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Poster Presentation Abstracts

213 Abstract Title:	**Modulatory Action of Acetylcholine in Mechanosensory Processing in Drosophila melanogaster: Behavior, Development, and Sensory-motor Circuit Physiology**
Author(s):	E. Somasundaram, Department of Biology, U of Kentucky C.A. Malloy, Department of Biology, U of Kentucky A. Omar, Department of Biology, U of Kentucky R.L. Cooper, Department of Biology, U of Kentucky

Abstract: The model organism, Drosophila melanogaster uses many of the same neurotransmitters as mammals and other organisms. Acetylcholine (ACh) is one of these neurotransmitters. In this model, ACh is the primary transmitter used in peripheral sensory neurons projecting to the Central Nervous System (CNS) and is the primary excitatory transmitter within the CNS. A number of stereotyped sensorimotor responses have been confirmed to be regulated by cholinergic sensory input in Drosophila, each of which are mediated by distinct circuits guided by individual neuronal populations within the peripheral nervous system. One such subset of sensory neurons are the Class IV multidendritic (md) sensory neurons that are known to be integral in mediating response to noxious stimuli, including tactile touch. Here, we have utilized an optogenetic approach to activate and suppress select cholinergic sensory neurons and observed the acute response to tactile touch. We utilized a well-characterized assay to assess the response of 3rd instar Drosophila larvae to a 20mN tactile stimulus upon alteration of activity. Additional work to assess the role of activity of individual subsets of cholinergic neurons, including Class IV md neurons, in modulating this response is underway. The ability to temporally alter activity of subsets of neurons at various developmental stages allows for additional questions to be evaluated. This sensorimotor circuit can be utilized to assess the role of activity in formation and maintenance of a defined neural circuit. Behavioral, morphological and electrophysiological assessments can be utilized after long-term, chronic alteration of these subsets of neurons to identify persistent changes in neural circuit function.

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32nd Annual BGSFN Spring Neuroscience Day
Poster Presentation Abstracts

214 Abstract Title:	**Pharmacological Identification of Cholinergic Receptor Subtypes in Modulation of a Drosophila melanogaster Sensory-motor Circuit**
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Abstract: Acetylcholine (ACh) is an abundant neurotransmitter found in many species. In mammals, it is known to be integral in modulating neural circuits underlying important processes such as learning, memory, and reward processing. In Drosophila melanogaster, ACh exhibits comparable importance. It is the neurotransmitter used in sensory neurons and is the primary excitatory neurotransmitter within the CNS. The receptors that facilitate synaptic transmission at cholinergic synapses are divided into two broad subtypes: the ionotropic nicotinic acetylcholine receptors (nAChRs) and the metabotropic muscarinic acetylcholine receptors (mAChRs). This receptor classification is shared in multiple species; however, the pharmacological and functional characterization of these receptors within the Drosophila nervous system has lagged behind its model counterparts. To further the classification of these receptors in the nervous system of an important model organism, we have used a behavioral and electrophysiological approach to identify cholinergic receptor subtypes within the Drosophila CNS that may be crucial in modulating defined neural circuits. We have exposed intact Drosophila 3rd instar larvae to various concentrations of ACh agonists and antagonists by way of feeding to observe modulation of locomotion. In addition, we have utilized a well-characterized electrophysiological approach to assess the efficacy of a defined sensory-CNS-motor circuit in the presence of cholinergic agonists and antagonists exposed directly to the CNS of a semi-intact larval preparation. Preliminary results suggest that exposing the CNS directly to ACh and agonists, nicotine and muscarine, enhances electrical activity of a sensory-CNS-motor circuit. Conversely, acute feeding of nicotine and acetylcholine suppresses locomotion suggesting both receptors may modulate distinct motor circuits.

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Poster Presentation Abstracts

215 Abstract Title:	**The Effect of CO2, Intracellular and Extracellular pH on Mechanosensory Proprioceptor Responses in Crayfish and Crab**
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Abstract: We present experimental models and paradigms for students in a neurophysiology teaching lab to explore the effect of CO2 and relationship of intra/extracellular pH on neuronal function. The basic concepts of lab have a physiological relationship with human disorders, chronic obstructive pulmonary disease (COPD), sudden infant death syndrome (SIDS), and are active areas of neurobiological research. In furthering our understanding in basic cellular responses to pH/CO2 is fundamental in addressing physiological and pathological processes. The models we use are the proprioceptors of blue crab walking leg joints and crayfish muscle receptor organ. The paradigm monitors electrical activity associated with the nerves during activation while exposing the preparations to CO2 bubbled saline, pH 5 saline, and 20mM propionic acid saline. The results demonstrate extracellular pH 5 did not greatly alter activity; CO2 bubbled saline and propionic acid caused activity to cease. We suggest a drop in intracellular pH is either blocking stretch activated mechanosensory channels or ion channels associated with production of action potential. Future studies with intracellular recordings will examine if graded depolarizations and action potentials can be induced by electrical stimulation. Either stretch activated channels or voltage gated Na+ channels are targeted for the effect of intracellular pH. These findings are relevant as some mechanosensory ion channels belong to degenerin/epithelium sodium channels and a group of these are acid-sensing ion channels and activated by low extracellular pH. Furthering our understanding in basic cellular responses to pH/CO2 is fundamental in addressing physiological and pathological processes related to all animals.

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216 Abstract Title:	**(2016) An Undergraduate Education Module Based on a Research Question: The Effects of Neighboring Muscle Injury on Proprioception Responses in Crayfish and Crab**
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Abstract: Proprioception is an important sensory modality to detect limbs positions in reference to the body. The proprioceptive neurons monitor the direction, velocity of movement and static positions of body parts. We report on the development of an experimental module to understand how raising the extracellular concentration of K + ions can alter proprioceptive responses. In addition, the exercises relates to authentic health issues and future research endeavors related to hyperkalimia as well as the effects of deep tissue injury (DTI) which indirectly affects neuronal function. We use the chordotonal organ in the most distal joint of the walking leg in the blue crab (propodite-dactylopodite, PD organ) and the muscle receptor organ (MRO) of the crayfish abdomen as the two experimental models. Raising [K+] in incremental steps (from 20 to 60 mM) as well as diluted skeletal muscle homogenates with estimated concentration of K+, we noted the response are impaired around 20 mM K and neural activity is silenced at 30 mM. Thus, a DTI of a large skeletal muscle in an intact organism could have an impact on non-injured neighboring neurons. This could relate to altered sensory-motor neuronal function to non-injured bodily parts in the presence of a DTI. The MRO preparation is a standard neurophysiology protocol for teaching how to record extracellular neural activity and to explore sensory physiology; whereas the PD organ is not as widely used but is very amenable for student driven neurophysiology courses. Other species of crabs can also be used for the same protocol. University level student observations and comments are reported.

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32nd Annual BGSFN Spring Neuroscience Day
Poster Presentation Abstracts

217 Abstract Title:	**Examining the Pharmacology of Stretch Activated Ion Channels in Mechanosensory Proprioceptor Responses in Crayfish, Crab and Drosophila**
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Abstract: The physiology of mechanosensory transduction is diverse in that there are many types of receptors which transduce mechanical forces into an electrical neural impulses across taxa. Receptors that are sensitive to mechanosensation are used to monitor both external and internal forces and are essential in transferring information that allow for appropriate behavioral responses to stimuli and body positioning. Invertebrates serve as models in understanding the physiology of mechanoreception due to the diversity of receptor types and the relative ease with which one can utilize these models in experimentation. The family of stretch activated ion channels is broad and is currently being characterized by gene/protein sequences and pharmacological profiles. Common types of mechanoreceptors are those associated with chordotonal organs (COs), which monitor joint movements within the limbs of arthropods. The MRO in the crayfish abdomen is a well-described model system but only preliminary studies have been conducted for examining pharmacology of these receptors. In addition, the pharmacology of the COs in the limbs of crabs has not previously been investigated. This preparation offers unique properties as the sensor ending are embedded in an elastic strand with cell bodies and endings relatively exposed. We examined the effect of amiloride, ruthenium red and a low extracellular pH in an attempt to profile the pharmacology of these receptors. For comparison, we also examine cuticular mechanosensors in larval Drosophila known to be sensitive to amiloride. Our goal is to enhance understanding of the physiology of COs which can serve as models for mechanosensation.

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218 Abstract Title:	**Acute and chronic effects of inhibiting mTOR by rapamycin on development, behvaior, and physiology in larval Drosophila**
Author(s):	S.J. Potter, Department of Biology, U of Kentucky R.S. Potter, Department of Biology, U of Kentucky S.L.E. Blumich, Department of Veterinary Sciences, U of Leipzig J. Sifers, Department of Biology, Alice Lloyd College E. Yocum, Department of Biology, Kentucky Wesleyan College R.L. Cooper, Department of Biology, U of Kentucky

Abstract: Rapamycin is a compound that can specifically block mTOR signaling and is therefore used in experimental biology. It is being utilized clinically as an immunomodulator after transplantation procedures and treatment for some forms of cancer. Due to its many possible effects on different molecular pathways, it could have any number of impacts on synaptic transmission. This issue has not, however, been addressed in a developing system. We hope to address it by feeding second and third instar Drosophila larvae varying concentrations of rapamycin and monitoring larval stages, pupation, and survival. Typical larval behavioral assays being examined are mouth hook movement while eating and body wall movement while crawling on apple juice agar plates. Behaviors in the adults fed rapamycin include climbing, righting response, and movement assays. The results to date suggest 2nd instar larvae are more susceptible to rapamycin as compared to 3rd instar, based on a higher death rate. Adults fed rapamycin climb less over time and tend to fall off the wall when climbing. Dose-response studies are being established. This study is significant as we are starting to address the acute and long-term action of inhibiting the mTOR pathway on neuronal function and potential mechanisms to account for altered physiological function.

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219 Abstract Title:	**Modification of the serotonergic system in larval Drosophila: development, behavior and physiology**
Author(s):	E. Greene, Department of Biology, U of Kentucky B. Demers, Department of Biology, U of Kentucky Z.R. Majeed, U of Salahaddin, Erbil, Iraq; Dept. Biology, U of Kentucky J. Morgan Department of Biology, U of Kentucky R. L. Cooper, Department of Biology, U of Kentucky

Abstract: The neurotransmitter serotonin (5-HT) is an important modulator in animals that controls both behavior and physiology. Many antidepressant drugs function by increasing the amount of serotonin in the synaptic cleft. Although this excess of serotonin may provide mood relief, increased excitation of the serotonergic system could have harmful effects on development, specifically fetal development. Many studies have been conducted on this particular topic by selectively activating the serotonergic system via temperature sensitive activation and feeding fluoxetine (prozac) using transgenic organisms. This specific investigation focuses on using optogenetics as a more rapid, invariable way to selectively manipulate the 5-HT system. Drosophila melanogaster is used as the model system because of the powerful genetic tools available such as the UAS- Gal4 system, which can be used to manipulate various populations of neurons including serotonergic neurons. The optogenetic tool, channelrhodpsin 2 (ChR2), is also used to increase the activity of serotonergic neurons in a spatiotemporal controlled manner in behaving larvae. I hypothesize that exciting the serotonergic system using optogenetics will provide a more rapid, cleaner approach to demonstrate the effect of altered neural circuits in third instar Drosophila larvae controlling locomotion. Specifically, selective over excitation of the serotonergic system during development will effect the late stage larvae's ability to produce normal body wall contractions.

