbens is associated with motivated behavior, we tested the hypothesis that astrocytes in this region modulate the motivation for ethanol. We found that nucleus accumbens core (NAcore) astrocyte density was positively correlated with increased motivation for ethanol after abstinence. To probe this finding, we sought to impair the ability of astrocytes to communicate. Blockade of astrocyte and neuronal gap channels increased the motivation to self-administer ethanol, whereas blocking neuronal gap channels alone did not. Thus, we sought to selectively stimulate NAcore astrocytes. Do to this, we used astrocyte-specific Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) that were coupled to $G\alpha q$. Following exposure to the otherwise inert DREADD agonist CNO, intracellular calcium increased in vitro, but only in astrocytes that were expressing the $G\alpha q$-coupled DREADDs. Stimulation of these astrocyte-specific receptors in vivo reduced the motivation of rats to self-administer and moderately, but significantly increased responding for intracranial self-stimulation. These data indicate that distinct limbic subregions differentially respond to ethanol consumption and that rat NAcore astrocytes play an important role in modulating

the reinforcing efficacy of ethanol. Together, these data suggest that stimulating astrocytes in this manner may be therapeutically efficacious and also possess low abuse liability.

3 Undergraduate Award Finalist

The chemokines CXCL9 and CXCL10 are produced by distinct cell types in the brain during chronic Toxoplasma gondii infection

Benjamin A. TRAN, Nikolas W. Hayes, and Tajie H. Harris

Center for Brain Immunology and Glia, Department of Neuroscience, University of Virginia, Charlottesville, VA, USA

Toxoplasma gondii, an intracellular protozoan parasite, persists in host neural and muscle tissues during the chronic stage of infection. IFN-γ is a critical mediator of resistance to toxoplasmic encephalitis (TE). CXCL9 and CXCL10, IFN-γ –dependent ligands of the CXCR3 receptor, promote the migration of immune cells in the brain during chronic *T. gondii* infection. While previous studies have shown the importance of CXCL9 and CXCL10, the identity of cell types producing these chemokines has remained elusive. Using a CXCR3 ligand reporter mouse (REX3), which reports CXCL9 and CXCL10 with red fluorescent protein (RFP) and blue fluorescent protein (BFP) respectively, we identified the CXCR3 ligand producing cells in the brain during *T. gondii* infection using immunohistochemistry. In preliminary studies, we have found that 93% of CXCL9 producing cells are IBA-1$^+$ (microglia and macrophages), while the remaining cell types have not been identified. On the other hand, CXCL10 is produced by several cell types, including astrocytes, IBA-1$^+$ cells, and endothelial cells. Thus, *T. gondii* infection induces CXCL9 and CXCL10 production in distinct cell types. These results suggest that each of these ligands may play a non-redundant role during chronic *T. gondii* infection.

4 Undergraduate Award Finalist

Modularity and multimodal connections in the inferior colliculus prior to experience

Joseph A. BALSAMO, Mark L. Gabriele

Dept. of Biology, James Madison University, Harrisonburg, VA

The midbrain houses the inferior colliculus, a known auditory relay hub. Well established is the tonotopic arrangement of its central nucleus (CNIC) and the organization of its many converging inputs. Considerably less is known about its external nucleus or lateral cortex (LCIC). In contrast to the CNIC, the LCIC lacks a clear frequency order, exhibits a modular organization, and receives a multimodal input array. Previous studies in a variety of adult species demonstrate somatosensory inputs to this region arising from the spinal trigeminal (Sp5) and dorsal column nuclei. These projection distributions, unlike the characteristic layering exhibited by CNIC afferents, terminate as a series of discrete patches reminiscent of modular Eph-ephrin expression (EphA4, ephrin-B2) in the developing LCIC. As a first-step in determining the guidance roles this signaling family may play in LCIC circuit formation, the present study examines the development of Sp5 inputs to the multimodal IC prior to auditory experience. Fluorescent tract-tracing approaches in a developmental series of fixed mouse tissue preparations reveal the presence of pioneer somatosensory fibers in the nascent IC. Ipsilateral and contralateral Sp5 fibers reach the midbrain by birth and target aspects of the LCIC. Sp5 fibers were also noted in the ipsilateral cochlear

nucleus. At present it remains unclear whether Sp5 distributions in mouse ultimately exhibit patchy or discrete IC modular terminal fields as has been described in other adult species. If so, the relative alignment of these inputs with previously described modular neurochemical markers (e.g. ephrin-B2, EphA4, GAD, PV, CO, NADPH, AChE, WFA) should provide insights regarding the anatomical substrate of the LCIC and signaling mechanisms that establish its multimodal circuitry.

5 Graduate Award Finalist

Regulation of circadian rhythms through activation of dopamine receptor expressing SCN neurons

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In mammals, circadian rhythms or periodic oscillations of approximately 24 hours, are orchestrated by a population of neurons within the suprachiasmatic nucleus (SCN). The SCN receives direct photic input through the retino-hypothalamic tract and can be synchronized by environmental light cycles. In the absence of external cues the periodic behaviors governed by the SCN such as the sleep-wake cycle, temperature regulation, and feeding persist with an endogenous period around 24 hours. Non-photic signals can also influence the circadian pacemaker. The neuromodulator dopamine is involved in reward/punishment recognition and movement. These motivational and arousal states show daily oscillations which suggest that dopamine could have a significant role in the proper maintenance of the circadian rhythms. Furthermore, patients suffering from alterations of the dopamine system in Parkinson's disease, depression and addiction to drugs of abuse demonstrate abnormal sleep patterns. Discovering the link between dopamine and the circadian system could provide insight into how these symptoms develop and allow a better understanding of neural circuitry governing non-photic entrainment. In the present study, we show that D1 dopamine receptor (D1R) expressing SCN neurons are present through adulthood and are functionally connected to dopamine release. We use a chemogenetic approach to selectively manipulate D1R expressing SCN neurons and demonstrate that activation of this neuron population resets the circadian clock. Through a series of retrograde and anterograde tracing experiments we plan to determine the source of dopaminergic innervation to the SCN which will provide further insight into the neural circuitry involved in dopamine's modulation of the central oscillator. Taken together, these data indicate that dopamine can act directly on the SCN through activation of D1R expressing neurons.

6 Graduate Award Finalist

Axon initial segment disruption in multiple sclerosis and EAE

CLARK KC[1] , Josephson A[1], Brookins S[1], Thummala S[1], Joslyn M[2], Oh U[3], DeVries G[1,2], Dupree JL[1]

1 Department of Anatomy and Neurobiology, Virginia Commonwealth University School of medicine, Richmond VA; 2 Molecular Biology and Genetics Program, VCU; 3 Hunter Holmes McGuire Veterans Affairs Hospital; 3 Department of Neurology, VCU, Richmond VA

Multiple Sclerosis (MS) is an inflammatory autoimmune disease of the central nervous system (CNS) exhibiting both demyelination and axonal pathology. Currently, axonal pathology is believed to be consequent to demyelination in MS. Here, we challenge this longstanding paradigm by presenting convincing data demonstrating that axonal pathology is a primary target in disease, independent of demyelination. Immunocytochemical analysis shows breakdown of the axon initial segment (AIS) domain as a primary event in MS and its murine inflammatory model; experimental autoimmune encephalomyelitis (EAE). The AIS is the region of the axon, distal to the soma, spanning about 40 μm in length. Due to its high density of Na^+ and K^+ channels, it is the site of action potential initiation and modulation. Maintaining AIS integrity is, therefore, vital to proper neuronal function. Our data show that cortical layer V AISs are significantly disrupted in EAE induced mice, and the number of AISs in MS tissue is reduced. Since demyelination occurs in both EAE and MS, we determined the effect of AIS stability in the cuprizone model of demyelination/remyelination. Although cortical myelin loss was extensive in the cuprizone mice, no change in AIS number or length was observed, indicating that AIS stability is not dependent on the myelin sheath. Although AIS disruption was independent of demyelination, it coincided with microglial activation. Moreover, we have observed an upregulation of neuronal m-calpain, a calcium activated cysteine protease whose substrates include critical AIS proteins. Interestingly, in calpain positive neurons, clustered AIS proteins are not observed, while calpain negative neurons reveal robust AIS protein labeling. Finally, we present evidence that a novel anti-inflammatory, free radical scavenger, known as didox prevents this AIS loss, reverses AIS shortening and attenuates m-calpain upregulation. Overall, we have identified the breakdown of the axon initial segment as a primary axonal insult, contrary to the commonly accepted paradigm of MS, revealed a key mediator of this axonal pathology, and determined a method of protection for this AIS disruption.

7 Graduate Award Finalist

Time-sensitive molecular mechanisms underlying post-TBI remodeling of the inhibitory synaptic network.

Pavel N. LIZHNYAK, Pierfrancesco De Domenico, and Andrew K. Ottens

Department of Anatomy and Neurobiology, Virginia Commonwealth University School of Medicine, Richmond VA

Understanding the molecular processes that underlie the pathobiology of traumatic brain injury (TBI) is instrumental to developing more effective diagnostics and treatments. Of particular interest in our laboratory are the regenerative mechanisms initiated during a critical post-acute period following TBI. Using a controlled cortical impact model, we induced focal brain injury in male rats and collected tissues for proteomic and immunofluorescence microscopy between 2 and 14 days post-TBI. We employed a non-targeted, data-independent mass spectrometry method to quantify TBI-induced proteome dynamics within spared somatosensory cortex adjacent to the focal injury. In this study, we tested the hypothesis that an analogous molecular mechanism that initiates inhibitory network development would be activated during the post-acute repair period following TBI. We examined the peptide data related to inhibitory-specific isoforms of synaptic proteins to include synaptotagmin-2 (SYT2), neuroligin- 2 (NL-2), gephyrin, and KCC2 to evaluate post-translational changes. Results demonstrate novel, temporally resolved changes at 2 and 4 days, respectively post injury, in ubiquitin and phosphorylative motifs of

NL-2, a key initiator of inhibitory network remodeling. Coinciding at 4 days post-TBI, we resolved the reduction of KCC2 chloride transporter levels at the membrane, reverting inhibitory neurons to a development-like GABA-induced excitatory state. KCC2 level recovery then coincides with a transient de-polymerization of gephyrin at 7 days following injury, suggesting prominent plasticity of the inhibitory network. Immunofluorescence results affirm the temporal reduction in punctate NL-2 staining of inhibitory synapses as well as remodeling as suggested by Syt2 staining. Study findings reveal a temporal process by which the cortical inhibitory network is reorganized following TBI that reflects similar machinery employed during development. Further, these data define a transient critical period during post-acute recovery that represent a plausible boundary for therapeutic protection or enhancement of network repair as well as post-translational events by which to study the efficacy of novel pre-clinical interventions.

8 Graduate Award Finalist

Dual inhibition of FAAH and MABL reveals a CB_1 receptor-mediated discriminative stimulus

Robert OWENS, Dr. Bogna Ignatowska-Jankowska, Dr. Patrick Beardsley, Dr. Jenny Wiley, Dr. Benjamin Cravatt, Dr. Aron Lichtman

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The observation that dual blockade of endogenous cannabinoid degradative enzymes monoacylglycerol (MAGL) and fatty acid amide hydrolase (FAAH) elicits THC-like psychotropic effects suggest FAAH & MAGL function as dual brakes in curtailing the pharmacological effects of endogenous cannabinoids. To test this hypothesis, we examined whether blockade of FAAH and MAGL would serve as an interoceptive stimulus in mice, using the drug discrimination paradigm. Drug discrimination is used to infer the subjective/psychotropic effects of drugs. Here we tested whether elevating levels of the naturally occurring endogenous cannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) via inhibition of both degradative enzymes would serve as an interoceptive stimulus in mice, N = 7-8. After, we determine the relative contribution of FAAH or MAGL in generating the SA-57 interoceptive stimulus. Towards this end, we employed the dual FAAH & MAGL inhibitor SA-57 as the training drug. Mice were trained using a double alternation between SA-57 and vehicle (DDVVDD) and acquired the task over 40 days. The discriminative stimulus effects of 10 mg/kg SA-57 were dose-related (ED_{50} (95% CI) = 4.49 (3.77 – 5.35) mg/kg) and were antagonized by the CB_1 receptor antagonist rimonabant (3 mg/kg), but not the CB_2 receptor antagonist SR144528 (3 mg/kg). CP55, 940 as well as the dual FAAH/MAGL inhibitor JZL195 substituted for SA-57, and rimonabant blocked the substitution of both. Low doses of SA-57 (<3 mg/kg), which completely inhibit FAAH, but not MAGL, did not substitute for SA-57. The FAAH inhibitor PF-3845 (10 mg/kg) did not substitute for SA-57. However, the MAGL inhibitor MNJ110 substitutes for the SA-57 discriminative stimulus. These findings suggest that an elevation in AEA after FAAH inhibition is not sufficient to elicit SA-57-like subjective effects. However, elevations in 2-AG after MAGL inhibition may be sufficient. This study represents the first example that elevating levels of naturally occurring marijuana-like molecules serves as an interoceptive stimulus. Thus, FAAH, and more likely MAGL serve as brakes in curtailing the psychotropic effects of endogenous cannabinoids.

9 Post-doc Award Finalist

Matricryptins derived from collagen XIX induce inhibitory synapse formation

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[1]Virginia Tech Carilion Research Institute, Roanoke VA, [2]Department of Biological Sciences, Virginia Tech, Blacksburg VA

Although inhibitory synapses comprise only ~20% of the total synapses in the mammalian cerebrum, they play essential roles in controlling neural activity. In fact, perturbing inhibitory synapse assembly or function has been associated with autism, epilepsy and schizophrenia. Although many types of inhibitory synapses exist, these developmental disorders have been strongly linked to defects in inhibitory synapses formed by parvalbumin (PV)-expressing interneurons. Despite their importance we lack a complete understanding of the mechanisms that underlie the formation of these inhibitory synapses. With that in mind our attention has been drawn to collagen XIX, an unconventional collagen expressed by interneurons during synaptogenesis. Based on video EEG/EMG recording, we found that mice lacking collagen XIX exhibit spontaneous generalized motor and absence seizures and are more susceptible to drug-induced seizure induction, both phenotypes associated with defects in inhibitory signaling. Combined with impaired responses to pre-pulse inhibition assays and a striking lack of nest-building activity in collagen XIX mutant mice, we found that they exhibit schizophrenia-related behaviors. Further more we show that these collagen XIX-deficient mice exhibit defects in PV^+ synapse formation in subiculum, visual cortex and prefrontal cortex. Like other unconventional collagens, the C-terminal domain of collagen XIX is proteolytically shed and functions as a matricryptin (*i.e. a fragment of an ECM molecule that exhibits a unique function from the full length molecule from which it was released from*). Since other matricryptins have been shown to be synaptogenic, we speculated that collagen XIX-derived matricryptins (termed NC1[XIX]) are synaptogenic. Our *in vitro* assays show that NC1[XIX] induces the formation of functionally active inhibitory nerve terminals and is sufficient to rescue synaptic defects in the absence of full-length collagen XIX. Moreover, the synaptogenic activity of NC1[XIX] can be blocked with RGD-containing peptides, indicating that integrins are required for NC1[XIX] function. Taken together, these results reveal a novel set of mechanisms governing the formation of inhibitory synapses in the mammalian cerebrum during developmental disorders.

10

Planar cell polarity genes control anterior-posterior axon guidance decisions in spinal commissural neurons

Ashley M. PURDY and Gregory S. Walsh

Department of Biology, Virginia Commonwealth University, Richmond, VA

In zebrafish, a single pioneering axon of a dorsally located glutamatergic commissural interneuron, termed CoPA (**C**ommissural **P**rimary **A**scending) is the first among ascending commissural axons to pathfind and form the spinal commissure; its axon travels ventrally to cross the midline and then projects dorsally and anteriorly. This single pioneering axon enables researchers to view axon pathfinding mechanisms on a single-cell level. It is known

that the growth cones of CoPAs use chemoattractant and chemorepellant cues to guide their axon pathfinding decisions. One pathway that guides their anterior growth is the planar cell polarity (PCP) signaling pathway, but it is not fully known how PCP signaling regulates anterior guidance. We exploited this system by examining CoPA pathfinding in various PCP mutants to determine if anterior-posterior (A-P) guidance of CoPAs is dependent on PCP signaling. We found that Fzd3a, Vangl2, and Scribble homozygous mutants all exhibited anterior pathfinding defects, with approximately half of all affected CoPAs migrating incorrectly posteriorly. We also wanted to determine if anterior pathfinding that occurs in the absence of midline crossing requires PCP signaling. By using a translation-blocking *dcc* morpholino to prevent CoPA midline crossing, we discovered that CoPA axons in Fzd3a and Scribble mutants show severe defects in A-P guidance. We also discovered a novel correlation between pre-crossing and post-crossing directional bias of CoPAs. We found that A-P guidance of post-crossing fibers is highly correlated with pre-crossing directional bias, which suggest that PCP influences A-P guidance of CoPAs prior to and after midline crossing. Our data suggests 3 major conclusions: 1) pre-crossing commissural fibers are responsive to anterior guidance cues, 2) anterior guidance of commissural axons is independent of midline crossing, and 3) this anterior guidance is dependent on PCP, even in the absence of midline crossing.

11

Serum Factor Alters Cav3.2 Ion Channel Current Density and Gating Kinetics

J. Grayson EVANS[1] and Slobodan M. Todorovic, M.D., Ph.D[1,2,3]

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T-type Calcium channels play a critical role in regulating neuronal excitability and modulating sensory transmission. The Cav3.2 channel isoform is highly expressed in peripheral nociceptors as well as in the pain-processing regions of the dorsal horn of the spinal cord (Nelson et al. 2005; Jacus et al. 2012). Augmenting Cav3.2 channel currents has been shown to induce hyperexcitability in nociceptive neurons in vitro and hyperalgesia in vivo (Jagodic et al. 2007, Orestes et al. 2013). These studies strongly suggest that potentiation of the Cav3.2 channel results in abnormal nociceptive transmission, which could contribute to a variety of clinical pain syndromes. Therefore, it is important to identify endogenously produced molecular species that modulate Cav3.2 channel currents. Using the patch-clamp technique and stably transfected human embryonic kidney cells (HEK-293) expressing the Cav3.2 channel, we have begun to characterize a factor found in fetal bovine serum (FBS) that profoundly affects Cav3.2 channel gating kinetics. Specifically, when compared to baseline recombinant currents, 1% serum produces maximal increases in current magnitude (350%; $p<.001$), conductance (150%, $p<.001$), rate of macroscopic inactivation (47.1%; $p<.001$) and deactivation (74.3%; $p<.001$). Furthermore, 1% serum induces a hyperpolarizing shift in voltage-dependence of activation (V50) (-4.77mV; $p<.001$) with minimal effect on voltage-dependence of channel inactivation. Future studies will focus on identifying this serum factor in order to evaluate its potential role in nociceptive signal modulation as well as cellular excitability.

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Morphine enhances the effect of HIV on proliferation of primary human neural progenitor cells; role for μ-opioid receptor (MOR) splice variants

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Growing evidence suggests that opiate drug abuse exacerbates HIV-associated neurocognitive disorders, (HAND). The interactive effect of opiates and HIV-1 is thought to work at two levels; directly on neurons, and indirectly through other CNS cells that express opioid receptors. We previously reported evidence of the vulnerability of murine and immortalized NPCs to HIV-1/Tat and morphine co-exposure. Human NPCs (hNPCs) also were demonstrated to express μ-opioid receptors (MOR) and HIV co-receptors CCR5 and CXCR4, suggesting possible cellular mechanisms for the deleterious effect of morphine and HIV in these cells. Subsequently, we reported the selective expression of MOR-1 and MOR-1K spice variants in primary CNS cells, and in HIV-1 infected brain samples, suggesting differential roles of splice variants in HIV mediated neuropathology. The current studies investigated the functional consequences of HIV-1 and morphine co-exposure in the proliferation and survival of primary hNPCs *in vitro*. We also aim to investigate the regulation of MOR splice variants in hNPCs exposed to HIV-1. We developed a primary model of hNPCs derived from 10-week fetal brain tissues that are composed primarily of Sox2 and nestin-expressing cells. We demonstrated that HIV-1 supernatant (5.0 - 500 pg/mL HIV-1 p24), significantly decreased the percentage of $BrdU^+$ hNPCs, as early as 12 h after treatment. Morphine co-exposure further exacerbated the effect of HIV-1 on BrdU incorporation. Considering these findings, we then examined changes in cell growth and doubling time of hNPCs. HIV treatment significantly reduced hNPC cell density, shifting the cell growth curve to the right, and prolonging the hNPC doubling time. Morphine co-exposure, as seen in the BrdU study, further enhanced the HIV-1 effect, demonstrating the vulnerability of hNPCs to HIV-1/morphine interactions. There was no evidence that HIV-1 ± morphine increased hNPC cell apoptosis/death at 12-48 h, via propidium Iodide flow cytometry and DEAD Red staining. Lastly, we found that MOR-1 and MOR-1K are selectively up-regulated in immortalized and primary hNPCs exposed to HIV-1 supernatant. Our findings provide initial evidence of HIV and morphine interaction in primary hNPCs and suggest that specific MOR splice variants may respond differently to HIV-1.

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Novel roles for spon1 in establishing circuits and behaviors associated with circadian photoentrainment

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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 Spon1 (also called F-Spondin): an extracellular matrix protein whose expression is dramatically enriched in the SCN compared with adjacent hypopthalamiuc nuclei. Spon1 appeared as a good candidate synaptic targeting cue for ipRGCs since it binds and signals through the same receptors as Reelin. Behavioral analyses of *spon1*$^{-/-}$ mutant mice reveal reduced activity and defects in circadian photoentrainment, suggesting significant defects in the circuitry associated with circadian behavior. Here we have applied anatomical approaches to further assess the formation and maintenance of the retino-hypothalamic tract in novel targeted mouse mutants that lack Spon1.

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Lrrtm1 is necessary for the development of corticogeniculate inputs in the mouse dorsal lateral geniculate nucleus

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The dorsal lateral geniculate nucleus (dLGN) of the mouse has emerged as a powerful model for studying the development and function of sensory inputs in dorsal thalamic nuclei. Thalamic relay neurons within mouse dLGN are innervated by retinal axons prior to and during the first week of postnatal development. While we know a great deal about the mechanisms that direct the topographic targeting of retinal projections and their segregation into eye-specific domains, we lack an understanding of the molecular cues that drive retinal nerve terminal assembly in dLGN. To identify potential synaptogenic cues in dLGN, we performed a transcriptional screen to identify synaptic organizing molecules in the developing dLGN. Here we report that one such candidate synaptogenic factor expressed in dLGN is Leucine-Rich Repeat Trans Membrane protein 1 (LRRTM1), a postsynaptic transmembrane adhesion molecule that binds presynaptic neurexin to induce excitatory synapse formation in the hippocampus. Here we discovered that *lrrtm1* mRNA is generated by thalamic relay neurons and is upregulated as retinal terminals mature into their adult-like morphology. To test whether LRRTM1 is important for the formation and/or maturation of retinogeniculate synapses we assessed the formation of retinal terminals in targeted mutant mice lacking LRRTM1. To our surprise we observed no defects in the distribution, density or morphology of glutamatergic retinal inputs in the dLGN of these mutant mice. However, since dLGN relay neurons also receive glutamatergic inputs from layer VI of primary visual cortex we also investigated whether LRRTM1 was required for the assembly of these excitatory corticogeniculate inputs. Immunohistochemistry and western blotting with antibodies against vesicular glutamate transporter 1 (VGluT1), a vesicle associated transporter present only in corticogeniculate terminals in mouse dLGN, revealed significant abnormalities in the assembly of corticogeniculate synapses in dLGN in LRRTM1-deficient mice. While further studies are needed to investigate whether these defects in corticogeniculate terminal formation are due to decreased cortical projections, decreased VGluT1 synthesis or transport by cortical

neurons, or defective enrichment in nerve terminals, these studies are the first to identify a role for LRRTM1 in thalamic circuit assembly.