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32nd Annual BGSFN Spring Neuroscience Day
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220 Abstract Title:	**Manipulation of various neural circuits and the effect on behavior in Drosophila using optogenetics: NGSS-Neurons, genetics, and selective stimulations**
Author(s):	B. Demers, Biology, U of Kentucky F. Koch, U of Leipzig, Leipzig, Germany Z. Majeed, Biology, U of Salahaddin, Erbil, Iraq J. Morgan, Dept. Electrical Engineering, U of Kentucky H. Andersen, DuPont Manual High School R. L. Cooper, Dept. Biology & Center for Muscle Biology, U of Kentucky

Abstract: The objective of this module is to explain and address principle concepts in neurobiology. The goal of neurobiology is to show how neural circuit activity controls corresponding behavior in animals. We use Drosophila melanogaster as a model system and powerful genetic tool such as UAS-Gal4 system to manipulate various populations of neurons: glutanergic neurons, serotonergic neurons, GABAergic neurons as well as cholinergic neurons. The optogenetic tool, channelrhodpsin 2 (ChR2), is employed to increase the activity of each population of neurons in a spatiotemporal controlled manner in behavior of larvae and adult flies. Various behavioral assays are used to observe the effect of specific neuron population activation on crawling behavior in larvae and climbing behavior in adult flies. Students will be exposed to important aspects in control of neural circuits, which control the animal's behavior. Also, by doing these activities the students will become well acquainted with the actions of different neurotransmitters in the nervous system. A pre- and post- assessment survey on the content was provided to the high school and college students who participated in the experimentation. A large improvement on the students' understanding of content and concepts was gained by conducting this module in the high school and college classes.

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32nd Annual BGSFN Spring Neuroscience Day
Poster Presentation Abstracts

221 Abstract Title:	**Optogenetic and pharmacological alteration in the GABAergic system within Drosophila melanogaster affects development, feeding behavior and locomotion.**
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Abstract: Drosophila melanogaster is a model system due to a relative simple nervous system and the ability to make modifications genetically for studies on identifiable neurons within the live animal. Key questions in understanding the function of the central nervous system (CNS) in physiologic and pathologic conditions can be addressed in this system quickly and cheaply to develop a foundation which can be followed up in mammalian systems. Neuromodulators and neurotransmitters play a significant role in the activity of the CNS. Many studies address the effects of enhanced activity on synapse formation and axon growth but few address the effects of reducing activity or the effects of GABA, an inhibitor modulator in the brain. Our central research question is on the role of GABA, on the development and maintance of neural circuits and in relation to animal behavior. We examined the effects of administering varying levels of GABA to the organisms through tests on development, behaviors, olfaction, survival and physiology. The developmental time to pupation and to eclosion as well as behavioral tests were also investigated. The larval development is slowed in a dose-dependent manner with feeding GABA. Locomotive behavior is not as sensitive as mouth hook movements in the third instar larvae to low concentrations of food tainted with GABA. The optogenetic stimulation of GABA neurons in larvae produces locomotive alterations. We are now addressing the role of various GABAergic receptor subtypes in larval CNS related to development of neural circuits and behavioral changes. This study may help to address the roles GABAergic transmission throughout all animals.

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32nd Annual BGSFN Spring Neuroscience Day
Poster Presentation Abstracts

222 Abstract Title:	**Cyclosporine A Protects Synaptic and Non-Synaptic Mitochondrial Bioenergetics 24h Following Severe Controlled Cortical Impact Injury**
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Abstract: Following traumatic brain injury (TBI), mitochondria play an essential role in maintaining cellular homeostasis and survival. Therefore, mitochondria are promising therapeutic targets for prevention of cellular death and dysfunction following TBI. Mitochondria are heterogeneous, consisting of both synaptic and non-synaptic populations, which have distinct properties. It is essential mitochondria targeted pharmacotherapies protect both synaptic and non-synaptic populations to be considered optimally effective. One of the most promising mitochondrial targeted TBI therapies is the mitochondrial permeability transition pore (mPTP) inhibitor, cyclosporine A (CsA). However, previous studies have focused on CsA protection of total mitochondria. This study is the first to examine the effects of acute CsA administration on isolated synaptic and non-synaptic mitochondria following TBI, as well as the first to study the effects of CsA on mitochondrial bioenergetics 24h following TBI in adult rodents. Our results indicate that synaptic mitochondria sustain more damage 24h following severe controlled cortical impact injury (CCI) than non-synaptic mitochondria, and that administration of CsA (20mg/kg, i.p.) 15min following injury is able to significantly attenuate mitochondrial respiratory dysfunction in both populations. However, following injury CsA treatment results in synaptic mitochondria that are still significantly impaired compared to non-synaptic mitochondria. Further characterization of both synaptic and non-synaptic mitochondria in TBI, as well as their responses to pharmacotherapy following injury is essential in order to better understand the contribution each population makes to TBI pathology, and to the successful development of mitochondria directed therapeutic strategies.

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32nd Annual BGSFN Spring Neuroscience Day
Poster Presentation Abstracts

223 Abstract Title:	**Neurocognitive Findings Associated with Complicated Meningioencephalocele of the Dominant Temporal Lobe**
Author(s):	K. J. Dunham, Department of Neurology, U of Kentucky A. J. Anderson-Mooney, Department of Neurology, U of Kentucky

Abstract: Objective: Temporal lobe meningoencephalocele is an unusual middle fossa herniation often requiring neurosurgical intervention (Wind, Caputy, & Roberti, 2008). Little neurocognitive data is available regarding the effects of the pathology and/or surgical repair. Thus, we review longitudinal findings in a patient with left temporal meningioencephalocele, highlighting surgical complications, neurocognitive outcomes, and key considerations for assessment methodology and clinical recommendations. Method: A 58-year-old, right-handed woman with 13 years of education presented with longstanding left ear CSF leak after an extended history of otitis media with hearing loss. Imaging revealed tegmental and middle fossa floor erosion with temporal herniation into left middle ear space, thought to be chronic. Surgical repair involved a small area of resection, and recovery was complicated by subdural hematoma (left greater than right with mild mass effect) requiring evacuation. The patient subsequently developed persistent concerns with memory, attention, practical executive functioning, and expressive language. Results: Despite significant improvements in the 3-year interval between assessments, profound impairment in sustained attention and difficulty adjusting to variable task demands remained, along with relative weaknesses in mental arithmetic, mental flexibility, language repetition, delayed list recall, and manual dexterity. Some degree of cognitive interference related to mild depression was evident. Conclusions: Significant recovery was demonstrated after a complicated history of left temporal meningioencephalocele. Deficits on follow-up were generally mild and congruent with clinical history, though prominent higher-level dysexecutive change remained disabling. This highlighted the importance of thoroughly assessing higher-order executive functions in a predominantly left temporal pathology, findings from which significantly shaped clinical recommendations for this patient.

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32nd Annual BGSFN Spring Neuroscience Day
Poster Presentation Abstracts

224 Abstract Title:	**Signaling and Expression of a Truncated, Constitutively Active Human Insulin Receptor**
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Abstract: Insulin signaling is indispensable for key metabolic pathways in the periphery. Several studies have demonstrated that insulin signaling is also important for brain function. Early stage clinical trials report a positive impact of intranasal insulin on memory recall in young subjects and patients with mild cognitive decline or Alzheimer's disease (AD). However, the underlying molecular mechanisms involved are not well understood. Here we sought to investigate the role of insulin in neuronal physiology by overexpressing a constitutively active human insulin receptor (Lebwohl et al., 1991) in rat pheochromocytoma (PC12) and primary hippocampal neurons. Cells were transfected with either pCI-ires-dsRed, a mammalian expression plasmid encoding a red fluorescence protein (ds-Red), or pCI-HA-IRβ-ires-ds-red, the construct with a truncated human insulin receptor beta subunit (IRβ), via either electroporation (PC12 cells) or a targeted lentiviral delivery system (neurons). The expression of IRβ receptor in PC12 cells was corroborated by the expression of the red fluorescent protein. Photomicrographs of mixed primary hippocampal cultures confirmed expression of the lentiviral plasmid in neurons. The expression level and effect of IRβ overexpression on insulin signaling was confirmed in PC12 cells by performing immunoblots using antibody against HA-tagged IRβ and measuring pAkt/Akt ratio. Our data show that overexpression of insulin receptor enhances neurite outgrowth and increases the pAkt/Akt ratio in PC12 cells. Overexpression of truncated receptor increased insulin signaling compared to control. This initial characterization provides insights into future intervention approaches to combat reduced insulin signaling in AD and/or aging.

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225 Abstract Title:	**Effects of Environmental Conditions on c-fos Expression in Rat Nucleus Accumbens After Remifentanil**
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Abstract: Previous studies have shown that adolescents raised in impoverished conditions are more likely to develop drug abuse in adulthood. In addition, both stress-causing living conditions (impoverishment/isolation) and drugs of abuse may lead to an increase in the c-fos transcription factor in the reward circuit of the brain, particularly in the nucleus accumbens. The aim of the current study was to quantify the number of c-fos positive cells in the nucleus accumbens of enriched and isolated rats exposed to the opioid remifentanil. Thirty-two male Sprague-Dawley rats were raised in either enriched or isolated conditions for one month, after which they received 10 i.v. infusions of 3 µg/kg remifentanil or saline through the jugular vein. Eighty-five minutes after the last infusion, rats underwent perfusions. After immunohistochemistry was performed on tissue containing the nucleus accumbens, the average number of c-fos positive cells per slice was obtained using ImageJ. Using a 2x2 between subjects ANOVA, with drug and environment as factors, this research demonstrated a main effect of environment on c-fos expression in the nucleus accumbens, with isolated rats expressing more c-fos positive cells than enriched rats. However, there was no significant effect of drug treatment, suggesting that remifentanil did not increase total c-fos as expected. This study demonstrated the cellular consequences of being raised in different living conditions, as it showed that individuals raised under high levels of stress may be at risk of altered cell signaling and gene expression in the reward system of the brain.