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A Novel Role for RelB Phosphorylation in Glioblastoma Multiforme

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Glioblastoma multiforme (GBM) is the most common and devastating primary brain tumor. Despite aggressive multimodal treatment that includes surgery, chemotherapy, and radiation, the median survival time after diagnosis is a mere 12 months. Although the mechanisms underlying GBM remain poorly understood, recent research has suggested that the interaction of tumor cells with the inflammatory microenvironment might play a role in cancer progression. Specifically, aberrant NF-kB activity has been implicated in GBM. NF-κB proteins are most known for their role in immune and inflammatory responses, however they also control cell proliferation, differentiation, apoptosis, and oncogenesis. Classically, NF-κB proteins are activated either via canonical or non-canonical pathways. Canonical signaling results in p65/p50 heterodimer formation, while non-canonical signaling results in RelB/p52 heterodimers. However, recently a novel "RelB-canonical" pathway of NF-κB signaling that involves RelB/p50 dimers has been found to be active in dendritic cells. Interestingly, our data indicate that interleukin-1β supports GBM progression through this RelB-canonical pathway. Despite the seemingly important role of RelB/p50 in GBM, the mechanism of preferential signaling through the RelB-canonical pathway in GBM remains to be elucidated. Our preliminary data indicate that RelB is induced and phosphorylated in response to interleukin-1β. Our aim is to determine if this phosphorylation facilitates RelB-canonical signaling, thus regulating GBM progression.

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Investigation of Three Noninvasive Sensory Recordings: Pattern ERG, Diffuse Optical Tomography, and ABR

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Developments in neurophysiology help us to understand how and why our bodies behave the way they do. Our lab has taken keen interest in understanding the structure and function of the eyes, auditory brainstem, and brain imaging. We aim for developing techniques for these areas that are of low cost and high quality.

- Pattern ERG- We have designed and tested a device to record evoked potentials from retinal ganglion cells as an early detection for blindness.
- Diffuse Optical Tomography- Through simulations, we observed reconstructions of arrangements of infrared light sources and detectors for brain imaging.
- Auditory brainstem recording-We focused on creating an Android App for hearing localization.

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Selective vulnerability to HIV-1 Tat in an nNOS positive subset of hippocampal interneurons

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One of the areas of the brain that is known to be strongly affected in neuro-acquired immunodeficiency syndrome (neuroAIDS) is the hippocampus, which has a large variety of interneuron subtypes and complex circuitry with varying protein expression profiles, functions, and metabolic demands. Attenuation of spatial learning and memory, a characteristic of HIV-associated neurocognitive disorders (HAND), is linked to hippocampal pathology. HIV Tat is known to play a significant role in key pathologic processes thought to underlie neuroAIDS. We hypothesize that some of these diverse hippocampal interneuron subtypes may respond differentially to the presence of HIV-1 Tat. To test this hypothesis, neuronal vulnerability was explored in GFAP-driven, doxycycline-inducible Tat transgenic mice, while transgenic mice lacking only the *tat* transgene served as controls. The layers of the hippocampus CA1 region were probed in brain slices using antibodies for neuronal markers, including parvalbumin (PV), neuronal nitric oxide synthase (nNOS), neuropeptide Y (NPY), and neuronal nuclear marker (NeuN), combined with fluorescent tagged secondary antibodies. Cell nuclei were counterstained with Hoechst. No significant differences were observed between groups for PV expression in any layer of CA1. The percentage of nNOS-immunoreactive interneurons without NPY immunoreactivity was significantly decreased in the pyramidal layer of the CA1 region of the hippocampus, as was the percentage of nNOS-immunoreactive interneurons in the stratum radiatum of CA1 in Tat expressing animals compared to controls. The findings indicate a subset of CA1 nNOS-expressing interneurons is selectively vulnerable to HIV-1 Tat and suggest that a subset of hippocampal interconnections is preferentially susceptible to neuroAIDS. This finding suggests novel structural and functional deficits in hippocampal circuitry that underlie HAND.

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RelB/p50 complexes drive mesenchymal glioblastoma multiforme

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In the past 25 years, little improvement has been made in patient survival after a diagnosis of glioblastoma multiforme (GBM). The robust induction of angiogenesis, extreme resistance to radiation, and a highly aggressive, invasive phenotype make GBM one of the most lethal and difficult human cancers to treat. Recently, a mesenchymal subtype of GBMs has been identified that displays the most infiltrative behavior, is the most resistant to standard therapies , and expresses markers, such as a secreted protein YKL-40, which correlate with the worst patient prognosis. It

has long been known that the interaction of tumor cells with the inflammatory microenvironment plays a role in cancer progression. Indeed, extensive necrosis and inflammation are hallmarks of GBM pathology. Our preliminary data suggests that chronic inflammation induces the production of mesenchymal GBM markers. We have identified that two proinflammatory cytokines, interleukin-1β (IL-1β) and oncostatin M (OSM), are specifically overexpressed in patients with mesenchymal GBM. Furthermore, we found that IL-1β treatment of GBM cells activates expression of an NF-kB family member, RelB, while OSM promotes dimerization of RelB with p50. We show that RelB/p50 activation by chronically elevated IL-1β and OSM drive the inflammatory gene expression program which promotes mesenchymal GBM aggressiveness and leads to increased resistance to treatment.

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Treating Obesity and Other Metabolic Disorders through a Sympathetic Approach

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Obesity, along with its many associated health disorders, is one of the foremost healthcare issues facing the modern world. In attempts to stave the ongoing increases in obesity rates, a number therapies for this affliction have been developed, many of which work by altering the endocrine system. However, treatments targeting the endocrine system have been met with rather limited success. Here, we suggest a new avenue for treating obesity by targeting the sympathetic nervous system. We report that wild-type mice placed on weight loss-inducing ketogenic diets exhibit fluctuations in the release of norepinephrine by sympathetic neurons to both white and brown adipose tissue. These fluctuations in norepinephrine appear to correspond to periods of weight loss and stoppage of weight loss across a 12 day time period. Moreover, we show that application of β3-adrenergic receptor agonists enhance weight loss in response to the ketogenic diet while the respective antagonists or complete ablation of the sympathetic nervous system can reduce weight loss responses. These data provide a foundational framework for developing novel therapies to treat obesity.

20 Oral Presentation

Noxious stimuli suppress nutrient sensation in neuron pair ASI in *Caenorhabditis elegans*

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Satiety quiescence in *Caenorhabditis elegans* is a behavioral state defined as a lack of movement or feeding following worm satiation. ASI, an amphid neuron pair, are essential for worms to enter satiety quiescence. Using GCaMP expressed in ASI, we previously found the presentation of nutrients directly activates ASI. However, the presence of nutrients does not always stimulate feeding behavior. To understand how feeding is controlled by external cues, we treated worms with nutrients mixed with noxious stimuli such as a high concentration of NaCl (4 M - (Chatzigeorgiou, Bang, Hwang, & Schafer, 2013)) or glycerol

(1M –(Hilliard, Apicella, Kerr, Suzuki, Bazzicalupo, & Schafer, 2005)). Our preliminary results are that these noxious stimuli override the ASI activation by nutrients, suggesting feeding, a potential result after sensing nutrients via ASI, can be suppressed in the presence of noxious stimuli. Because these noxious stimuli are mostly sensed by another pair of amphid neurons, ASH, we tested our hypothesis that ASH act upstream of ASI and suppress the ASI activation (by nutrients) in the presence of noxious stimuli. Not only does stimulation of ASH via noxious stimuli suppress ASI's response to nutrients, in a genetic ablation of ASH, ASI responds to nutrients even when noxious stimuli are present. Our next step will be to determine how ASH suppress ASI; we will determine the neurotransmitter(s) involved and whether ASH directly or indirectly suppress ASI, using channel rhodopsin (ChR2) expressed in ASH as a more direct route of stimulation.

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Heme Oxygenase 1 and Lipocalin 2 as potential modulators of vascular disruption after traumatic brain injury.

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Heme Oxygenase 1 (HO-1), the inducible form of Heme Oxygenase, degrades heme into biliverdin, CO, and iron. It is a heat shock protein, robustly induced by CNS vascular hemorrhage following traumatic brain injury (TBI). Notably, HO-1 releases highly oxidative iron, which can promote local pathology, as well as up-regulate transcription, potentially affecting neural plasticity during postinjury recovery. Lipocalin 2 (LCN2) is a scavenging/trafficking siderocalin, which traps bound iron and relocates it to intracellular functional sites. LCN2 also persistently binds and activates matrix metalloproteinase-9 (MMP-9), a secreted gelatinase which affects local injury/repair mechanisms. Microarray analysis revealed significant elevation of HO-1 and LCN2 mRNA in rat cortex and hippocampus after fluid percussion TBI, suggesting that HO-1 and LCN2 act in concert to influence recovery within these brain regions. We hypothesized that trauma-induced neuroplasticity at sites of vascular damage is mediated by a pathway involving focal HO-1 expression and iron generation, which then drives LCN2 induction and MMP-9 activation. Twenty four hours after moderate central fluid percussion TBI, HO-1 protein expression in cortex and hippocampus was documented by Western blot (Wb) and immunohistochemistry (IHC). In parallel imaging experiments, HO-1 and LCN2 were examined for co-expression. Wb analysis revealed that diffuse TBI elevated HO-1 by 153% in hippocampus, and 455% in cortex relative to sham injured controls, all statistically significant effects. Wide field immunofluorescence microscopy showed HO-1 elevation in white matter tracts and at the injury site, both areas known to exhibit hemorrhage and neurodegeneration after injury. Interestingly, HO-1 was also elevated in lateral cortex and hippocampal subsectors, regions not associated with hemorrhagic bleeds. Confocal imaging demonstrated primary HO-1 localization within a subset of GFAP positive astrocytes at 24 hr post injury, in both the lateral cortex and hippocampus. HO-1 was also localized within IBA1 positive microglia surrounding gross hemorrhage and necrosis. LCN2 IHC experiments in the same tissue revealed LCN2 and HO-1 co-expression in the same subset of reactive astrocytes. These results show that TBI induces focal HO-1 up-regulation at hemorrhagic sites and in regions without overt vascular disruption. This HO-1

response involves both astrocytes and microglia, and is correlated with local LCN2 increase. Ongoing mapping of postinjury HO-1 and LCN2 response will determine their potential molecular interaction during recovery and role in MMP regulation.

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Matrix metalloproteinase-9 and osteopontin contribute to the regulation of trauma-induced synaptogenesis in the olfactory bulb

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Traumatic brain injury (TBI) damages a variety of CNS circuits, including multiple sensory pathways, resulting in persistent behavioral deficits. Such deficits are attributed to diffuse axotomy, producing circuit deafferentation with loss of synaptic organization. Olfactory receptor neurons (ORNs) are particularly vulnerable to TBI axotomy, resulting in synaptic deafferentation of olfactory bulb (OB) glomeruli. Typically, damaged ORN axons regenerate over time during OB reactive synaptogenesis, restoring connections. After TBI, OB synaptogenesis can be attenuated, with persistent anosmia occurring in up to 25% of patients. This anosmia can considerably impact quality of life. Although the underlying attenuation of OB synaptic plasticity is not well understood, our studies in other brain regions suggest that matrix metalloproteinases (MMPs) may regulate TBI-induced synaptic repair. Secreted gelatinase MMP-9 increases in OB after direct transection of olfactory input or ORN cell death, consistent with its role in reshaping local synaptogenic environment. Recently, the cytokine osteopontin (OPN) was identified as a substrate for MMP-9 proteolysis, which generates OPN peptides with exposed integrin binding domains. These domains are known to signal immune driven glial reactivity after CNS insult. We hypothesized that MMP-9/OPN interaction in the deafferented OB supports glial signaling to mediate successful synaptogenesis, and that after TBI, aberrant MMP-9 expression changes OPN/glial interaction, resulting in poor synaptic recovery. Using FVB/NJ wild type (WT) and FVB MMP-9 knockout (KO) mice subjected to moderate central fluid percussion TBI, we analyzed OB OPN fragment generation at 1, 7, and 21d post-injury relative to paired sham-injured cases by Western blot (WB). These time points were selected to represent acute degenerative (1d) and regenerative (7, 21d) phases of OB reactive synaptogenesis. MMP-9 activity of injured and sham WT mice was also assessed by gelatin zymography. Results showed significant injury-induced elevation of MMP-9 activity at both 1d (68%) and 7d (~3 fold), while 21d MMP-9 activity was not different from controls. WB OPN analysis revealed generation of a 47kD integrin binding fragment that was significantly elevated over controls at 7d, temporally correlated with a 7d maximal elevation of OB MMP-9 activity. Interestingly, when synaptic regeneration is active at 21d, we found normalized MMP-9 lysis and a significant reduction in 47kD OPN fragment compared to sham cases. Further, in mice with KO of MMP-9, expression of the 47kD OPN fragment was significantly attenuated at 7d relative to WT, suggesting that OB MMP-9 plays a prominent regulatory role in OPN signaling after TBI. Because MMP processing of OPN signal is linked to effective presynaptic terminal clearance for synapse regeneration, we analyzed the effect of TBI on OB presynaptic vesicle marker synapsin-1 at 7 and 21d post-injury. Our initial WT findings support the predicted synapsin-1 reduction

(~35%) at the end of the degenerative phase, however, at 21d after TBI, the period of active synaptic regeneration, we found a persistent, significant reduction of WT OB synapsin-1. Interestingly, with MMP-9 KO, this 21d synapsin-1 reduction is no longer detected, supporting a role for MMP-9 in presynaptic terminal regeneration during OB synaptic repair. To further explore OPN expression in specific OB cell populations, we have begun in vitro studies of mixed OB neuronal/glial cultures from WT and MMP-9 KO mice. When WT cultures were subjected to OPN immunocytochemical analysis, we found OPN localized within IBA1+ microglia, but not in GFAP+ astrocytes or TUJ1+ neurons. In MMP-9 KO cultures there were fewer IBA1+ microglia expressing OPN and microglial reactivity was notably attenuated. Collectively, these data suggest that MMP-9 lysis of OPN contributes to critical OB cell signaling after TBI, likely mediating the axonal regeneration component of synaptogenesis and acting through reactive microglial pathways.