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32nd Annual BGSFN Spring Neuroscience Day
Poster Presentation Abstracts

226 Abstract Title:	**Restricting feeding to the active phase in middle-aged mice attenuates adverse metabolic effects of a high-fat diet without reducing caloric intake**
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Abstract: High-fat diet (HFD) adversely affects metabolism and cognition. In young adult mice, time-restricted feeding (TRF) of HFD lessens the negative metabolic effects. Because obesity is prevalent in the middle-aged population, we tested whether TRF ameliorates HFD-induced impairments of metabolism and cognition in 12 month-old male C57BL6 mice. Groups of mice (n=15 ea) were fed: 1) HFD (60% fat) ad-libitum (AL), 2) low-fat diet (LFD, 10% fat) AL), or 3) HFD-TRF, i.e., HFD access for 8 hours at night, for 21 or 25 weeks. Diet affected body weight gain ($p<0.0001$). HFD-TRF mice gained less than HFD-AL mice (~20% vs 55%, respectively), but lower caloric intake which occurred only at weeks 3 & 5 did not account for this difference. Average daily cage activity assessed with motion detectors varied among the groups ($P=0.042$) and was lower in HFD-AL than LFD-AL mice ($P<0.05$) but HFD-TRF mice did not differ from either group. Diet did not alter performance on the novel object and novel location tests. After 16 weeks, a glucose tolerance test was conducted after an overnight fast by collecting tail vein blood samples at 0, 15, 30, 60, and 120 min after glucose injection (2 g/kg, i.p.). Glucose tolerance, assessed as incremental area under the curve (iAUC), was lower in HFD-TRF than in HFD-AL ($p<0.02$) but not different between HFD-TRF and LFD-AL. After euthanasia, livers but not fat pads from HFD-TRF mice weighed less than those from HFD-AL mice ($p<0.0001$), but did not differ from those of LFD-AL mice. In conclusion, TRF attenuates the metabolic effects of a HFD in middle-aged mice and may be an attractive strategy for ameliorating human obesity because it does not require caloric restriction.

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32nd Annual BGSFN Spring Neuroscience Day
Poster Presentation Abstracts

227 Abstract Title:	**Cholinergic modulation of locomotion and feeding behaviors in Drosophila melanogaster larvae**
Author(s):	C.A. Malloy, Department of Biology, U of Kentucky E. Somasundaram, Department of Biology, U of Kentucky A. Omar, Department of Biology, U of Kentucky R.L. Cooper, Department of Biology, U of Kentucky

Abstract: We investigated the role of acetylcholine (Ach) in the Drosophila melanogaster larval CNS to identify how this important neurotransmitter modulates locomotion and feeding behaviors. We used a multitude of genetic approaches in order to deduce the role of cholinergic neuronal signaling in mediating these behaviors and to gain a better understanding of modulation of neural circuits that drive these two distinct behaviors this model. In addition, we hope to employ novel techniques to identify the role of chronic manipulation of cholinergic neuronal activity in formation and maintenance of sensory-CNS-motor neural circuits. In Drosophila, Ach is a neurotransmitter within the CNS and is the neurotransmitter for sensory neurons but not motor neurons, as in mammals. A distinctive advantage of Drosophila larvae is the short developmental time (~4 days) in which the development of the CNS can be investigated. The alteration in neural activity related to circuits is particularly important during neural development for formation and stabilization of neural connections. In addition, the proposed experimental design allows for a multitude of options for future experimentation including investigation of regulation of olfaction and response to light upon altering the cholinergic system. All of these are testable for proof of concept and will provide the degree of inhibition or activation that results in the defined genetic techniques employed in sensory responses. This study will help to establish the role of cholinergic neuronal activity in regulating simple motor behaviors and will help to assess activity-dependent neural circuit formation and maintenance.

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228 Abstract Title:	**Intracellular Changes in Nicotinic Acetylcholine Receptors in Response to Ligand Exposure: A Single Molecule Approach**
Author(s):	A.M. Loe, Department of Chemistry, U of Kentucky C.I. Richards, Department of Chemistry, U of Kentucky

Abstract: Although tobacco consumption is the leading preventable cause of death, addiction to nicotine prevents many users from quitting. It is well documented that chronic nicotine exposure induces a variety of physiological changes in nicotinic acetylcholine receptors (nAChRs), leading to an incomplete link between nAChRs and nicotine addiction. The nAChRs are pentameric ligand-gated ion channels consisting of alpha (α2-10) and beta (β2-4) subunits. Exposure to nicotine and its primary metabolite, cotinine, have been shown to alter the trafficking and assembly of nAChRs, as well as shift the overall stoichiometry of α4β2 to favor expression of the high sensitivity (α4)2(β2)3 version. We have developed a novel method to resolve organelle specific changes in the stoichiometry of an individual receptor using cell-derived nanoscale vesicles that encapsulate a single nAChR in the native cell membrane. Once nAChRs originating in the endoplasmic reticulum or plasma membrane are separated, we use single molecule photobleaching to evaluate stoichiometry differences under nicotine exposure. In these studies, a green fluorescent protein (GFP) is encoded in the sequence of each alpha subunit of the isolated nAChR. Since GFP stochastically bleaches when exposed to continuous excitation, the number of single molecule bleaching steps detected correlates to the number of alpha subunits present in the nAChR pentamer, and therefore the stoichiometry. Detecting changes in stoichiometry within the endoplasmic reticulum supports the theory that nicotine affects nAChRs intracellularly. Our single molecule method using organelle specific membrane derived vesicles provides strong evidence that nicotine acts on nascent, unassembled subunits within the endoplasmic reticulum.

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229 Abstract Title:	**Inhibition of astrocytic calcineurin/NFAT signaling in a mouse model of vascular cognitive impairment and dementia**
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Abstract: Astrocytes are one of the most abundant cell types in the brain, and play a vital role in maintaining healthy nervous tissue. Calcineurin (CN), an exquisitely Ca2+-sensitive protein phosphatase that has been associated with several neurodegenerative diseases, appears at elevated levels in activated astrocytes associated with aging, injury, and disease. The signaling between CN and the transcription factor NFAT (Nuclear Factor of Activated T-cells) regulates several critical pathways in astrocytes, including those involved in excitotoxicity, inflammation, and neuronal death. Inhibition of the astrocytic CN/NFAT pathway in a mouse model of Alzheimer's disease was associated with reduced glial activation and improved synaptic and cognitive function (Furman et al. 2012). However, no studies that we know of have investigated the role of astrocytic CN/NFAT signaling in vascular cognitive impairment and dementia (VCID). Here, we used adeno-associated virus (AAV 2/5) vectors containing an astrocyte-specific promotor, Gfa2, and VIVIT, a potent NFAT inhibitor, to selectively inhibit astrocytic NFAT signaling in a diet-induced mouse model of VCID. AAV-treated mice were maintained on either control or methionine-enriched and folate-deficient diet for a minimum of 11 weeks to induce hyperhomocysteinemia (HHcy) associated with vascular pathology. HHcy diet was associated with a significant reduction in hippocampal synaptic strength and long-term potentiation (LTP). Both of these deficits were ameliorated by AAV-Gfa2-VIVIT, suggesting that astrocytic CN/NFAT signaling contributes to synaptic dysfunction during VCID. Other hallmarks of VCID, including cognitive decline and cerebral hypoperfusion, are being assessed in a second cohort of HHcy mice using the radial-arm water maze (RAWM) task and MRI. Histochemical analyses will also be performed to characterize the relationship between the astrocytic CN/NFAT pathway and a variety of vascular abnormalities, including microhemorrhages and microinfarcts.

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230 Abstract Title:	**Analysis of Hairy-related 9 (her9) During Vertebrate Ocular Development**
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Abstract: The hairy-related 9 (her9) gene—belonging to the hairy/enhancer of split (hes) superfamily of Basic-Helix-Loop-Helix-Orange (BHLH-O) transcription factors which are involved in many developmental processes—is expressed during vertebrate embryonic retinal development and in the regenerating adult retina. Her9 was found to be upregulated in the retina of a transgenic line of zebrafish that exhibits constitutive rod photoreceptor specific degeneration and regeneration. Her9 is expressed throughout the developing central nervous system of the zebrafish, including the retina. Fluorescent in situ hybridization (FISH) experiments were conducted in a transgenic line of zebrafish that expresses the GFP reporter in vascular endothelial cells. We found that her9 expression co-localizes with markers for retinal vasculature. Pharmacological manipulation of several signaling pathways starting during the appearance of the primordial eye field revealed that her9 expression in the retina is sensitive to the Retinoic Acid (RA) signaling pathway. To investigate the function of her9, we generated her9 mutant fish lines using the CRISPR/Cas9 system. A guide RNA specific to her9 developed in our lab was co-injected with Cas9 mRNA into 1-cell stage zebrafish embryos. These founders were crossed to generate F1 embryos that, upon sequencing, were found to have an insertion or deletion causing a frameshift mutation and no gene product. If our experiments confirm that her9 plays a role in vasculogenesis, this may lead to key therapeutic treatments for eye diseases involving defects in retinal vasculature such as age-related macular degeneration (AMD), diabetic retinopathy (DR), and retinitis pigmentosa (RP).

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231 Abstract Title:	**Social Cues and Diet Restriction Act Through Similar Neural Mechanisms to Affect Aggression in the Honey Bee**
Author(s):	C.C. Rittschof, Department of Entomology, U of Kentucky C.M. Grozinger, Department of Entomology, Pennsylvania State U, State College, PA G.E. Robinson, Department of Entomology, U of Illinois, Urbana, IL

Abstract: Honey bees exposed to aggression-inducing social cues undergo a shift in brain energy metabolism that causes an enhanced response to future threats. The function of this metabolic shift is largely unknown. In a variety of organisms, diet restriction also causes changes in brain energy metabolism, and these changes are largely the opposite of those seen in the aggressive honey bee brain. Thus we hypothesized that diet restriction and aggression-inducing social cues alter neural function using similar brain metabolic mechanisms. Furthermore, based on observed patterns, we predicted that diet restriction would cause decreased aggression in the honey bee. Contrary to our predictions however, we found that long-term diet restriction caused increased aggression in the honey bee. Short-term diet restriction had no effect on aggressive behavior, suggesting the effects of diet are distinct from acute changes in hunger state. We also measured general activity levels for diet restricted bees and found that activity increased predominantly in response to short-term diet restriction. Our results suggest that social cues and diet restriction may act through similar mechanisms to affect aggressive behavior. Surprisingly, however, in the honey bee, diet restriction appears to induce a brain metabolic state that is the opposite of that observed in other organisms. This could reflect an adaptation to a carbohydrate rich nectar diet. In future studies we will evaluate whether honey bee brain mitochondria show patterns of fuel use distinct from those observed in the context of diet restriction in other species.

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32nd Annual BGSFN Spring Neuroscience Day
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232 Abstract Title:	**Impact of single or repeated dose intranasal zinc-free insulin in young and aged rats on cognition, signaling, and brain metabolism**
Author(s):	K.L. Anderson, Department of Pharmacology and Nutritional Sciences, U of Kentucky H. Frazier, Department of Pharmacology and Nutritional Sciences, U of Kentucky S. Maimaiti, Department of Pharmacology and Nutritional Sciences, U of Kentucky V. Bakshi, Sanders-Brown Center on Aging, U of Kentucky Z.R. Majeed, Department of Biology, U of Kentucky L.D. Brewer, Department of Pharmacology and Nutritional Sciences, U of Kentucky N.M. Porter, Department of Pharmacology and Nutritional Sciences, U of Kentucky A.-L. Lin, Sanders-Brown Center on Aging, U of Kentucky O. Thibault, Department of Pharmacology and Nutritional Sciences, U of Kentucky

Abstract: To combat decreased insulin signaling in the brain of Alzheimer's disease patients, several groups have used intranasal insulin in clinical and pre-clinical settings with results showing improved memory. However, it is unclear how intranasal insulin facilitates memory in animal models of aging. It is also unclear if all insulin formulations act similarly. We characterized the impact of acute intranasal insulin across different brain regions at different times following delivery. We tested the effect of Apidra® or saline on 8 different brain regions in young and aged F344 rats. Insulin activity was quantified using Western blots. The pAkt:Akt ratio significantly increased starting at 30m, mostly in the ventral brain regions. Acute and chronic Apidra® was also tested on memory retrieval using the Morris water maze in young and aged F344 rats. Saline or chronic Apidra® was delivered daily for 8 days with MWM starting on the fifth day. On the 9th day, half the saline animals received a single Apidra® dose. Neither chronic nor acute Apidra® was able to improve memory. In a subset of animals, neurotransmitter levels were determined by MR spectroscopy (MRS) and cerebral blood flow was measured by MRI. Changes in MRS and MRI signaling rule out effects mediated solely by blood flow dynamics. Our results identify mechanisms by which chronic Apidra® can challenge neuronal integrity.

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233 Abstract Title:	**Sensitivity of presynaptic pH on synaptic transmission: Differences in evoked and spontaneous release.**
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Abstract: The presynaptic terminals package transmitter into vesicles based on a proton gradient. We address issues related to altering this gradient in influencing synaptic responses. We are addressing two issues: (1) the influence of pHi on vesicular packaging of neurotransmitter; (2) response of glutamate receptors on postsynaptic targets with altering extracellular and intracellular pH. These investigations are being addressed at the model crayfish and Drosophila neuromuscular junctions (NMJs). These two projects are interrelated as transmission at glutamatergic synapses is retarded in the presence of CO2 which cannot be fully accounted for by a reduced pHi within the presynaptic nerve terminal or within the postsynaptic muscle fiber since the EPSPs increase in amplitude with rebound acidification after a pulse of NH4Cl. High (40 mM) proprionic acid acidifies both the pre- and post-synaptic cells. When used the frequency and amplitude of mini's increases despite a slight membrane depolarization. However, evoked transmission is blocked. Examining low pH on mammalian glutamatergic neurons with Fura 2 (Ca2+ indicator) in culture indicated Ca2+ release from ER as a potential mechanism to explain some of the observations for the increase in frequency of minis. The use of high [CO2] containing saline blocks evoked and mini's as well as the sensitivity of glutamate receptors. These NMJs are glutamatergic and the evoked (non-spiking) synaptic potentials and spontaneous (quantal) events are readily measured. Addressing the mechanisms underlying these observed phenomena may help in understanding synaptic depression after high frequency stimulation and feedback process in synaptic transmission. These studies tackle fundamental principles which are likely present in glutamatergic neurons for all animals.

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234 Abstract Title:	**Genetic Variants and Risk for Alzheimer's Disease: ABCA7**
Author(s):	J. B. Vasquez, Department of Physiology, U of Kentucky S. Estus, Department of Physiology, U of Kentucky

Abstract: Genome-wide association studies (GWAS) identify single nucleotide polymorphisms (SNPs) that associate with Alzheimer's disease (AD). Adenosine triphosphate binding cassette A7 (ABCA7) reached significance within several of these studies warranting investigation into this association. Our overarching hypothesis is that AD-associated SNPs modulate ABCA7 RNA expression and/or splicing to impact disease risk. Here, we analyzed ABCA7 expression in a set of human brain samples as a function of AD status and AD SNPs. We report that ABCA7 expression is increased in AD and in individuals that carry the protective rs3764650T allele. Additionally, rs200538373 has been reported to modulate ABCA7 splicing increasing AD risk. Elucidating the mechanism of action for this increased AD risk may help to target this pathway effectively with pharmacological agents. In preliminary results, we found that the minor allele of rs200538373 is associated with a 14 bp extension of exon 41 into intron 41, thereby altering the codon reading frame and introducing a premature stop codon. Additionally, we quantified expression of both isoforms using PCR to discern the extent that the atypical isoform is modulated by AD status, other SNPs, or cell type differences in the samples. Moving forward, we will use minigenes containing each allele to determine directly whether the AD-associated SNP modulates splicing. Currently our findings suggest a model that increased ABCA7 expression reduces AD risk and that the increase observed in AD reflects an inadequate compensatory change.

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Poster Presentation Abstracts

235 Abstract Title:	**Reversal of aging-related neuronal Ca2+ dysregulation and cognitive impairment by adeno-associated viral delivery of FKBP1b (12.6) to the hippocampus**
Author(s):	J.C. Gant, Department of Pharmacological and Nutritional Sciences, U of Kentucky K.C. Chen, Department of Pharmacological and Nutritional Sciences, U of Kentucky I. Kadish, Department of Cell, Developmental and Integrative Biology, U of Alabama at Birmingham E.M. Blalock, Department of Pharmacological and Nutritional Sciences, U of Kentucky O. Thibault, Department of Pharmacological and Nutritional Sciences, U of Kentucky N.M. Porter, Department of Pharmacological and Nutritional Sciences, U of Kentucky P.W. Landfield, Department of Pharmacological and Nutritional Sciences, U of Kentucky

Abstract: Brain Ca2+ regulatory processes are altered during aging, disrupting neuronal and cognitive functions. In hippocampal pyramidal neurons, the Ca2+-dependent slow afterhyperpolarization (sAHP) exhibits an increase with aging, which correlates with memory impairment. The increased sAHP results from elevated L-type Ca2+ channel activity and ryanodine receptor (RyR)-mediated Ca2+ release, but underlying molecular mechanisms are poorly understood. Previously we found that expression of the gene encoding FK506-binding protein 12.6/1b (FKBP1b), a small immunophilin that stabilizes RyR-mediated Ca2+ release in cardiomyocytes, declines in hippocampus of aged rats and Alzheimer's disease subjects. Additionally, knockdown/disruption of hippocampal FKBP1b in young rats augments neuronal Ca2+ responses. Here, we tested the hypothesis that declining FKBP1b underlies aging-related hippocampal Ca2+ dysregulation. Using microinjection of adeno-associated viral vector bearing a transgene encoding FKBP1b into the hippocampus of aged male rats, we assessed the critical prediction that overexpressing FKBP1b should reverse Ca2+-mediated manifestations of brain aging. Immunohistochemistry and qPCR confirmed hippocampal FKBP1b overexpression 4-6 weeks after injection. Compared to aged vector controls, aged rats overexpressing FKBP1b showed dramatic enhancement of spatial memory, which correlated with marked reduction of sAHP magnitude. Further, simultaneous electrophysiological recording and Ca2+ imaging in hippocampal neurons revealed that the sAHP reduction was associated with a decrease in parallel RyR-mediated Ca2+ transients. Thus, hippocampal FKBP1b overexpression reversed key aspects of Ca2+ dysregulation and cognitive impairment in aging rats, supporting the novel hypothesis that declining FKBP1b is a molecular mechanism underlying aging-related Ca2+ dysregulation and unhealthy brain aging and pointing to FKBP1b as a potential therapeutic target.

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32nd Annual BGSFN Spring Neuroscience Day
Poster Presentation Abstracts

236 Abstract Title:	**Computer Aided Diagnosis and Scoring for Parkinson's Disease utilizing Voice Recordings and Dopamine Transporter Scans**
Author(s):	W. Kimmerer, Sayre School

Abstract: A combination of standard deep brain stimulation and neural grafts of sural Schwann cells into various parts of the brain are analyzed here for their effect on vocal function in terms of the /a/ phoneme for patients with Parkinson's Disease. Feature extraction was performed on voice recordings and a subset of these features was used to compare patient's voices before and after surgery along with trial testing of machine learning algorithms for UPDRS estimation. Further machine learning analysis was performed on Dopamine Transporter Scans (DaTScans) taken from the PPMI database. Feature extraction techniques primarily focused on intensity and thresholding-based segmentation. Patients with neural grafts in the SN were observed with a decrease in symptoms in many areas, although this effect was highly variable. The patient with a neural graft in the NbM however was observed with high improvements across all features except Shimmer, with HNR (a measure of hoarseness) being particularly improved. This neural graft location shows promise (limited by sample size) as a possible target for treatment of PD voice symptoms. Machine learning performed on DaTScan data was shown to be highly discriminative for diagnosis with accuracy of up to 95% and a sensitivity of 96%. Finally machine learning was, as hypothesized, shown to be too difficult on a small dataset voice dataset with a Mean Absolute Error (MAE) for UPDRS prediction of 7-8. A similar MAE was obtained from UPDRS prediction on the DaTScan dataset.

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32nd Annual BGSFN Spring Neuroscience Day
Poster Presentation Abstracts

237 Abstract Title:	**Intranasal Delivery of Human Insulin-like Growth Factor-1 Mediates Regional Alterations to the mTOR Signaling Pathway in the Hippocampus Following TBI**
Author(s):	E.L. Littlejohn, Spinal Cord & Brain Injury Research Center, Dept. of Physiology, U of Kentucky D.M. Sama, Spinal Cord & Brain Injury Research Center, Dept. of Physiology, U of Kentucky T.M. Stewart, Spinal Cord & Brain Injury Research Center, Dept of Physiology, U of Kentucky K.E. Saatman, Spinal Cord & Brain Injury Research Center, Dept. of Physiology, U of Kentucky

Abstract: Every year nearly 2 million traumatic brain injuries (TBI) occur and are the leading cause of death and disability among adolescents and there is currently no treatment for TBI survivors. Insulin-like Growth factor 1 (IGF1), an endogenous growth factor that promotes neuronal survival and plasticity, has significant potential as a therapeutic candidate for TBI. We have shown that IGF1 improves cognitive function following brain trauma in animal models of TBI. However, the intricate signaling mechanism through which this potential therapeutic target modulates brain plasticity in the setting of TBI remains unclear. In the nervous system, PI3-K/Akt signaling predominates in mediating many of IGF1 functions, including precursor proliferation and differentiation and neuronal survival. In a transgenic mouse model with IGF1 overexpression restricted to astrocytes, we show that increased IGF1 levels in the hippocampus by means of injury-induced astrogliosis leads to increased activation of Akt. Akt activation results in the phosphorylation of multiple downstream signaling molecules including mammalian target of rapamycin (mTOR). Following brain injury, mTOR is transiently activated in the hippocampus. We hypothesized that increased brain levels of IGF1 would potentiate posttraumatic activation of the mTOR signaling pathway, a pathway associated with growth and differentiation. To this end, following severe controlled cortical impact (CCI) we delivered hIGF1 or its vehicle through intranasal administration, a clinically relevant method, daily to injured (n=6 CCI/treatment) and sham (n=3/treatment) mice. To evaluate if the effects of hIGF1 on mTOR activity were dose dependent, mice receiving 150ug hIGF1 daily were compared to groups receiving 75ug, 10ug, 1ug, and vehicle daily following injury. At 72hrs following injury, immunohistochemical labeling of pS6, a well characterized downstream effector of mTOR, was quantified in the granule cell layer, molecular layer, and the hilus of the dentate gyrus. Analysis of pS6 at the injury epicenter (3 sections/animal) suggests that hIGF1 stimulates activity of the mTOR pathway following TBI.