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23 Oral Presentation

The Role of K63-linked Polyubiquitination in the Activation of the Type I Interferon Response by IRF1

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The interferon regulatory factor (IRF) family of transcription factors controls immune and inflammatory signaling as well as myeloid and lymphoid cell maturation and differentiation. Several IRF including IRF3, IRF5, and IRF7 require phosphorylation and ubiquitination for activation and nuclear translocation. IRF1 is required for expression of chemokines which recruit monocytes to sites of inflammation, but in stark contrast to other IRF1 family members, it has long been thought to be constitutively active upon transcription. We have recently discovered that in response to interleukin 1, IRF1 is modified with K63-linked polyubiquitin chains. Significantly, we show that although K63-linked polyubiquitination of IRF1 occurs on multiple lysines, lysine 78 is uniquely required for IRF1 activation of the Interferon β promoter. In contrast, K78 is not required for IRF1 activation of an interferon response element reporter, which lacks the NF-κB and AP-1 elements of the IFNβ promoter, indicating that the K63-linked polyubiquitination of K78 may facilitate assembly of complex enhanceasomes. K78 is not required for nuclear translocation or DNA binding, and the majority of K63-linked polyubiquitinated IRF1 resides in the cytoplasm. We hypothesize that K63-linked polyubiquitination of IRF1 facilitates its activation by recruiting a cofactor such as a kinase which is required for activation of IRF1 or a binding partner such as NF-κB P65.

24 Oral Presentation

Environmental tobacco smoke exposure alters rat cerebellar development in behavior and synaptic transmission

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The incidence of childhood exposure to environmental tobacco smoke (ETS) was unchanged over the last decade per the US CDC. Recent epidemiological findings show a link between childhood ETS and risk for behavioral deficits and disorders. However, a neurobiological basis linking ETS exposure and mental health issues remains unclear. The broad range of human deficits found correlated with ETS exposure, particularly in boys, suggests possible cerebellar vulnerability that is consistent with fMRI data in children with ADHD and conduct disorders. Cerebellum is now known to be critical in refining higher-order behavior, to include control over actions, attention, and impulses. Thus, we hypothesized that late-forming, lateral cerebellar circuitry is susceptible to ETS-induced aberrant synaptic organization to result in behavioral control deficits.

To assess this hypothesis we exposed Sprague-Dawley male rat pups to daily ETS (0 or 100 ug/m^3 total suspended particulate from postnatal day 8 (PD8) through PD23. At PD22 behavioral testing was performed to quantify activity, attentional control in a novel environment and light/dark box. In addition, using fluorescence microscopy, we assessed organizational perturbation within lateral cerebellar circuitry induced with ETS exposure relative to room air at PD24.

We found that juvenile ETS exposure induced sex-dependent deficits in risk-aversion and attention tasks. Furthermore, males demonstrated a reduction in excitatory granular input along with heighted inhibition of Purkinje cells that provide the comparator function needed to refine higher-order behaviors. Our findings show a neurobiological basis for male susceptibility to behavioral control deficits underlain, in part, by inhibited lateral cerebellar function following juvenile ETS exposure. Future studies will assess the effect of ETS exposure within frontal cortical domains and the underlying biochemical networks involved in this process to help us to further understand the neurodevelopmental impacts of ETS exposure in relation to mental health disorders.

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Sex-specific neurophysiological adaptions to muscle unloading

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Hindlimb suspension affects a muscle's ability to produce a contractile force and the muscle's endurance. Forty young adult Wistar rats were divided into the following four groups: 1) male control, 2) female control, 3) male unloaded, 4) female unloaded. The unloaded condition was imparted by a hindlimb suspension model that held the rats up by their tails to prevent their hindlimbs from being able to touch the ground and bear any weight. After the two week intervention period, Soleus muscles was removed and used for an ex vivo procedure to quantify neuromuscular function. By using unique stimulation protocols, muscle contraction was induced either directly or indirectly (by way of nerve terminal excitation) and muscular force was quantified by a force transducer. This allowed the assessment of neuromuscular transmission failure. Parameters quantified

were peak force production, specific tension (force relative to muscle mass), Time to peak force, neuromuscular block, and fatigue over the 5 min stimulation protocol. The data was analyzed with a two-way ANOVA (main effects for sex and unloading), and in the event of significant ($p<0.05$) effects, a Tukey post-hoc was used to identify significant pair-wise differences. The results showed that when combined, fatigue is greater during indirect (nerve stimulated), than direct (muscle directly stimulated) indicating that in in vivo conditions the fatigue observed in continuously contracting skeletal muscle is more closely related to nervous system failure than failure of the muscle's contractile apparatus. Hindlimb suspension affected the females more than the men whether the muscle was stimulated directly or by the nerve. Specifically, unloading significantly increased the neuromuscular block over the five minute fatigue train only in the females. In summary, the muscle fatigue is likely due to fatigue in the neuron's ability to stimulate the muscle. Females are also more affected by the hindlimb suspension than males.

26 Oral Presentation

Novel Neurofascin Isoform: Potential Mediator of Microglia-AIS Interaction

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Multiple Sclerosis (MS) is a debilitating disease of the central nervous system characterized by profound demyelination and inflammation. Axonal injury, another prominent feature of MS, is considered a major contributor of chronic disability in patients. While most axonal injuries associated with MS are thought to be a secondary consequence of demyelination, findings from our lab strongly suggest that disruption of the axon initial segment (AIS), the region of the axon responsible for action potential initiation, is targeted for disruption in an inflammatory mouse model of MS through a mechanism independent of myelin loss. This breakdown was, instead, shown to correlate with increased microglial activation associated with specific microglial-AIS contact. Understanding this disease-associated contact could provide insight into the inflammation-induced AIS disruption. We hypothesize neurofascin, a member of the L1 subgroup of the immunoglobulin superfamily and known mediator of extracellular axolemmal adhesion, to be a potential mediator of this contact. Neurofascin is enriched at the AIS and plays an important role in maintaining stability of the domain via homophilic and heterophilic interactions. Neurofascin has multiple isoforms, and the expression of these alternatively spliced isoforms is cell and tissue specific. To begin to test our hypothesis, we have isolated microglia from the neocortex, the brain region that exhibited AIS loss, microglial activation and microglial-AIS interaction. By Western blot analysis we observe a neurofascin band of a unique molecular weight indicating the isolation of a novel isoform of the neurofascin gene. IHC analyses demonstrate neurofascin antibody reactivity in microglia, and this reactivity is enhanced with disease progression. qPCR confirmed the presence of neurofascin mRNA transcript in isolated microglia, suggesting this protein is not present as a result of phagocytosis. We, therefore, propose that a novel isoform of neurofascin is present specifically in microglia and mediates contact with the AIS; potentially resulting in disruption of the domain.

cIAP2 regulates EAE associated neuroinflamation

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Inhibitors of apoptosis (IAPs) modulate cell death and play critical role in signal transduction that promotes inflammation. Recently, Smac mimetics, which are IAP antagonists, have attracted a lot of attention and are currently under development as novel cancer therapeutics. Cellular Inhibitor of Apoptosis 2 (cIAP2), a member of IAP family, positively affects both NF-κB and MAPK activation in response to many inflammatory stimuli and also controls inflammasome and ripoptosome activation. In addition to known functions of cIAP2, cIAP2 also polyubiquitinates and activates Interferon Regulatory Factor1 (IRF1). Accordingly, $IRF1^{-/-}$ mice are resistant to experimental autoimmune encephalomyelitis (EAE), which is a mice model for multiple sclerosis. We hypothesized that $cIAP2^{-/-}$ mice should also be protected from the disease due to deficiency in IFR1 activation. Surprisingly, induction of EAE in $cIAP2^{-/-}$ mice resulted in rapid and exaggerated disease. Consistent with high clinical score, we found increased level of pro-inflammatory cytokines such as TNF α, IL-1, IL-6 and chemokines including CCL5 and CXCL10 in $cIAP2^{-/-}$ mice. Currently, we are trying to understand the molecular mechanism driving the disease in these mice.

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Effects of introducing a visual distracter on cognitive flexibility in aged rats

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Previous research in our lab has indicated that the addition of a visual distracter during a two choice sustained attention task enhances performance on a novel attention task when compared to animals that did not receive the distracter. The present study was designed to investigate if these findings are also observed in aged animals. Twenty rats trained on a standard two choice sustained attention task for the majority of their lives. At age 20 months, 10 rats continued on the same attention task with a stable house light (non-distracter condition) and 10 rats were introduced to a flashing house light while performing the task (distracter condition) for 20 sessions. Blocks of a novel signal discrimination task were then randomly dispersed within blocks of the sustained attention task for both groups. For 20 consecutive sessions 40% of the blocks contained the novel signal discrimination task. Then for the next twenty consecutive sessions 70% of the blocks contained the signal discrimination task. We did not observe differences in performance of rats that were exposed to the distracter compared with those that were not. The present results suggest that this form of cognitive flexibility may be limited in aged animals.

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Embryonic avian axons undergo degeneration after injury and stress

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Axon degeneration is a hallmark of many sensory impairments like glaucoma, CNS conditions like Parkinson's and Alzheimer's, and following traumatic neural injuries. Recently, SARM, a multidomain protein has been found to play a significant role in the process of Axon degeneration, and its inhibition is suspected to improve many of these conditions. While ongoing studies in drosophila and mouse are appropriate for studies of peripheral neuropathy and underlying mechanisms of this process, they are often deficient in modeling human sensory or CNS disease. Songbirds (*Taeniopygia guttata*) allow for the study of an animal, which like humans has exceptional vision as well as sophisticated memory. We have developed a novel embryonic culture system for avian neurons to first characterize wallerian degeneration. Recent data indicate that avian neurons are sensitive to the chemotherapy drug taxol as well as physical injury.

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Synaptic adaptations to muscle unloading in male and female rats

Kaitlin KRESSIN, Colleen Leathrum, Dr. Michael Deschenes

The Neuroscience Department at the College of William and Mary, the Kinesiology and Health Sciences Department at the College of William and Mary, The Foundation for Aging and Exercise Science Research, and the National Institute of Health

Differences in aging and activity level can encourage pre-synaptic and post-synaptic remodeling of neuromuscular junctions (NMJs). Despite this plasticity, proper pre-synaptic and post-synaptic coupling must be maintained in order for effective cell to cell communication. In this study, the morphological profiles of NMJs of the Soleus muscle of male and female adult rats subject to unloading and their controls were analyzed. Immunofluorescent techniques were used to stain for nerve terminal branching, pre-synaptic vesicles, and post-synaptic receptors. Confocal microscopy was used to capture images of NMJs for later quantitative analysis. Data were subjected to a two-way ANOVA and in the event of a significant ($p < 0.05$) F ratio, a post hoc analysis was performed to identify pairwise differences.