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238 Abstract Title:	**Preliminary experience in the validation of an observational pain scale in patients with post-stroke aphasia**
Author(s):	C.D. Soares, Department of Psychology, U of Kentucky P. Panuganti, Department of Neurology, U of Kentucky S. Aroor, Department of Neurology, U of Kentucky A. Shrivastava, Department of Neurology, U of Kentucky J.H. Smith, Department of Neurology, U of Kentucky

Abstract: Individuals with neurologic disorders may not be able to communicate basic needs, including self-reporting pain. Following prior research supporting the utility of the Pain Assessment Checklist for Seniors with Limited Ability to Communicate (PACSLAC-II) in assessing pain in advanced dementia patients; this study will aim to expand the generalizability of this instrument to patients with post-stroke aphasia. The current research was a cross-sectional cohort study on acutely hospitalized stroke patients. We analyzed the results from the first 7 enrolled patients. The a priori sample size target is 37. Participants consisted of acute stroke patients obtained from Chandler Hospital at the University of Kentucky. Participants were to be 18 years of age or older with less than 14 days between onset of ischemic stroke accompanied by aphasia. Stroke severity and aphasia severity were assessed using the NIH Stroke Scale (NIHSS). Pain was administered to stroke patients in a blinded fashion by a mechanical pain apparatus while being videotaped. Each patient underwent three separate trials, consisting of 0, 2, or 4.5 pounds of pressure in randomized order. The recordings were then rated by 2 nurses utilizing the PACSLAC-II instrument. The PACSLAC-II features a 31-item checklist featuring six categories of nonverbal, observational measures to assess pain. It was hypothesized that the PACSLAC-II would demonstrate good content validity in patients with acute stroke aphasia. Our preliminary cohort had a median age of 71 (interquartile range (IQR): 46-79), and was 57% female. All patients were right-handed, and 6/7 were non-Hispanic white. 2/7 (28.5%) of the patients had a history of non-narcotic analgesic use at baseline, and no patient had narcotic exposure. The median total NIHSS score was 17 (IQR: 11-19), and the median NIHSS language subscore was 2 (IQR: 2-3). Patients were evaluated as part of this study at a median duration of 7 (IQR: 2-11) days following stroke onset. Only a single patient was able to self-report pain intensity using a numerical rating scale. The most commonly used items on the PACSLAC-II were items 4. Increased Eye Movement (12.3 %), 16. Gasping or Breathing Loudly (9.1%), and 3. Pain Expression (9.1%). Items 21 (Guarding Sore Area), 23 (Limping), 24 (Clenched Fist), 28 (Not Wanting to be Touched), and 29 (Not Allowing People Near) were not used by any rater. Overall, the median PACSLAC-II score was 3 (IQR = 2-5.25, range = 0-12). The lowest score (0) was recorded in the expected zero pain group, and the highest score (12) was recorded in the expected highest pain group. Qualitative appraisal of the image data supported our primary hypothesis, but we did not compare PACSLAC-II scores for statistical significance between groups, as we had not yet reached our target sample size. Our preliminary results are encouraging, suggesting that the PACSLAC-II has construct validity in the assessment of experimental pain in individuals with moderate-severe post-stroke aphasia.

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32nd Annual BGSFN Spring Neuroscience Day
Poster Presentation Abstracts

239 Abstract Title:	**A comparative study of sleep and circadian rhythms between the house mouse (Mus musculus) and African spiny mouse (Acomys cahirinus**
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Abstract: The study of circadian and sleep behavior in different organisms can provide valuable insight for understanding behavioral, physiological and environmental influences on these processes. Interestingly, two species of African spiny mice, Acomys russatus (Golden spiny mouse) and Acomys cahirinus (Cairo spiny mouse) have been reported to exhibit different circadian rhythm patterns in locations where the two species overlap. Both species are primarily nocturnal when not in direct competition, but in areas of overlap A. cahirinus exhibit nocturnal behavior, while A. russatus becomes more diurnal. However, very few studies on the circadian activity of these species are available and nothing is known of their sleep behavior, which can be the dominant force in driving other diurnal variables. Therefore, we have begun to study one of these species (A. cahirinus) in greater detail alongside the well-studied house mouse (Mus musculus) using a well validated, non-invasive, piezoelectric system, that picks up all movements during wake, and the breathing rhythms during sleep. In these studies, we found A. cahirinus and M. musculus to be primarily nocturnal, but with clearly distinct behavioral patterns. Specifically, the activity of A. cahirinus sharply increases right at dark onset, which is common in nocturnal species, but surprisingly, decreases sharply just one hour later. These differences may be related to foraging differences between these species, or may be related to the socialized behavior of A. cahirinus and its poorer adaptation to isolation as compared to Mus musculus. In order to confirm the exact sleep patterns of A. cahirinus in the original cage and experimental cage, we set up four IR cameras surrounding the cage to record activity and electroencephalogram (EEG) recording. With IR camera recording in the single and group cage conditions, we found that A. cahirinus is more active before the middle of the night period than after middle of the night period in both single and group cages, and this decreased activity in the latter half of the night is much greater than Mus musculus. With an EEG recording approach, we confirmed that after middle of the night time period, wake percentage is less than before the middle of the night time period. During IR camera recording observations, we also found that A. cahirinus do not close their eyes during sleep periods of the day or night. Eye closure and sleep has not been systematically studied across mammals, but is clearly a rare behavior, that we will investigate further.

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Poster Presentation Abstracts

240 Abstract Title:	**Noninvasive Sleep Monitoring in Large Scale Screening of Mouse Knockouts (KOMP2) Produces High Hit Rate with Implications for Sleep and Behavioral Studies**
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Abstract: Genomic manipulations can aid in identifying genes that influence sleep and hence may provide insight into functions and regulation of sleep. Our current study employs a non-invasive, high throughput piezoelectric system to characterize sleep-wake phenotypes in a large population of control and single-gene knockout mice; recorded as part of the Knockout Mouse Phenotype Program (KOMP2) at the Jackson Laboratory (JAX). A piezoelectric sensor pad placed at the bottom of the mouse cage records gross body movements. The pressure signals thus generated are classified by an automated classifier into sleep and wake. The system characterizes traits that range from sleep time over 24 hours, as well as during the light and dark phase, and distribution of sleep bout lengths. The piezoelectric system has been validated with EEG and human observations, and demonstrates a classification accuracy of over 90%. To date, we have recorded over 5000 mice representing 300 knockout lines, and more than 1200 controls, both males and females. More than 40 of the 300 knockout lines compared to control mice demonstrated altered sleep phenotypes. Some genes were found to specifically alter total sleep amounts or sleep fragmentation (sleep bout lengths) primarily in the light phase, others in the dark phase. Additionally, sex differences were also found for many of the knockout mouse strains and control mice. C57BL6/NJ female mice exhibited shorter bout length and less total sleep compared to males. Several genes were also found that alter the breathing rate. A number of novel genes influencing multiple sleep traits have been identified thus far, and these data will also be compared and correlated with non-sleep traits

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241 Abstract Title:	**Combining Multiple Schedules of Reinforcement with Glutamate Biosensors to Examine the Effects of Cocaine and Food on Prelimbic Glutamatergic Signaling in Freely-Moving Rats**
Author(s):	S.R. Batten, Behavioral Neuroscience & Psychopharmacology, Dept. of Psychology, U of Kentucky J.S. Beckmann, Behavioral Neuroscience & Psychopharmacology, Dept. of Psychology, U of Kentucky

Abstract: Drug-specific reward and associated effects on neural signaling are often studied between-subject, where one group self-administers drug and a separate group self-administers a natural reinforcer. However, exposure to drugs of abuse can cause long-term neural adaptations that can affect how an organism responds to drug reward, natural reward, and their reward-associated stimuli. Thus, to isolate drug-specific effects it is important to use models that expose the same organism to all of the aforementioned. Multiple schedules provide a means of dissociating the rewarding effects of a drug from the rewarding effects of food along with their cues, within a single animal. Herein, we used glutamate biosensors implanted into the prelimbic cortex of freely-moving animals to assess glutamatergic and behavioral changes in rats performing under a cocaine-food multiple schedule. Our results show that the average amplitude of prelimbic glutamate release is greater for food compared to cocaine. The use of frequency distribution analyses shows that the frequency of glutamate release is greater for responses associated with food compared to cocaine. Our results suggest that combining glutamate biosensors with multiple schedules provides a practical means for assessing differential glutamatergic signaling associated with cocaine and food.

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242 Abstract Title:	**Brain Imaging Validated Neuropsychological Indicators of Cognition in Persons with and without Brain Injury**
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Abstract: Traumatic brain injury (TBI) is a major public health concern that affects 1.7 million people every year in the US. Mild TBI (mTBI) accounts for 80% of all reported cases. Military related mTBI, primarily from blasts, has been called the signature injury from the conflicts in Iraq and Afghanistan and has presented unique challenges for treatment. Veterans who sustain mTBI have an increased probability of developing neurological and psychiatric comorbidities post-trauma, such as posttraumatic stress disorder (PTSD). The underlying pathophysiological mechanisms may lead to long-term adverse outcomes. The current investigation utilizes brainwave signatures, or Event Related Potential (ERPs) to elucidate how persons with brain injury affect neural activity underlying cognition, which can lead to potential biomarkers for tailored diagnosis and treatment. 25 combat veterans performed a delayed-match-to-sample task while brain electrical signals were recorded from their scalps. Subjects included those with and without mTBI. Additionally, each subject completed a battery of neuropsychological tests related to attention, memory, executive functioning, and information processing efficiency. Preliminary results suggest a strong, statistically significant correlation between the brainwaves associated with attention, memory, and executive functioning (frontal and parietal ERP P300) and three neuropsychological tests that measure these same constructs. Individuals who showed enhanced frontal ERP P300 responses were faster and more accurate on an information processing task (Symbol Search; $p<0.01$). Reduced mean voltage of the P300 wave in parietal sites (particularly right parietal) were correlated with longer times and increased errors on attention/vigilance and visual scanning tasks. The current findings reveal that these three neuropsychological tests are excellent neuroimaging validated indicators of brain mechanisms sub-serving cognitive functions, i.e. processing speed, visual search, and attention/vigilance.

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Poster Presentation Abstracts

243 Abstract Title:	**Characterization of Calpain-5 in the adult and developing zebrafish retina**
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Abstract: Autosomal dominant neovascular inflammatory vitreoretinopathy (ADNIV) is a devastating inherited autoimmune disease of the eye that displays features commonly seen in other eye diseases, such as retinitis pigmentosa and diabetic retinopathy. A gain of function mutation in Calpain-5 (CAPN5), a calcium dependent cysteine protease, has been implicated as the cause of ADNIV. Capn5 is evolutionarily conserved amongst most vertebrates and has been shown to be expressed in the retina and CNS of the rat. Very little is known about the normal function of Capn5 in the adult retina, and there are conflicting results regarding its role during embryonic development. A Capn5 knockout mouse produced viable offspring with a subset that were runted at birth and died after 4 months; however, a second Capn5 null mutant allele resulted in pre-implantation embryonic lethality. Thus, there is a need for additional animal models to better understand the role of Capn5 during development and in the pathogenesis of ADNIV. Here, we lay the foundation for studying the function of Capn5 in the zebrafish model by first characterizing the expression of capn5 in the developing embryo and in the adult retina. Using a combination of RT-PCR, whole-mount, and fluorescent in situ hybridization we show that capn5 is expressed in the CNS and retina during development, that capn5 expression in photoreceptors is cone specific, and that rod photoreceptor degeneration induces expression of capn5 in neighboring cones.

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244 Abstract Title:	**Haploinsufficiency of Calcium and Integrin Binding Protein 2 (CIB2) Increases Susceptibility to Noise-Induced Hearing Loss**
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Abstract: Calcium and integrin binding protein 2 (CIB2) is localized to the mechanosensory stereocilia of inner ear hair cells and is essential for hearing. Mutations in CIB2 underlie Usher syndrome type I and non-syndromic deafness in humans. We have recently generated a knock-in mouse model that carries a p.F91S mutation in Cib2, recapitulating a deafness-associated point mutation in humans. While homozygous knock-in mice are deaf, the heterozygotes seem to have normal hearing thresholds. However, CIB2 is expected to function as a Ca2+ buffering protein inside inner ear hair cells. Considering acoustic stimulation leads to a large Ca2+ influx into inner ear hair cells, the decreased amount of wild type CIB2 may increase the susceptibility of these cells to acoustic trauma in heterozygous Cib2 mutants. Therefore, in this study, we used auditory brainstem responses to measure the hearing thresholds of heterozygous Cib2 knock-in mice and their wild type littermates before and after exposure to damaging noise. We found that hearing thresholds of heterozygous Cib2-deficient mice do not recover to the same extent as those of wild type mice following exposure to a moderate wide band noise of 100 dB SPL intensity. These results suggest that heterozygous CIB2-deficient mice are, in fact, more susceptible to noise-induced hearing loss than their wild type littermates. We conclude that CIB2 haploinsufficiency may represent a risk for noise-induced or age-related hearing loss in humans.

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245 Abstract Title:	**Modeling the Ultradian Sleep-Wake Cycle in Rodents**
Author(s):	H. Wang, Department of Biomedical Engineering, U of Kentucky S. Sunderam, Department of Biomedical Engineering, U of Kentucky

Abstract: Mathematical models are widely used in the analysis of sleep and its underlying dynamics. Validated models are useful in both replicating experimental results based on well understood physiological background as well as testing hypotheses regarding the neural circuitry in sleep dynamics. Motivated by the similarities in brain circuitry and electrophysiological rhythms, rodent sleep models are expected to shed light on various aspects of human sleep propensity. Despite the common features, rodent sleep, unlike a circadian rhythm-modulated sleep-wake cycle in humans, is polyphasic, and contains multiple bouts of sleep and wakefulness per day. Furthermore, within each sleep bout, there are alternating bouts of REM sleep, non-REM sleep, and wakefulness on shorter timescales. Physiologically based mathematical models successfully replicate the shorter timescale of wakefulness within sleep, but not the longer one corresponding to prolonged wakefulness. We adapt an existing “flip-flop” model of human sleep to capture the ultradian alternation of sleep and wakefulness on the longer timescale in rodents. The resulting model reproduces both the mean durations of alternating sleep and wake bouts as well as the circadian trends in their bout durations documented in our experiments on mice.

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246 Abstract Title:	**Effects of Ambient Temperature on Sleep in Control and Epileptic Mice**
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Abstract: Besides recurring seizures, disordered sleep is common in individuals with epilepsy and may present as reduced sleep depth with fragmentation, and altered proportions of different sleep stages. Sleep loss can in turn precipitate seizures, thus sustaining a vicious cycle. On the other hand, changes in ambient temperature (Ta) are known to elicit thermoregulatory responses that also influence the sleep-wake cycle. Vigilance state and changes in sleep quality are both critical factors in determining seizure likelihood. Manipulation of Ta offers a simple and minimally obtrusive way to titrate sleep and study the consequences on seizures in epilepsy with possible relevance in the treatment of other neural disorders as well. As a first step toward sleep modulation in epilepsy, we characterized the effects of acutely elevated temperature on vigilance states in control mice (n=10). Elevated temperatures significantly decreased time spent in Wake, while significantly increasing time in both rapid eye movement sleep (REM) and non-REM sleep (NREM). While REM bouts increased in both number and duration with Ta (but not significantly), increases observed in NREM bout duration with Ta ($p<0.05$) accompanied a reduction in the number of NREM bouts (not significant). We also assessed the temperature effects on sleep dynamics and seizure frequency/timing in the pilocarpine mouse model of chronic temporal lobe epilepsy with spontaneously recurring seizures (n=4). The data indicate that elevated temperature affects vigilance dynamics in epileptic animals as well. Ambient temperature change can therefore serve as a noninvasive means for modulating sleep with potential benefit.

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247 Abstract Title:	**Restoration of Cellular Bioenergetics After Mitochondrial Transplantation into the Injured Spinal Cord**
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Abstract: Traumatic spinal cord injury (SCI) results in excitotoxicity, reactive oxygen and nitrogen species production, and necrotic cell death which can contribute to the development of secondary pathophysiological cascades which are responsible for increasing the spread of injured tissue. Importantly, many branches of these cascades can stem from mitochondrial dysfunction, thus a single mitochondrial therapeutic can be far reaching in its potential benefits after SCI. Mitochondrial transplantation has been investigated in various models of injury both in vitro and in vivo, and while these studies show promise in alleviating tissue damage, no studies have examined the effects of transplanting mitochondria into spinal cord tissue after traumatic injury. We hypothesized that transplanting exogenous genetically-labeled mitochondria after SCI in vivo can replace damaged endogenous mitochondria, providing a multi-mechanistic approach to restore cellular bioenergetics and reduce oxidative stress. Our data indicate that mitochondrial transplantation increases overall respiration of the acutely injured cord in a dose-dependent manner. Specifically, tissues transplanted with 50ug mitochondria show significantly increased respiration compared to vehicle-treated injured or contusion injury alone after 24hr. Further, we sought to visually identify transplanted mitochondria and found that exogenous fluorescently-labeled mitochondria colocalize within a variety of host cell types by 24 hours, which can still be visualized up to 10 days post transplantation. Ongoing studies are examining the effect of mitochondrial transplantation on long-term functional recovery and tissue sparing. Given the encouraging results as a therapeutic for SCI, this novel approach may be applied to other central nervous system traumas and diseases involving mitochondrial pathophysiology.

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Poster Presentation Abstracts

248 Abstract Title:	**NFAT4 Is Up-Regulated in Astrocytes in Aging Dog Brain Model**
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Abstract: We focus on the role of the inflammatory response within brain that happens in Alzheimer's disease, traumatic brain injury, and other neurodegenerative diseases. We are particularly interested in the role of astrocytes in this process, as our previous work has implicated astrocyte activation in each of these injury processes and we have shown that blocking a particular signaling pathway associated with astrocyte activation, the calcineurin-NFAT pathway, in astrocytes can ameliorate the effects of neuronal injury. Although there are several NFAT isoforms, there is one particular isoform, NFAT4, which appears to be up-regulated in astrocytes in injured tissues. Most of our analyses have been carried out in rodent models of disease. To demonstrate the broader implications of these findings, we investigated the role of the calcineurin-NFAT pathway in a more advanced model, the aging canine brain. We have a bank of canine brain tissue, from which we can draw samples for analyses. Focusing on NFAT4, we carried out Western analyses to determine the amount of NFAT4 expressed globally in cortex, and immunostaining to look at patterns of expression as well as overall levels in samples from aged versus young brains. Our results demonstrate that NFAT4 expression is increased in most aged canine brains, but some young brains also have high NFAT4 expression. One feature of the stained tissue is that the astrocytes surrounding and feeding into the vasculature are particularly well-labeled with NFAT4 antibody and GFAP antibody, while in other regions there are some astrocytes that express high levels of NFAT4 and lower levels of GFAP and some astrocytes that express high levels of GFAP and lower levels of NFAT4. Taken together, these data suggest there is heterogeneity in the astrocyte population, but NFAT4 is up-regulated in aged canine brain, consistent with our previous observations in rodent models.

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249 Abstract Title:	**Clarifying Diagnosis of Posterior Periventricular Nodular Heterotopia Using Neuropsychological Assessment**
Author(s):	J.P. Harp, Department of Psychology, U of Kentucky A.J. Anderson-Mooney, Department of Psychology and Kentucky Neuroscience Institute, U of Kentucky

Abstract: Objective: Periventricular nodular heterotopia (PNH) is a neurodevelopmental disorder often related to Filamin A gene mutation (FLNA). This results in poor neural migration, manifesting in epilepsy with mild cognitive impairment in females and spontaneous abortion or early death in males. Recent research identified some PNH patients without FLNA mutation, dubbed posterior PNH. Typical presentation includes asymmetric ventricular nodules and cerebellar, hippocampal, colpocephalic malformations (Mandelstam et al., 2013). Unlike PNH-FLNA, posterior PNH exhibits no sex-based differences, but is often accompanied by cognitive impairment and epilepsy. This presentation discusses the pathophysiology of PNH and illustrates a typical case of posterior PNH condition seen in clinic. Participants and Methods: A 22-year-old, left-handed, Ukrainian-American male university student presented with refractory epilepsy and practical cognitive difficulties. Seizures appeared with fever at age 9 and persisted, usually absence with occasional falls. Imaging revealed bilateral PNH, cerebellar dysgenesis, reduced cortical volume, and possible mesial temporal sclerosis. Results: Examination revealed forward-tilting posture; tremulous upper face; vague, slowed speech; and spatial disorientation. Data revealed borderline impaired IQ and deficits often observed with periventricular lesions, such as generalized slowing and significantly weaker visuospatial than verbal performances. Additional impairments included encoding and retrieval, naming, verbal fluency, mental flexibility, and construction. Close examination of imaging revealed primarily posterior, asymmetric nodules accompanied by multiple pathognomonic abnormalities. Conclusions: Imaging and neuropsychological testing led to diagnosis of posterior PNH. Patient was deemed a poor surgical candidate due to non-localized seizure focus. This rather typical presentation of an unusual disorder provides differential diagnostic value with implications for treatment planning in posterior PNH.

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Poster Presentation Abstracts

250 Abstract Title:	**Sex Differences in Pioglitazone Analgesia for Painful Diabetic Neuropathy**
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Abstract: Blood levels of methylglyoxal (MG), a glucose metabolite, are elevated in patients with diabetes, and even further elevated in patients with painful diabetic neuropathy (PDN). We found intraplantar injection of MG produces dose-dependent pain-like behaviors in mice. We found that intraplantar injection of MG dose-dependently elicits pain-like behaviors in mice (e.g. licking and lifting of the hind paw) as well as hyperalgesia (mechanical and heat hypersensitivity). Pioglitazone, a thiazolidinedione and PPARγ receptor agonist, is FDA approved for the treatment of type II diabetes and dose dependently reduced MG-induced hypersensitivity in male mice (Donahue, unpublished). We are now testing the hypothesis that pioglitazone is more effective at reducing MG-induced hyperalgesia in female mice than in male mice (Sorge et al, 2015). We will be using von Frey filaments to test mechanical sensitivity, and a hot plate assay to test heat sensitivity. Mice of each sex will be given varying amounts of pioglitazone intraperitoneally. Our preliminary studies indicated an optimal dose and injection volume of MG to be 100ug/5ul for mechanical tests and 100ug/10ul for thermal tests. 30 minutes after injection of pioglitazone or vehicle, MG will be administered into the paw. Heat latencies will be recorded at 20, 30 and 40 minutes post-MG. Mechanical thresholds will be recorded at 15, 30, 60, 90, 120, and 180 minutes. We predict that pioglitazone will reduce mechanical and heat hyperalgesia to a greater extent in female mice than in male mice, and that low doses of pioglitazone will inhibit MG-induced hyperalgesia in female but not male mice.