The results showed that a main effect of unloading was found only with the number of nerve terminal branches. There was not an effect of sex found for any variable. Unloading resulted in a larger number of nerve terminal branches in females, however no other statistical significances were found. Interestingly, our lab has found remodeling of the myofiber profiles with unloading in previous studies, but the lack of similar change in the NMJ morphology suggests that perhaps NMJs withstand a greater degree of unloading than myofibers.

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Genetic and molecular characterization of Sarm1-mediated axon degeneration within avian models

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Within neurodegenerative disorders, inappropriate wallerian degeneration leads soma degradation. Recent research has identified the Sarm1 protein as both highly conserved among avian species and necessary to promote the synchronous degeneration of an axon. Indeed, interference of Sarm1 significantly attenuates all axon degeneration, both after axotomy or after chemo-induced fragmentation. Gene sequencing, annotation, and analysis creates the first description of Sarm1 regulation and evolution within Avian species. Targeted Sarm1 inhibition through CRISPR gene modification allows precise and highly accurate genomic editing within the Zebra finch (*taeniopygia guttata*). Together, these two research efforts help construct a functional model of axon degeneration within Songbirds.

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Nitroreductase-Mediated Cell Ablation: Investigating Glial-GlialInteractions During Peripheral Nerve Development and Maintenance.

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Currently, 20 million people in the United States are suffering from peripheral neuropathic disorders characterized by muscle weakness and atrophy due to damaged axons, myelin sheaths (demyelination), or both. Due to the complexity of such peripheral functions, the construction and maintenance of a peripheral nerve involves an intricate process with interactions of multiple cell types. A specific cell type, called glial cells, originally thought only to contribute to support of neurons has recently been shown to play an important role in nerve development and maintenance. The goal of this study is to determine how peripheral glial cells, namely Schwann cells (SC) and perineurial glia (PG), interact with each other during development and injury. To investigate this interaction, two novel transgenic zebrafish lines were created allowing for conditional ablation of SC or PG. Cell-specific promoters (*Sox10* and *Nkx2.2a* for SCs and PGs, respectively) were used to drive expression of the *E. coli* nitroreductase (NTR) gene fused with a fluorescent reporter into SC or PG. Introducing the pro-drug Metronidazole (Mtz) into the zebrafish water supply leads to death of NTR+ cells. Optimal Mtz concentration and exposure times were investigated and cell ablation efficiency was determined by examining fluorescent reporter expression levels. Following successful PG ablation, future experiments will investigate SC migration and myelination defects. Similar studies will be conducted with the SC ablation line with a focus on alterations in PG migration and differentiation. Immunohistochemistry will also be used to confirm successful cell ablation following Mtz treatment. Using the novel SC and PG conditional ablation zebrafish lines will allow us to develop a greater understanding about the relationship between peripheral glial cells during development and may be helpful in identifying mechanisms involved in demyelinating neuropathies.

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Differentiation into motor neurons stimulates mitochondrial biogenesis

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Mitochondrial biogenesis (mitobiogenesis) is the process by which cells increase their mitochondrial mass and includes transcription of genes encoded by both the mitochondrial and nuclear genomes. The use of human pluripotent stem cells (hPSCs) has allowed us to study previously inaccessible cells like neurons. Previous research suggests a switch from glycolysis to mitochondrial oxidative phosphorylation during the spontaneous differentiation of hPSCs into cells of all three germ layers. This process is reversed during the reprogramming of somatic cells into induced pluripotent stem cells (iPSCs). However, the mechanisms underlying this switch remain unclear. We hypothesized that mitobiogenesis is increased during motor neuron differentiation. To test this, we differentiated commercially available human neural stem cells (hNSCs) and iPSCs into motor neurons in low (5%) oxygen conditions. During this process hNSCs increased mRNA and protein expression of genes expressed by post mitotic spinal motor neurons. Electrophysiological recordings including whole cell voltage and current clamp confirmed the maturation of neurons. These cells also increased expression of peroxisome proliferator-activated receptor gamma, co-activator 1-α (PGC-1α), an upstream regulator of transcription factors involved in mitobiogenesis, as well as its downstream targets. This was correlated with increased protein expression of electron transport chain subunits but no change in mitochondrial mass. Our findings suggest that mitochondrial biogenesis, but not mitochondrial mass, is increased during differentiation of hNSCs into a motor neurons.

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The autotaxin-LPA axis modulates histone acetylation and gene expression during oligodendrocyte differentiation

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During development, oligodendrocytes (OLGs), the myelinating cells of the central nervous system (CNS), undergo a stepwise progression during which OLG progenitors, specified from neural stem/progenitor cells, differentiate into fully mature myelinating OLGs. This progression along the OLG lineage is characterized by well-synchronized changes in morphology and gene expression patterns. The latter have been found to be particularly critical during the early stages of the lineage, and they have been well-described to be regulated by epigenetic mechanisms, especially by the activity of the histone deacetylases HDAC1 and 2. The data presented here, identify the extracellular factor autotaxin (ATX) as a novel upstream signal modulating HDAC1 activity and gene expression in cells of the OLG lineage. Using the zebrafish as an *in vivo* model system as well as rodent primary OLG cultures, this functional property of ATX was found to be mediated by its lysoPLD activity, which has been well-characterized to generate the lipid signaling molecule lysophosphatidic acid (LPA). More specifically, ATX's lysoPLD activity was found to modulate HDAC1 regulated gene expression during a time window coinciding with the transition from OLG progenitor to early differentiating OLG. In contrast, HDAC1/2 regulated gene expression during the transition from neural stem/progenitor to OLG progenitor appeared unaffected by ATX and its lysoPLD activity. Thus, and taken together, our data suggest that an ATX-LPA-HDAC1 axis regulates OLG differentiation specifically during the transition from OLG progenitor to early differentiating OLG and via a molecular mechanisms that is evolutionarily conserved from at least zebrafish to rodent.

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Physiologic electric fields regulate the astrocytic response to injury.

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Traumatic injury to the central nervous system (CNS) induces reactivity in surrounding astrocytes that can have both positive and negative influences on repair. Astrocyte reactivity is characterized by hypertrophy of the soma; realignment of cellular processes; up-regulation of the cytoskeletal elements glial fibrillary acidic protein (GFAP), vimentin, and nestin; proliferation; and migration to the lesion border. While reactive astrocytes play a necessary role in the injury response, they ultimately inhibit regeneration by blocking sprouting axons. Altering this reactive phenotype could facilitate regeneration by selectively enhancing growth-promoting behaviors while attenuating inhibitory ones. However, it is unclear what physiologic change at the injury site induces the many cellular behaviors characteristic of astrocyte reactivity. Injury currents that induce a 10-fold increase in the physiologic electric fields (EF) have been measured in mammalian skin, cornea, and bone where they have been shown to direct cellular behaviors essential to the reparative responses in those tissues. A similar increase in physiologic EFs has been reported in the mammalian CNS upon injury, but the extent to which these injury-induced EFs can drive astrocyte reactivity has not been fully elucidated. With this in mind, our lab has become interested in chronicling the injury currents in the mammalian CNS following a stab injury, and in determining whether the intensity and duration of these EFs is sufficient to drive the reactive phenotype. Using a vibrating probe electrode to measure the injury currents, we found that a stab wound in the rat cortex induces an injury current similar to those demonstrated in other injured mammalian tissues. We then demonstrated that these physiologic EFs drive multiple behaviors in isolated astrocytes *in vitro* that are characteristic of the reactive phenotype see *in vivo.* EF exposure induces hypertrophy of the cell soma with increased expression of the cytoskeletal elements GFAP, vimentin, and nestin, as well as other proteins associated with reactivity. We found that EFs induce a marked increase in proliferation that peaks 48 hours after onset of exposure. Furthermore, EFs affect astrocyte migration, directing them toward the anode as early as 3 hours after field onset. Together, these unique observations demonstrate that the intensity and duration of the physiologic EFs found after injury *in vivo* are capable of driving reactive behaviors of astrocytes *in vitro*. This suggests that injury-induced EFs may be an important stimulus of reactive astrocytosis and represents an ideal target to induce a more regenerative response.

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JNJ- 39393406, a novel alpha7 nAChR positive allosteric modulator, attenuates nicotine drinking in mice

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Individuals with schizophrenia diagnosis have a significant reduction of alpha7 nicotinic acetylcholine receptors (nAChR) in brain and recent reports link genetic variations in the CHRNA7 gene with tobacco dependence in this population. Our previous studies show that local administration of a selective alpha7 nAChR antagonist into the nucleus accumbens shell or anterior cingulate cortex significantly increases nicotine self-administration, suggesting that a poverty of alpha7 nAChR function promotes nicotine use. Hence, these preclinical feasibility trials assessed if an alpha7-selective positive allosteric modulator, JNJ-39393406, would inhibit oral nicotine self-administration in mice. These studies further determined JNJ-39393406 mechanism of action by comparing nicotine ingestion behavior of C57BL/6J wild type (WT) and alpha7 nAChR subunit knockout (a7KO) mice, and assessed if JNJ-39393406 effects on self-administration are shifted in heterozygous knockout mice (a7HET), which have a 50% reduction in alpha7 nAChR expression, to suggest that dosing be adjusted for individuals with schizophrenia who share this phenotype. Mice (n = 10/genotype) were given 24hr access to 0, 50, 100 and 200 ug/ml nicotine in 2% saccharin solution in their homecages. Upon stable nicotine drinking, JNJ-39393406 (0, 0.3, 1, 3 and 10 mg/ kg in 20% 2-hydroxypropyl-beta-cyclodextrin solution) was administered using a Latin-square, within-subject design via intra-gastric gavage prior to the dark cycle when mice are active. Experimenters were blinded to dosing. JNJ-39393406 significantly reduced mg of nicotine consumed and nicotine preference in WT mice. There was no shift in the dose-effect curve of a7HET mice to suggest that clinical doses be adjusted for smokers with schizophrenia. There was no effect of JNJ-39393406 evident in a7KO mice, suggesting that JNJ-39393406-associated reductions in nicotine intake were modulated via alpha7 nAChRs. These data suggest that positive allosteric modulation of alpha7 nAChRs is sufficient to reduce nicotine consumption and provide preclinical evidence to support JNJ-39393406 for smoking cessation in healthy individuals and those with schizophrenia.

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37 Oral Presentation

Alpha4 nAChRs and septum ERK signaling regulate age-associated changes in anxiety-like behavior.

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Independent previous findings have shown that a4 subunit containing nicotinic acetylcholine receptor (a4b2*nAChR) expression declines with age and that pharmacological inhibition of a4b2*nAChRs reduces anxiety-like behavior in rodents. To study the age-dependent effects of reduced basal expression of a4b2*nAChRs on anxiety-like behavior, these studies assessed 6-8 month (ADULT) and 22-24 month (AGED) wild type (WT) and mice heterozygous for an a4 nAChR subunit null mutation (a4HET, which have a 50% reduction of a4b2*nAChR expression) in the light-dark and open field tests. AGED mice and a4HET ADULT mice showed significantly reduced anxiety-like behavior

compared to ADULT WT controls in these assays. Surprisingly, AGED a4HET mice showed elevations in anxiety-like behavior compared to ADULT a4HETs. Similar to aging, selective antagonism of a4b2*nAChRs with dihydro-beta-erythroidine (DHbE) increased anxiety-like behavior in ADULT a4HETs. Following challenge injection of either saline or a behaviorally relevant dose of DHbE, the lateral septum and anterior cingulate cortex were extracted from ADULT and AGED mice of both genotypes for western blot analysis. In parallel to behavioral studies, vehicle-injected AGED WT mice and ADULT a4HET mice showed elevated levels of septal pERK and pCREB compared to vehicle-injected WT controls. DHbE injection blocked this elevation in ADULT a4HET mice. There was no effect of treatment, age or genotype on ERK or CREB levels in the anterior cingulate. Immunoprecipitation assays on the septum, amygdala, anterior cingulate cortex and hippocampus showed reduced a4b2*nAChR expression in a4HET mice but failed to repeat previous findings of reduced a4b2*nAChR expression with aging, perhaps due to detection of the intracellular pool of nAChRs using this assay. Together with previous data these findings suggest that reduced expression of a4b2*nAChRs that may occur with normal aging supports anxiolysis-like phenotype but that inhibition of this pool of receptors may increase anxiety-like behavior in individuals that have a poverty of a4b2*nAChR expression. These studies further suggest that a4b2*nAChR expression may provide a valuable biomarker to determine if individuals may be at risk for developing adverse emotive side-effects of therapeutic drugs such as varenicline, which selectively inhibit a4b2*nAChRs.