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32nd Annual BGSFN Spring Neuroscience Day
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251 Abstract Title:	**Novel Synthetic Isoflavone for the Treatment of AUD: An Examination In Vitro and In Vivo**
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Abstract: An estimated 13.9% of Americans currently meet criteria for an alcohol use disorder (AUD). Ultimately, chronic alcohol use may result in neurological deficits, with up to 85% of alcoholics exhibiting signs of cognitive decline. However, biochemical and behavioral factors contributing to this decline have remained elusive. Our ongoing research program encompasses a multi-tiered screening and validation process of a synthetic product library to provide novel information about mechanisms underlying these deficits and to identify novel chemical scaffolds to be exploited in the development of pharmacological treatments for AUD. In collaborating with scientists at the University of Kentucky Center for Pharmaceutical Research and Innovation (CPRI), our group has screened more than 40 synthetic compounds from the CPRI synthetic products repository for their ability to attenuate ethanol (100 mM)-induced cytotoxicity in a rodent organotypic hippocampal slice culture model. CS86, a novel isoflavone derivative, demonstrated potent cytoprotective effects in this assay. Trolox (100 μM), a potent antioxidant, was also found to reduce ethanol (100mM)-induced cytotoxicity. Preliminary in vivo studies have demonstrated CS86 dose-dependently decreases ethanol consumption in a 2-bottle choice drinking paradigm. These findings highlight the potential applications of this novel scaffold for use in the treatment of alcohol use disorder.

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252 Abstract Title:	**Inhibition of the Astrocytic NFAT4 Quells Glutamatergic Hyperactivity in a Mouse Model of Alzheimer's Disease**
Author(s):	P. Sompol, Sanders-Brown Center on Aging, U of Kentucky M.M. Pleiss, Sanders-Brown Center on Aging, U of Kentucky I. Artiushin, Sanders-Brown Center on Aging, U of Kentucky L.A. Simmerman, Spinal Cord and Brain Injury Research Center, U of Kentucky S.D. Kraner, Sanders-Brown Center on Aging, U of Kentucky S. Batten, Anatomy and Neurobiology, U of Kentucky G. Quintero, Anatomy and Neurobiology, U of Kentucky G.A. Gerhardt, Anatomy and Neurobiology, U of Kentucky C.M. Norris, Sanders-Brown Center on Aging, U of Kentucky

Abstract: Calcineurin (CN) and its transcription factor substrate, Nuclear Factor of Activated T-cells (NFAT), are associated with cognitive decline in Alzheimer's disease (AD). Several recent studies have reported that the NFAT4 isoform is specifically associated with activated astrocytes, but the role(s) of this isoform in AD remains unclear. Here, we found an increase in nuclear localization of NFAT4 in astrocytes from postmortem human AD brain tissue and in a common mouse model of AD (i.e. 5xFAD). Direct targeting of astrocytic CN/NFAT signaling, using AAV vectors expressing the NFAT inhibitor VIVIT under the control of a GFAP promoter (Gfa2), led to a reduction in nuclear NFAT4 levels in intact 5xFAD mice in parallel with a reduction in dendritic degeneration, elevated synaptic strength, and improved memory function. Whole-cell voltage clamp analyses of CA1 pyramidal neurons indicated that the astrocytic CN/NFAT pathway modulates the balance of AMPA/NMDA receptor mediated signaling 5xFAD mice, but does not significantly contribute to other synaptic changes (e.g. the appearance of silent synapses). Strikingly, AAV-Gfa2-VIVIT reduced the frequency of spontaneous AMPA receptor-mediated currents and spontaneous glutamate spikes in AD mice, suggesting that astrocytic CN/NFAT signaling may drive glutamate-mediated hyperactivity. Together, our findings reveal a novel modulatory role of astrocytic NFAT4 signaling in astrocyte-neuron interactions, synaptic transmission and cognitive function. Inhibition of astrocytic NFATs, especially the NFAT4 isoform, may provide a unique way to prevent or treat a complex neuronal disease such as AD.

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Poster Presentation Abstracts

253 Abstract Title:	**Investigating the Effects of Hyperglycemia on the Developing Zebrafish Retina**
Author(s):	K.E. Shady, Department of Biology, U of Kentucky H.E. Henson, Department of Biology, U of Kentucky A.C. Morris, Department of Biology, U of Kentucky

Abstract: Neonatal hyperglycemia is induced by a number of factors, including administration of glucose to low birth weight infants, insulin resistance, drug treatments, or stress from birth. Studies show elevated glucose during early development may yield long-term consequences; however, little research has been conducted concerning how neonatal hyperglycemia may affect eye development, particularly of the neural retina. To understand the effects of neonatal hyperglycemia on the developing retina, we have generated hyperglycemic zebrafish embryos. Zebrafish are ideal organisms for observing retinal development because embryos develop externally, and the zebrafish retina is rich in cones, similar to human retinas. Zebrafish can also regenerate their retina. To produce hyperglycemic zebrafish, 24 hours post-fertilization embryos were treated with a control fish water, or fish water containing 300mM glucose for 48 hours. At 72 hours post-fertilization, embryos were collected for analysis. Using a colorimetric glucose assay, we determined that glucose-treated zebrafish embryos had an approximate 3-fold increase in glucose levels compared to controls. In addition to having smaller eyes, immunostaining of zebrafish retinal sections with cell-type specific antibodies showed a reduction in the number of rod and cone photoreceptors in glucose-treated embryos. To determine whether apoptosis is responsible for the loss of photoreceptors, we performed a TUNEL assay to specifically label apoptotic cells. Future experiments will involve characterizing the cell death pathway, screening for other mechanisms of cell death, and determining if retinal damage is permanent or if the zebrafish retina can subsequently regenerate.

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32nd Annual BGSFN Spring Neuroscience Day
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254 Abstract Title:	**Can Lobinaline Reduce Ethanol Consumption in C57BL/6J Mice?**
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Abstract: Recent data suggests that 7% of American teens and adults have an alcohol (ETOH) use problem, although many fewer receive treatment (SAMHSA, 2013). One barrier to treatment is the need for well-tolerated pharmacotherapies that can aid in recovery and abstinence. In our previous work, drugs that inhibit or modulate the activity of cholinergic receptors (AChRs) reduced ETOH consumption in C57BL/6J mice (Farook et al., 2009, Lewis et al., 2009). Lobinaline, the major alkaloid from the Lobelia cardinalis plant and an inhibitor of the dopamine transporter protein, also appears to work on AChRs and so the present study was designed to assess the effects of lobinaline on voluntary ETOH consumption in mice. C57BL/6J mice are an inbred strain that readily consume ETOH when provided. We used a “Drinking-in-the-Dark” (DID) test in which the mice are given limited access to alcohol for 4 hours during their night cycle. DID is used as a model of binge drinking. Baseline ETOH consumption was first established to a 20% ETOH solution daily. Following baseline, subjects received subcutaneous injections of either lobinaline (5 or 25mg/kg) or vehicle 10 minutes prior to ETOH access. There were 4 drug treatment sessions, separated by a vehicle-treatment session after each dose. The order of doses was counterbalanced across mice with the only exception that the first treatment for all lobinaline subjects was 5mg/kg. Lobinaline reduced ETOH consumption following treatment with both doses relative to baseline or vehicle treatment. These results provide some of the first data suggesting that lobinaline may have therapeutic potential for alcohol use disorders and further study is clearly warranted. This work was supported, in part, by a subcontract to UK (SB) from Naprogenix Inc. on an SBIR contract from NIAAA “Novel Pharmacotherapies for Alcoholism”.

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255 Abstract Title:	**Aged Animals Appear Cognitively and Behaviorally Hyporesponsive to Chronic Restraint (Psychosocial Stress) Compared to Young**
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Abstract: It is established that aging has detrimental consequences including a change in sleep architecture, a blunted circadian rhythm, and a decrease in cognition. Psychosocial stress (PS) is a non-painful stimulus associated in humans with major life changes including job loss, death of a spouse, and social isolation. It strongly influences multiple systems (e.g., corticosterone level, body temperature regulation, sleep and cognition). In prior work, we showed that acute PS resulted in typical cognitive deficit and hyperthermia responses in young animals, but that aged animals were hyporesponsive to this acute PS challenge. However, PS in humans is normally chronic, not acute, and the likelihood of experiencing PS increases with age. Nevertheless, little work has investigated the response of chronic PS in aged subjects. We hypothesized that aged animals will continue to be hyporesponsive to chronic PS. To test this, young (3mos) and aged (19mos) male Fischer344 rats were assigned to control or PS groups and implanted with wireless telemetry from Data Sciences International to monitor sleep and body temperature. Chronic PS (restraint, 3 h/day, 4 days/week, 4 weeks) effects on distress response, Morris water maze (MWM), body temperature, and corticosterone levels were collected. Chronic PS did not affect spatial MWM training, deep sleep duration, body temperature, or corticosterone levels at any age. PS resulted in decreased active period wake in aged animals. Conversely, aged animals were hyporesponsive to PS effects on the distress response and MWM probe trial. Taken together, the aged animals appear cognitively and behaviorally hyporesponsive to chronic PS.

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256 Abstract Title:	**μ- and ƙ-, but not δ-opioid receptor provide endogenous analgesia and prevent the transition from acute to chronic pain.**
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Abstract: Latent sensitization (LS) is a type of long-lasting pain vulnerability that develops after injury or stress. This mechanism “primes” the organism to become more vulnerable to future stressor(s) or injury, resulting in a lengthened period of sensitization of nociceptive neurons. Previous data from our lab shows that the μ-opioid receptor becomes constitutively active after injury to combat opponent processes of excitatory pain transduction. However, the contribution of other opioid receptor subtypes is unclear. In this study we examined the contribution of the μ-, ƙ- and δ-opioid receptor subtypes in the continued maintenance of endogenous analgesia in LS long after the hyperalgesia of postoperative pain has subsided. We performed intrathecal injection of multiple opioid subtype-selective antagonists after recovery from hyperalgesia, 21-28 days after surgical incision of the hindpaw. We found that the mu-selective agent CTOP and the ƙ-selective agents nor-BNI and LY2456302 but not the delta-selective agents naltrindole and TIPP [psi], produced a reinstatement of pain-like behavior. Our results reveal that long-term inhibition of hyperalgesia after surgical injury is maintained through μ- and ƙ-, but not δ-opioid receptor subtypes. Since clinical studies suggest a sexual dimorphism in the responsiveness to the analgesic effects of ƙ-selective agonists, we aim to investigate whether there are sex differences in ƙ- (and also μ- and δ-) mediated inhibition of latent sensitization. Because stress is inversely linked with opioid receptor function, we will also evaluate the effect of chronic stress on endogenous opioid receptor analgesia.