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α7 nicotinic acetylcholine receptor expression does not appear to modulate oral operant ethanol self-administration in female mice

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There is a high prevalence of comorbid nicotine and ethanol (EtOH) use. Nicotinic acetylcholine receptors (nAChRs), including α7 nAChRs, have been implicated in the behavioral and biological effects of EtOH. Mice lacking the α7 subunit (α7KO) are more sensitive to EtOH-induced locomotor activation, hypothermia, and loss of righting reflex, are less sensitive to EtOH-induced memory impairment, and consume less EtOH. α7KO mice show no alterations in EtOH metabolism, indicating that α7 nAChR regulation of EtOH effects is not due to altered metabolism. In addition to nAChR contributions to the effects of EtOH, it is also important to consider that EtOH effects vary depending on sex. While a number of EtOH effects have been linked to α7 nAChR activity in male mice, no known studies have investigated α7 nAChR contributions to EtOH self-administration (SA) in male or female mice. Thus, the goal of the present study was to determine if α7 nAChRs modulate oral operant EtOH SA in female mice. WT mice, α7KO mice and α7 heterozygous mice that have a 50% reduction of the α7 subunit (α7HT) were trained to orally self-administer EtOH during 16 hr overnight binge SA sessions once every 7 days for 9 weeks. Reinforcers earned and EtOH consumed (g/kg) were measured. A main effect of EtOH concentration and a session x EtOH concentration interaction was found for reinforcers earned and EtOH consumed, revealing that EtOH reinforcement was concentration-dependent and EtOH intake increased across sessions. There was no effect of genotype detected for any measure. This data suggests that α7 nAChRs in female mice may not

regulate oral operant EtOH SA; rather, it is likely that other nAChR subunits regulate EtOH reinforcement in female mice. Further studies are needed to determine if α7 nAChRs regulate EtOH SA in males. Overall, this study contributes to knowledge regarding nAChR regulation of EtOH reinforcement, which is important for the development of targeted therapies for alcohol use disorders.

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39 Oral Presentation

Effects of HIV-1 Tat on oligodendrocyte viability: iGluR-mediated Ca^{2+} dysregulation and GSK3β activation

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Myelin pallor is frequently reported in HIV patients, and can occur in the CNS prior to other evidence of disease process. Previous work from the lab showed that oligodendrocytes (OLs) are direct targets of HIV-1 Tat (transactivator of transcription). Tat induces a dose-dependent increase of intracellular Ca^{2+} level ($[Ca^{2+}]_i$) in cultured murine OLs, which can be attenuated by ionotropic glutamate receptor (iGluR) antagonists MK801 and CNQX. Interestingly, this Tat-induced $[Ca^{2+}]_i$ increase leads to increased death in immature ($O4^+$, MBP^-), but not mature ($O4^+$, MBP^+) OLs over 96 h. Glycogen synthase kinase 3β (GSK3β) has been long known to be an important downstream modulator of $[Ca^{2+}]_i$ change. Since the activity of GSK3β is developmentally regulated in oligodendroglial lineage cells, we hypothesized that the differential effects of Tat on immature/mature OL viability are mediated via GSK3β activation. At resting state, murine OL cultures that were enriched with immature or mature OLs, respectively, showed similar levels of GSK3β inhibitory phosphorylation at Ser9. After Tat treatment, this level was decreased in immature OLs, but not in mature OLs. Both the GSK3β inhibitor, valproic acid and sb415286, and the iGluR antagonists, MK801 and CNQX, blocks Tat-induced GSK3β activation and immature OL death. Together, these data strongly suggest that 1) Activity of GSK3β in OLs can be regulated by Tat-induced iGluRs activation and 2) OLs at different developmental stages show different responses to Tat, possibly due to different activation levels of GSK3β.

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Nociceptin/orphanin fq role in glial development: control of glutamate transporter expression in maturing astrocytes.

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Previous results from our laboratory showed that the timing of oligodendrocyte development and brain myelination are highly regulated by signaling through the

Nociceptin/Orphanin FQ receptor (NOPR). NOPR, a G-protein coupled receptor and the most recently discovered member of the opioid receptor family, is specifically activated by nociceptin, an endogenous heptadecapeptide produced by astrocytes. Interestingly, astrocytes also express NOPR, raising the possibility of an autocrine effect on these cells mediated by nociceptin. In support of this possibility, we now found that nociceptin plays a crucial role in regulating the expression of GLAST (EAAT1 in humans), a glutamate/aspartate transporter highly expressed in early astrocytes and radial glia. Deficiency of this transporter is associated with a variety of problems including increased seizure duration and severity, episodic ataxia, and altered gait and motor coordination. Our studies showed that treatment of cultured rat brain astrocytes with nociceptin results in a dramatic increase in the expression levels of GLAST, an effect abrogated by co-incubation of the cells with either BAN-ORL24 or J-113397, two highly specific inhibitors of NOPR.

Furthermore, the regulation of GLAST expression by the nociceptin system is also observed *in vivo*. Levels of this transporter were found to be significantly decreased in the brain of rat pups subjected to postnatal administration of a blood brain barrier permeable NOPR inhibitor, as well as in the brain NOPR knockout pup mice. These novel findings indicate that, in addition to a role in controlling oligodendrocyte development, nociceptin also plays a crucial function in maturing astrocytes and their capacity to support glutamate homeostasis in the developing brain. (Supported by VCU Center for Clinical and Translational Research Endowment Fund Grant)

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Traumatic brain injury-induced functional hippocampal asymmetry of spatial learning and memory tasks in C57BL6/J mice.

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Traumatic brain injury (TBI) is a devastating disease characterized by clinical cognitive impairments such as learning and memory deficits, reduced processing speed, and impaired attention. Left-right hippocampal asymmetry has been described in humans which allows for functional specialization of memory. Murine left-right asymmetries have, as of yet, only been demonstrated in hippocampal receptor morphology and long-term potentiation. Here we examined whether hippocampal asymmetry of memory function is also present in mice. To address this question we used a moderate/severe lateral fluid percussion injury over the left or right parietal cortex, and measured impairments in hippocampal-dependent spatial memory tasks of the Morris Water Maze (Fixed Platform and Reversal tasks). Both left and right lateral injuries produced comparable righting times, 391.8±54.3 and 415.0±81.3 seconds respectively, a reliable correlate of injury severity. We found that both left and right lateral injuries produced impairments in the expression, but not acquisition, of reference memory in the Fixed Platform task. Whereas left, but not right, lateral injuries produced impairments in both acquisition and expression of memory in a Reversal task with increased cognitive flexibility demands. As such, differences in Morris Water Maze task-related demands revealed lateralization of TBI-induced cognitive deficits in mice, suggesting increased clinical validity for the use of mice as models of human TBI.

42 Oral Presentation

Monoacylglycerol lipase inhibitors produce opioid sparing effects in a murine model of neuropathic pain

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Serious clinical liabilities associated with the prescription of opiates for pain control include constipation, respiratory depression, tolerance, abuse, and addiction. A recognized strategy to circumvent these side effects is to combine opioids with other antinociceptive agents. Accordingly, the combination of opiates with the primary active constituent of cannabis, D^9-tetrahydrocannabinol, produces enhanced antinociceptive actions, suggesting that cannabinoid receptor agonists can be opioid sparing. In the present study, we tested whether inhibition of monoacylglycerol lipase (MAGL), the chief enzyme responsible for degradation of the endogenous cannabinoid 2-arachidonylglycerol (2-AG), will produce opioid sparing effects in a murine model of neuropathic pain (constriction injury (CCI) of the sciatic nerve); thus, reducing opiated-associated side effects. CCI produces robust increases in sensitivity to light mechanical touch, or allodynia, as assayed with the von Frey test, and increased thermal sensitivity, or thermal hyperalgesia, as assayed with the hotplate test. The dose-response relationships of i.p. administration of morphine and the novel MAGL inhibitor MJN110 were tested alone and in combination at an equally effective ratio in reversing CCI-induced mechanical allodynia and thermal hyperalgesia. The respective ED50 (95% confidence interval) doses of morphine and MJN110 given alone were determined to be 2.43 and 0.43 mg/kg. Isobolographic analysis of equally effective dose combinations of MJN110 and morphine revealed synergistic anti-allodynic and anti-thermal hyperalgesia effects. The acute antinociceptive effects of the combination of morphine and MJN110 required μ-opioid, CB1, and CB2 receptors. Further, this combination did not reduce gastric motility, a significant untoward side-effect of opioid use. In subsequent experiments, we found that repeated administration of combinations of MJN110 and morphine (i.e., twice a day for six days) retained their full antinociceptive effects. These findings taken together suggest that MAGL inhibitors are opiate sparing yield diminished tolerance and constipation side effects.

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The Effects of 7,8-DHF on neuroprotection and neuroplasticity following a TBI

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Aside from preventing Traumatic Brain Injuries altogether, treatment options for TBI focus on the secondary biochemical processes that occur in response to the primary mechanical insult. These secondary injuries can lead to apoptosis and necrosis in the days and weeks that follow a TBI. Therefore, finding a treatment that can prevent, reduce, and repair the

damages of secondary damages is futile in the recovery of TBI patients. The flavonoid 7,8-dihydroxyflavone (7,8-DHF) has been identified as a TrkB agonist that mimics the effects of brain derived neurotrophin factor (BDNF). Upon binding to the TrkB receptor, signal cascades are initiated that can promote neuronal survival and neural differentiation. The use of 7,8-DHF in the treatment of TBI is favorable due to its ability to pass the blood brain barrier and long ½ life. In this study, we evaluated the dosage time frame of 7,8-DHF that would allow for the greatest impact in recovery and plasticity after a focal TBI. Adult Sprague-Dawley rats were subjected to a moderate cortical impact injury and administered a 5mg/kg dose of 7,8-DHF i.p. for five days starting on day 1, 2, 3, or 5 post injury. Fear Conditioning and Morris Water Maze (MWM) were used to evaluate cognitive function. Sensorimotor function was evaluated with beam walking and rotarod test. Biotinylated dextran amine (BDA) was injected into the contralateral cerebral cortex at 14 days after injury. Animals were sacrificed 28 days after injury. Brain sections were processed for Giemsa histological staining to assess cortical lesion volume and the total number of survival neurons. Parallel sections were also processed for BDA staining to assess changes of axon sprouting in the injured cortex. We found that administration of 7,8- DHF starting at early time post TBI improved functional recovery of both motor and cognitive functions. Histological examination showed a significant reduction of cortical lesion volume and higher number of survival neurons in the injured hippocampus when 7,8-DHF was given at the earlier time post injury. BDA staining showed that DHF treatment also enhanced intracortical axon sprouting. Collectively, the results have suggested that DHF provides not only neuroprotection but also promotes regeneration making it a promising treatment for TBI.

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Persistent inflammation following traumatic brain injury does not affect hippocampal neurogenesis

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Traumatic Brain Injury (TBI) is the leading cause of death in the United States with no effective long-term treatment options available. After the primary insult, a cascade of secondary events can cause lasting cognitive and functional deficits long after the initial injury. Neuroinflammation is one of the major secondary events which can last for prolonged period and is linked to the development of neurodegenerative disease post-TBI. It is known that proinflammatory condition is detrimental for neurogenesis. TBI-TBI-induced chronic neuroinflammation has shown negative effect on hippocampal neurogenesis in a focal injury model. In this study, we examined whether diffuse brain injury also induces a prolonged neuroinflammation which can impact neurogenesis. In this study, three months old adult male Sprague-Dawley rats received a moderal lateral fluid percussive injury. Animals were sacrificed at 3 months after injury. Brain sections were processed for immunostaining with inflammatory cell marker OX6, cell proliferation marker Ki67 and doublecortin (DCX) as a marker for neurogenesis. The number of immunostained cells in the hippocampus, while matter tract (corpus callosum) were quantified. Cell counting data revealed that at 3 months after TBI, there were a greater number of OX6+ cells in the injured animals compared to sham both on the ipsilateral and contralateral side. However, the number of Ki67+ cells and DCX+ cells in the ipsilateral

dentate gyrus is no different between the injured and sham animals. Our data suggests that diffuse brain injury induces a prolonged inflammatory cell response and this chronic response has no effect on hippocampal neurogenesis.

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Oxycodone dependence on startle, sensorimotor gating, and body weight in HIV-1 Tat expressing and non-expressing mice

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Abuse of prescription opioids, such as oxycodone (OXY), has increased in recent decades. HIV-infected individuals are at a high risk of developing opiate abuse and there is evidence of worsened cellular damage and behavioral deficits symptomatic of HIV-associated neurocognitive disorders (HAND) occasioned by the likely interaction of the opiates with the neurotoxic HIV-1 Tat protein. Previously, in agreement with clinical evidence of HAND-related sensorimotor gating deficits, we found significant deficits in prepulse inhibition of the startle reflex (PPI), an operational measure of sensorimotor gating, in transgenic mice that centrally expressed the HIV-1 Tat protein upon administration of a doxycycline (DOX)-containing diet. We subsequently hypothesized that chronic exposure to OXY sufficient to induce physical dependence would engender greater PPI deficits in Tat-expressing vs Tat-null mice. After a 2-week DOX diet, a 10-day escalating OXY dosing regimen (9-33 mg/kg, s.c.) was utilized to induce physical dependence in DOX-fed Tat(+), Tat(-), and C57BL/6J (i.e., B6) mice. Surprisingly, chronic OXY did not affect startle or PPI significantly in any group. Precipitating withdrawal with naloxone (1 mg/kg, s.c.) also did not affect PPI in any group. However, upon precipitated withdrawal from OXY, B6 and Tat(-) mice had a significant ($p<0.05$) loss in bodyweight and a significant reduction in the startle reflex as compared to vehicle challenge that, again, surprisingly, was not observed in Tat(+) mice. Together, these results suggest that Tat-expressing mice may be more resistant to opiate withdrawal effects. Ongoing studies will examine the effects of acute administration of OXY on startle/PPI, as well as ascertain whether there are DOX- or naloxone-specific effects.

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FGF22 and FGFBP1 promote neuromuscular development and maintenance

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Recent findings indicate that members of the fibroblast growth factor signaling module play important roles in the development and maintenance of synapses in the brain. In this study, we assessed the roles of fibroblast growth factor 22 (FGF-22), a ligand for FGFR2b, and FGF binding protein 1 (FGFBP1), an enhancer of FGF activity, at neuromuscular junctions (NMJs). We found that FGF deficiency delays maturation of NMJs. Specifically, there are more multiply innervated NMJs at 8 days of age in FGFBP1 knockout mice compared to control. In line with these findings, the apposition of pre- and post-synapses is reduced in mice lacking FGF-22 and FGFBP1. However, these developmental defects are

short-lived, as NMJs in FGF-deficient mice appear indistinguishable from those in controls at 21 days of age, a time when NMJs have fully developed. We then asked if these factors are needed to repair NMJs after severing innervating motor axons by crushing the peroneal nerve. The absence of FGFs significantly delayed axonal reoccupation of previously vacated sites on muscle fibers. During the course of this study, we also asked if FGF-22 and FGFBP1 are required to slow age-related structural damages at NMJs. FGF22 and FGFBP1 knockout mice displayed obvious signs of accelerated aging. Their NMJs were characterized by increased postsynaptic fragmentation, elevated levels of denervation, and swelling of axon terminals, compared to control animals of the same age. Not surprisingly, these mice exhibit motor deficits on a rotarod and hanging test. Altogether, our findings strongly suggest important roles for FGF-22 and FGFBP1 in maintaining and repairing NMJs.

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Abnormal social behavior and pain sensitivity in CA3-Restricted BDNF Knockout mice

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Brain-derived neurotrophic factor (BDNF) is an important neurotrophic factor, which contributes to synaptic transmission and plasticity, and presumably regulates brain circuits in health and disease. BDNF is highly expressed in the hippocampus, particularly in the CA3 region of the hippocampus. Mice with BDNF knockout restricted to the hippocampal area CA3 show no deficits in cognitive tasks and anxiety-like behaviors, but show dominance and exaggerated aggression towards the cagemate (Ito et al., 2011). To explore the role of BDNF in the hippocampus in social behaviors, we test two social behaviors and pain sensitivity using the BDNF knockout mice.

1) Social interaction with anesthetized conspecific: We developed a social interaction task, which measures duration of contacts with conspecific demonstrator in distress. Here, the demonstrator is immobilized by anesthesia in the home cage. KO mice showed little interaction with anesthetized cagemates (familiar demonstrator), whereas WTs actively explored their partner. The difference did not appear when the anesthetized mice were unfamiliar to the subject.

2) Sociability Test: Next, to validate the task performed in (1), we employed a standard "sociability test" using the 3 chamber paradigm (Moy, Nadler, et al., 2004). The sociability was measured by duration of contact to either a cup with unknown conspecific or an empty cup. The test did not show difference between genotypes.

3) Formalin Test was performed to test perception of persistent pain. KO mice showed less licking of the formalin injected rear paw specifically during the sensitization period (15-25min after formalin injection).

We have identified task-specific alteration of social interactions in BDNF KO mice, which showed less contact to anesthetized familiar demonstrators, but not to the unfamiliar ones. In contrast, the sociability test did not show difference between genotypes. This discrepancy might result from the familiarity of demonstrators. The reduced contact to familiar demonstrator in distress and the lack of sensitization to persistent pain suggest

that BDNF deletion compromises neuronal circuits, which are responsible for perception of pain and drive empathy-like behaviors.

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Silent synapses in the prefrontal-amygdala synapses after psychological trauma

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Witnessing pain and distress in others can cause psychological trauma and increase odds of developing PTSD in the future, upon exposure to another stressful event. However, the underlying synaptic process remains unknown. Here we report that mice exposed to a conspecific receiving electrical footshocks exhibited enhanced passive avoidance learning when trained 24 h after the exposure. The exposure activated neurons in the dorsomedial prefrontal cortex (dmPFC) and basolateral amygdala (BLA) and altered synaptic transmission from dmPFC to BLA. It increased amplitude, slowed decay of NMDA receptor-mediated currents and generated silent synapses. Administration of sub-anesthetic ketamine immediately after the exposure prevented the enhancement of passive avoidance learning and silent synapse formation. These findings suggest that ketamine can prevent pathophysiological consequences of psychological trauma.

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Argonaute 2 Localization at the NMJ is Disrupted in Disease-afflicted Muscles

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MicroRNAs have been implicated in mediating stress-responses in skeletal muscles, including neuromuscular junctions (NMJs). These findings suggest that altering the expression of specific microRNAs could have a beneficial impact on injured, aging and disease-afflicted muscles. Because microRNAs require the RNA induced silencing complex (RISC) to impact cellular processes, we examined a key catalytic component of this complex, Argonaute 2 (Ago2), in both developing and adult mouse skeletal muscles. Using light microscopy, we found Ago2 initially dispersed throughout developing muscle fibers and gradually accumulating at maturing NMJs. In adult muscles, we found Ago2 enriched at the NMJ and intercalated between acetylcholine receptors (AChR). Supporting these findings, we discovered that Ago2 mRNA is also enriched in the synaptic region of muscles. These findings suggested that motor axons affect the level and distribution of Ago2 in muscles. To test this possibility, we treated cultured myotubes generated using the clonal myoblast cell line, C2C12, with the neuronal-derived splice variant of agrin. This factor is necessary to stabilize AChRs in developing and adult muscles. We found that Ago2 levels increase and remain elevated in cultured myotubes exposed to neural-agrin, further indicating that innervating motor axons regulate the function of microRNAs in muscles by affecting the levels and distribution of Ago2. In this regard, we discovered that Ago2 disperses from the NMJ as motor axons degenerated during the symptomatic stage of ALS in the SOD1G93A mouse model for the disease. Similarly, we found Ago2 scattered

throughout muscles fibers denervated after innervating motor axons were surgically severed. Together, these results suggest that Ago2 may play important roles in developing and adult NMJs. Importantly, our finding strongly suggest that microRNA-mediated interventions must include strategies to preserve the function of the RISC complex.

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Pur alpha: a potential therapy for ALS due to the C9ORF72 expanded repeat.

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that results in paralysis due to loss of motor neurons in the cortex and spinal cord. The most common genetic cause of ALS is an expanded hexanucleotide repeat (GGGGCC) in a non-coding region of the C9ORF72 gene, which encodes a protein involved in endocytosis and autophagy. Patients with this mutation have hundreds to thousands of copies of this repeat sequence. Repeat sequence RNA aggregates in nuclear foci that can be detected by fluorescent in situ hybridization (FISH). The mechanism by which the C9ORF72 repeat causes ALS is unknown, but a prominent hypothesis is that the repeat sequence in the RNA sequesters RNA-binding proteins. Disturbing the content of RNA-binding proteins in the cell can affect transcription, translation, and splicing of many mRNAs, resulting in expression of altered RNAs and reduction in RNA content.

We hypothesize that overexpression of Pur-alpha, an abundant RNA- and DNA-binding protein that binds to GGGGCC repeats, or a peptide with a generic Pur family repeat may be therapeutic in C9ORF72 ALS. Overexpression of Pur alpha in Drosophila or neuronal cells with 30 copies of the repeat resulted in a reduction in neurodegeneration (Xu et al., 2012). We used cells from C9ORF72 ALS patients to test the effects of Pur alpha on cellular pathology. We labeled autophagosomes in virally transformed lymphocytes from patients and controls with an antibody to p62, a protein that binds to the autophagosome membrane. These organelles are involved in removal of proteins that misfold and aggregate in the cytoplasm of neurons in ALS. Lymphoblasts from C9ORF72 patients had significantly more p62-labeled organelles than control cells. Transfection of Pur alpha or treatment of cells with the peptide resulted in a significant reduction in p62-positive puncta in C9ORF72 lymphoblasts. The peptide reduced the number of these autophagosomes to close to the level seen in control cells. Our results suggest that Pur proteins and the peptide may stimulate autophagy and ameliorate cellular pathology in this form of ALS. Additional experiments are underway to test the efficacy of the peptide in reducing cellular pathology in motor neurons differentiated from iPS cells of C9ORF72 ALS patients.

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Axon Initial Segment degeneration is reversible in a murine model of multiple sclerosis

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Experimental Autoimmune encephalomyelitis (EAE) is an inflammatory mouse model of multiple sclerosis (MS). In both EAE and MS, axonal domains, which are required for proper central nervous system function, are unstable and degenerate. One domain that is significantly disrupted in the cerebral cortex of mice induced with EAE is the Axon Initial Segment (AIS). The AIS is positioned immediately distal to the neuronal cell body and is responsible for modulating and initiating action potential. In a previous study from our lab, we have shown that the maintenance of the AIS is independent of myelin but that AIS disruption corresponds with inflammation consequential of EAE. In the early stages of EAE, we observe a shortening of AISs and in late stages of disease the number of AISs is significantly reduced. Following treatment with didox, an anti-inflammatory drug, at the early disease stage, AIS length is restored and the loss of AISs is inhibited. These findings demonstrate the potential of didox to inhibit disease progression and to reverse disease-related deterioration. In the present study we have further tested the capacity of didox to reverse axonal degeneration by administering didox to EAE-induced mice at late stages of disease when the AISs are significantly reduced. Preliminary immunocytochemistry results show that didox treatment of late stage EAE mice have higher average numbers of AISs when compared to untreated chronic EAE mice strongly suggesting that didox has the ability to reverse AIS degeneration observed in this inflammatory model of MS. These findings are important since they provide the first evidence that AIS degeneration, an axonal pathology observed in MS and other neurodegenerative diseases, is reversible through therapeutic intervention.

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The impact of altering cholinergic activity on NMJs in normal and stress conditions

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The neuromuscular junction (NMJ) undergoes significant structural and functional changes with aging and progression of diseases that affect the motor system. Recently, it was shown that a calorically restricted diet and physical exercise can slow and even reverse extensive structural alterations that occur at aging NMJs. Because motor neurons and skeletal muscles are more frequently used in both lifestyles, these findings suggest that increased synaptic activity slow aging of NMJs. To test this hypothesis, we used transgenic animals for the vesicular acetylcholine transporter (VAChT), a protein required to package acetylcholine into synaptic vesicles. These animals have increased (VAChT-Hyper) acetylcholine levels and release at synaptic sites. We first asked if altering cholinergic activity impacts the normal development of NMJs. Using light microscopy, we found no obvious differences in the size, architecture and rate of synaptic elimination between NMJs in 9 days-old VAChT-Hyper and control mice. At later ages, however, we discovered significant structural and molecular alterations. By 1 month of life, there is a significant increase in denervation of NMJs followed by fragmentation of the postsynaptic site in VAChT-Hyper transgenic compared to control mice. These findings indicate that presynaptic changes precede postsynaptic alterations and demonstrate that heightened

cholinergic activity accelerates structural changes at NMJs associated with aging and progression of amyotrophic lateral sclerosis (ALS). Consistent with this conclusion, we found aged and disease-related gene signatures in young adult VAChT-Hyper muscles. Finally, we provide evidence suggesting that increasing cholinergic activity contributes to ALS-related destruction of the motor system using a mouse model for the disease. Hence, fine tuning cholinergic activity in muscles may be a therapeutic approach to slow down the progression of age and ALS-related motor deficits.

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The human dopamine transporter

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In recent work, we employed a series of methylenedioxypyrovalerone (MDPV) analogs to determine the structural determinants for the potent nature of MDPV to inhibit uptake by the human dopamine transporter (hDAT). In hDAT-expressing *Xenopus laevis* oocytes clamped to −60 mV, MDPV and its analogs induced comparable outward currents (attributed to a block of the endogenous hDAT inward leak) that did not return to the original baseline after washout. We recorded DA-induced hDAT currents before and after application of either MDPV or its analogs, and obtained individual amplitude recovery profiles relative to the initial DA-induced currents, which correlated with the compounds' potency to inhibit DA uptake via hDAT. Interestingly, for all compounds, after washout of the second DA application, the hDAT-mediated shift in baseline returned to the elevated level. Moreover, for two MDPV analogs the second DA response recovered 100% of the first DA response even if the baseline had shifted. Furthermore, the shift in baseline produced by one of these analogs was not impeded by the presence of a high concentration of dopamine. These results suggest two distinct sites of action for drugs targeting DAT. We employed electrophysiology to characterize this secondary site of action, and homology models of hDAT based on the crystal structure of a bacterial leucine transporter (LeuT) combined with docking simulations to identify the secondary binding site of MDPV and MDPV analogs.

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54 Oral Presentation

Pharmacological Implications of $A_{2A}R$-D_2R heteromerization and the significance for Parkinson disease

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Recently, heteromeric GPCR complexes have become attractive targets for drug development since they exhibit distinct signaling and cell-specific localization from their

homomeric counterparts. Yet, the effect of heteromerization on the pharmacology of many GPCR homomers remains unknown. We have undertaken the task to examine the effect of heteromerization on G_s signaling through the adenosine 2A receptor ($A_{2A}R$) and G_i signaling through the dopamine receptor type 2 (D_2R) because the $A_{2A}R$-D_2R heteromer is an emerging therapeutic target for Parkinson's disease. We examined the effect of heteromerization on $A_{2A}R$ and D_2R homomeric signaling using electrophysiology and the *Xenopus* oocyte heterologous expression system. GIRK channels were used as reporters for G_i signaling because activation leads to direct $G_{beta\text{-}gamma}$-mediated stimulation of the GIRK current. We also coupled GIRK channels to G_s signaling by over expressing $G_{\alpha s}$. Our electrophysiological assay is innovative because it allows us to optimize the conditions of heteromerization and directly observe GPCR signaling at the G-protein level. We anticipate that specific ligand combinations targeting the $A_{2A}R$-D_2R heteromer will be more efficacious than individual drug administration targeting $A_{2A}R$ or D_2R homomers. Preliminary data have demonstrated that heteromer formation alone decreases dopamine-elicited G_i signaling through the D_2R and CGS-21680-elicited G_s signaling through the $A_{2A}R$. Furthermore, this reciprocal antagonism was predominately due to changes in efficacy versus potency. Currently, we are examining crosstalk by assessing whether $A_{2A}R$ agonists or inverse agonists will decrease or increase D_2R-mediated G_i signaling through the $A_{2A}R$-D_2R heteromer. Modulation of G_s signaling through the $A_{2A}R$ by D_2R ligands is also being examined. Characterization of the signaling pathway through the $A_{2A}R$-D_2R heteromer will provide insight into what ligands optimize dopaminergic signaling leading to the development of novel therapeutics for Parkinson's disease.

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Non-essential role for the NLRP1 Inflammasome complex following traumatic brain injury

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Traumatic brain injury (TBI) results in the immediate production of pro-inflammatory cytokines which participate in initiating an immune response. While the mechanisms regulating the adaptive immune response in secondary injury are well characterized, the role of the innate response is unclear. Recently, the NLR inflammasome has been shown to become activated following TBI, causing processing and release of interleukin-1β (IL-1β). The inflammasome is a multi-protein complex consisting of nucleotide-binding domain and leucine-rich repeat containing proteins (NLR), caspase-1 and apoptosis-associated speck-like protein (ASC). ASC has been shown to be upregulated after TBI and is critical in coupling the proteins during complex formation resulting in IL-1β cleavage. To directly test whether inflammasome activation contributes to acute TBI-induced damage, we assessed IL-1β, IL-18 and IL-6 expression and contusion volume in *Nlrp1*$^{-/-}$, *Asc*$^{-/-}$ and wild type mice 3 days after the controlled cortical impact (CCI) injury. Although IL-1β expression is significantly attenuated in the cortex of *Nlrp1*$^{-/-}$ and *Asc*$^{-/-}$ mice following CCI injury, no difference in contusion volume is observed at 3 days compared to wild type. We further demonstrate no motor behavior differences between the strains of mice at 3-14 days post-

CCI. These findings indicate that inflammasome activation does not significantly contribute to acute neural injury in the mouse CCI model.

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Physiological and Anatomical Cell Types of the Caudal Mesopallium

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In the avian auditory system, selective and invariant representations of learned conspecific vocalizations emerge in the caudal mesopallium (CM). Similar connectivity and gene expression patterns in the auditory pallium and mammalian auditory cortex suggest conserved circuit mechanisms for pattern learning and recognition. CM may be analogous or homologous to superficial cortical layers. To investigate mechanisms of invariant object recognition in CM, we characterized electrophysiological and morphological properties. Golgi-Cox staining revealed at least 4 cell types with spiny dendrites. One class has a pyramidal-like shape with a thick dendritic branch and smaller branches on the other sides. Other classes are distinguished by soma size/shape and spine density on dendritic processes. Whole-cell intracellular recording from NN neurons revealed at least 3 distinct classes of neurons within CM based on firing patterns. Phasic cells uniquely fire only 1-2 spikes during suprathreshold stimulation with broad spikes. Regular-spiking cells show marked frequency adaptation during stimulation with changes in spike height and shape over time. Fast-spiking cells display little to no frequency adaptation with a high firing rate and narrow spike shape. These three classes are likely to integrate synaptic inputs differently and may correspond to extracellular response properties found in previous studies. Variation in sag current, rebound spiking, and other physiological properties within neuron classes suggests additional subclasses. Biocytin reconstructions of recorded neurons suggests that phasic- and regular-spiking neurons correspond to specific Golgi-Cox classes.

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EphrinB3 blocks EphB3 dependence receptor functions to prevent cell death following traumatic brain injury.

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Eph receptor tyrosine kinases and their membrane-bound ligands, ephrins, have a variety of roles in the developing and adult central nervous system that require direct cell-cell interactions; including regulating axon path finding, cell proliferation, migration and synaptic plasticity. Recently, we identified a novel pro-survival role for ephrins in the adult subventricular zone, where ephrinB3 blocks Eph-mediated cell death during adult neurogenesis. Here, we examined whether EphB3 mediates cell death in the adult forebrain

following traumatic brain injury and whether ephrinB3 infusion could limit this effect. We show that EphB3 co-labels with microtubule-associated protein 2-positive neurons in the adult cortex and is closely associated with ephrinB3 ligand, which is reduced following controlled cortical impact (CCI) injury. In the complete absence of EphB3 (EphB3(-/-)), we observed reduced terminal deoxynucleotidyl transferase-dUTP nick end labeling (TUNEL), and functional improvements in motor deficits after CCI injury as compared with wild-type and ephrinB3(-/-) mice. We also demonstrated that EphB3 exhibits dependence receptor characteristics as it is cleaved by caspases and induces cell death, which is not observed in the presence of ephrinB3. Following trauma, infusion of pre-clustered ephrinB3-Fc molecules (eB3-Fc) into the contralateral ventricle reduced cortical infarct volume and TUNEL staining in the cortex, dentate gyrus and CA3 hippocampus of wild-type and ephrinB3(-/-) mice, but not EphB3(-/-) mice. Similarly, application of eB3-Fc improved motor functions after CCI injury. We conclude that EphB3 mediates cell death in the adult cortex through a novel dependence receptor-mediated cell death mechanism in the injured adult cortex and is attenuated following ephrinB3 stimulation.

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The neuroprotective properties of didox

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Didox has been shown to be effective in reversing the clinical symptoms of experimental allergic encephalomyelitis (EAE) an animal model for multiple sclerosis. The molecular mechanisms which are responsible for these clinical effects have not been shown. We now report that didox protects neurons via preservation of intact phosphorylated axons as well as preventing the proliferation of CD4+ cells and providing protection against oxidative stress. A cohort of EAE mice which had reached a clinical score of 3-4 were randomly assigned to two groups: a control group which received vehicle orally on a daily basis while the treatment group received didox orally (550mg/kg) for 6 days. The control group maintained a clinical score of 3-4 while the didox treated animals improved to a clinical score of 1-2. Immunocytochemical analysis of the lumbar spinal cord axons demonstrated that the control group had twice as many damaged axons as intact axons whereas the didox-treated animals had twice as many intact axons as damaged axons. The damaged axons in the didox treated group were at the same level as damaged axons found in naïve untreated animals. Splenocytes were harvested from naïve mice and stimulated with 3 different mitogens in the presence of increasing concentrations of didox. For each mitogen there was a dose dependent inhibition of proliferation; at 25 um didox no proliferation was noted. C6 glioma cells were exposed to oxidative stress (via cumene hydroperoxide) in the presence or absence of didox. There was a didox dose dependent protection of the cells at 10uM didox (72% protection) and 20 uM didox (91% protection). These data suggest that the improved clinical status of didox-treated EAE mice is due to the neuroprotective, antiproliferative and antioxidative properties of didox. (Supported by a Merit Grant from the VA)

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Pronounced hypoxia in the subventricular zone following traumatic brain injury and the neural stem/progenitor cell response.

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Traumatic brain injury (TBI) elicits identifiable changes within the adult subventricular zone (SVZ). Previously, we demonstrated that EphB3/ephrinB3 interaction inhibits neural stem/progenitor cell (NSPC) proliferation and downregulating this pathway following TBI plays a pivotal role in the expansion of the SVZ neurogenic compartment. It remains unclear, however, what early initiating factors may precede these changes. Using hypoxyprobe-1 (HPb) to identify regions of low oxygen tension or hypoxia (<1%), we found HPb uptake throughout the cortex (CTX), corpus callosum (CC) and SVZ within the first 24 h following controlled cortical impact (CCI) injury. At this early time point, HPb co-localized with EphB3 in the SVZ. NSPC specific markers also co-localized with HPb staining throughout the lateral wall of the ventricle. To determine the cell autonomous effects of hypoxia on EphB3/ephrinB3 signaling in NSPCs, we used an in vitro model of hypoxia to mimic 1% oxygen in the presence and absence of soluble aggregated ephrinB3 (eB3). As expected, hypoxia stimulated the uptake of 5-bromo-2'-deoxyuridine (BrdU) and reduced cell death. Coincident with these proliferative changes, both Hif1-α and phospho (p)-AKT were increased while EphB3 expression was decreased. Stimulation of EphB3 attenuated hypoxia-induced proliferation and prevented phosphorylation of AKT. Hif1-α accumulation, on the other hand, was not affected by EphB3/ephrinB3 signaling. These findings indicate that this pathway limits the NSPC response to hypoxic stimuli. These studies also suggest that early transient changes in oxygen tension following localized cortical injury may initiate a growth-promoting response in the SVZ.

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