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257 Abstract Title:	**Temozolomide decreases reactive cell proliferation in the subgranular zone of the hippocampal dentate gyrus following 4-day binge ethanol exposure**
Author(s):	C.R. Geil, Department of Pharmaceutical Sciences, U of Kentucky K. Nixon, Department of Pharmaceutical Sciences, U of Kentucky

Abstract: 30% of the US population will meet the diagnostic criteria for an alcohol use disorder (AUD) at some point in their lifetime. AUDs result in cognitive deficits in several brain regions including the hippocampus, a region responsible for learning and memory. However, cessation of alcohol consumption can result in recovery of brain mass and function. One possible mechanism of regeneration is reactive adult neurogenesis, which occurs following a binge model of an AUD in rats. Adult neurogenesis is a multi-step process originating with neural stem cell (NSC) proliferation in the subgranular zone (SGZ) of the dentate gyrus. The purpose of this study was to determine if temozolomide (TMZ; a DNA alkylating agent) could normalize reactive cell proliferation in the SGZ following a binge model of an AUD. Adult male Sprague-Dawley rats were given 25% (w/v) ethanol or isocaloric control diet via gavage every 8 hours for 4 days according to a modified Majchrowicz model. As NSC proliferation increases 5-7 days following ethanol exposure, 3.5 days after the last does of ethanol, rats were injected with TMZ every 12 hours for 3 days. 12 hours after the last injection, rats were given bromodeoxyuridine (BrdU; a proliferation marker) and killed 2 hours later. Brains were harvested, sliced, stained for BrdU using DAB immunohistochemistry, and BrdU-positive (BrdU+) cells in the SGZ were counted. A significant diet x drug interaction [$F(1, 23)=6.5$, $p<0.05$] was observed, such that TMZ blunted the binge ethanol-induced increase in BrdU+ cells. Specifically, the number of BrdU+ cells/section was significantly decreased in ethanol + TMZ (49 ± 6) compared to ethanol + saline (113 ± 12; $p<0.05$). Additionally, there was a significant reduction in BrdU+ cells in control + TMZ (16 ± 4) versus control + saline (37 ± 3; $p<0.05$). Importantly, TMZ decreased ethanol rats BrdU+ cell counts to control levels. Since TMZ was able to decrease reactive proliferation it will be useful in future work investigating the role of reactive proliferation in hippocampal recovery following ethanol exposure.

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258 Abstract Title:	**Mood-based impulsivity is reduced by environmental enrichment in female rats: Neural correlates using c-Fos immunoreactivity**
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Abstract: BACKGROUND: Negative urgency (NU) is a mood-based construct of impulsivity that refers to the tendency to act rashly in response to distress (Cyders and Smith, 2008). In humans, NU has been shown to be a predictor of drug abuse. Previous results from our laboratory demonstrated that male rats raised in an enriched environment, rather than an isolated environment, show reduced impulsive behavior in a reward omission task that models NU. The current study explored the effects of enriched and isolated housing in female rats using the same reward omission task and also determined if neural activity was altered. HYPOTHESIS: Females rats raised in an enriched environment would display less NU than females raised in an isolated environment. METHODS: Twenty-four female Sprague-Dawley rats in the PND21 were randomly assigned to one of 3 different housing conditions: (1) Enriched condition (EC), where 8 rats were housed in cage with 14 novel objects; (2) Social condition (SC), where 2 rats were rearing in NIH standard housing conditions; or (3) Isolated condition (IC), where rats were singly housed. Rats were first trained in an operant conditioning chamber to expect a non-contingent food reward upon presentation of a light. They then received operant training for food reward on an FR10. After acquisition, the Pavlovian (PV) and operant (OP) components were alternated and the number of responses in the OP were measured. Randomly, the expected food reward in the PV was omitted and responding in the OP was measured. NU was defined by the increase in responding observed following reward omission compared to responding following reward presentation. RESULTS: Female IC rats increased their OP rates following 2 trials of unexpected reward omission ($p<0.01$). In contrast, neither EC nor SC rats modified their response rate after the omission trials. Female IC rats also expressed greater c-Fos immunoreactivity in the prelimbic cortex compared to both female EC or SC rats. CONCLUSIONS: The current results indicate that female IC rats, but not EC nor SC rats, displayed a NU phenotype that was associated with increased neuronal activation in the prelimbic cortex. These results are important within the context of previous work showing that enrichment protects against drug abuse in preclinical models and involve the medial prefrontal cortex as a possible brain area involved in the neurobiological mechanism for understand the environment-dependent NU phenotype.

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259 Abstract Title:	**Identification of Novel mTBI Biomarkers Using Phage Display**
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Abstract: Mild traumatic brain injury (mTBI), often referred to as concussion, accounts for up to 90% of the brain injuries and represents a silent epidemic. mTBI is difficult to diagnose, as the current diagnosis relies on exclusion of structural damage detected by computed tomography scans combined with the presence of reported symptoms such as headache and confusion, which can result from other causes. To aid in the objective diagnosis and evaluation of mTBI, there is an urgent need for biomarkers indicative of the trauma. We employed a phage display method for genetic library screening in order to isolate sequences that preferentially bind to injured serum produced from a fluid percussion rat mTBI model. Bacteriophages displaying proteins of interest were screened against commercially available peptide libraries with three panning rounds of binding, elution, and amplification to identify sequences binding with the highest affinity. Animal subjects were euthanized and serum was extracted at 3h and 24h post-injury time points, and consensus sequences were identified in both injury time courses. A subsequent ELISA showed higher absorbance concentrations in the 3h injury time course in comparison to 24h, and consensus sequences displayed similar sham and injury levels. However, there were no significant differences between sham and injury models for selected sequences, and further work is needed to select for sequences that bind with a higher affinity to injury serum versus sham serum.

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260 Abstract Title:	**SUDEP and Functional Remodeling of Vagal Complex Activity in a Mouse Model of Temporal Lobe Epilepsy**
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Abstract: Sudden unexpected death in epilepsy (SUDEP) is associated with disturbances in cardiorespiratory function and autonomic nervous system (ANS) imbalance. GABAergic neurons of the nucleus tractus solitarius (NTS), located in the vagal complex of the caudal brainstem are an essential modulator of parasympathetic tone. Studies of genetic epilepsy models suggest that ANS dysfunction coincides with SUDEP susceptibility, but this has not been investigated in temporal lobe epilepsy (TLE). This study investigated the activity of GABAergic NTS neurons in the vagal complex and cardiac function in the pilocarpine-status epilepticus model of TLE. Pilocarpine (281 mg/kg) was administered to 5-6 week old mice to induce status epilepticus and eventual development of TLE. Mice were split into 3 groups for the following experiments: a survival study, in vivo electrocardiography (ECG), and in vitro electrophysiology. For electrophysiological recordings from identified GABAergic NTS neurons coronal brainstem slices were taken at 1 week, 6 weeks, and 12 weeks post-injection. Pilocarpine-treated mice displayed a 30% survival rate (versus 100% for vehicle-treated controls) by 150 days post-SE. Heart rate, RR interval, and heart rate variability were not significantly post-SE. One week post-SE, GABAergic NTS neurons had significantly increased spontaneous AP frequency compared to control mice (75% increase) and sEPSC frequency was significantly higher (247% increase) than in control mice ($p < 0.05$). Six weeks post-SE spontaneous APs were significantly higher (129% increase) compared to control mice ($p < 0.05$). Twelve weeks post-injection there was a significant increase in spontaneous AP firing in TLE mice (77% increase) and sEPSC frequency was significantly higher (83% increase) compared to control mice ($p < 0.05$). These results show an increased excitability of GABAergic NTS neurons leading to a tonic dampening of parasympathetic tone and ANS imbalance associated with TLE development.

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261 Abstract Title:	**Aging Increases Markers of Inflammation and Alters Brain-Gut Interactions**
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Abstract: Aging is the perhaps the greatest risk factor for the development of numerous health concerns, namely, neurological disorders such as Alzheimer's Disease (AD). Age is generally defined as the collection of deleterious changes to cells and tissues over time that increases one's risk of disease. These changes include increased inflammation, mitochondrial dysfunction, and alterations of the gut microbiota. Indeed, variations in gut microbiota have been implicated in the development of inflammation and neurological disease. We hypothesize that alterations of the gut microbiome from age may cause dysregulated brain-gut communication, promoting inflammation and ultimately, neurological disease. Thus, the objective of this study was to examine the influence of the aging process on the brain-gut axis and how these collectively affect overall neurological function. We used a multi-disciplinary approach to address brain-gut interaction in reflection on brain physiology and cognitive function, including neuroimaging, 16s genomic sequencing of the gut microbiome, cognitive and behavioral testing, and brain metabolomics assessment. In agreement with the hypothesis, our preliminary data indicate decreased cerebral blood flow, deleterious modifications of the gut microbiota, amplified markers of inflammation, and distorted cognitive function in old mice compared to young.

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262 Abstract Title:	**Vitamin D3 Preserves Cognition and Enhances Hippocampal Synaptic Function in Aging Rats**
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Abstract: Higher blood levels of vitamin D are associated with better health outcomes while vitamin D deficiency, particularly in the elderly, are frequently correlated with compromised health including an increased risk for accelerated cognitive decline. This suggests that optimal vitamin D levels may promote healthy brain aging. Using aging rodents, we attempted to model human vitamin D (25OHD) levels ranging from deficient to sufficient to test if long-term vitamin D3 manipulation (cholecalciferol) could preserve cognitive function and oppose markers of brain aging. Dietary manipulation of vitamin D3 was initiated in F344 rats at midlife because of the emergence of multiple markers of aging during this period of the lifespan. Rats were fed diets containing low, medium (typical amount) or high vitamin D3 (100, 1000, or 10,000 IU/kg diet, respectively) for 5-6 months and then evaluated in the Morris water maze to test hippocampal dependent learning and memory. Rats on high vitamin D3 achieved the highest 25OHD blood levels (in the sufficient range) and significantly outperformed low and medium groups on maze reversal, a particularly challenging task that detects more subtle changes in memory. In addition to calcium-related processes, hippocampal gene expression microarrays identified pathways pertaining to synaptic transmission, cell communication and G-protein function as being upregulated with high vitamin D3. The major finding of our study is that it demonstrates a causal relationship between 25OHD status and cognitive function. 25OHD levels, which may be considered in the "optimal range", appear to improve the likelihood of successful brain aging.

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263 Abstract Title:	**Spinal AMPA Receptor Signaling Contributes to Latent Pain Sensitization after Inflammatory Injury**
Author(s):	G. Sinha, Department of Physiology, U of Kentucky R.R. Donahue, Department of Physiology, U of Kentucky B.K. Taylor, Department of Physiology, U of Kentucky S. Doolen, Department of Physiology, U of Kentucky

Abstract: A large body of research indicates that peripheral inflammatory processes initiate maladaptive plasticity within the CNS termed central sensitization (increased responsiveness of CNS nociceptive neurons to normal or sub-threshold afferent input) that contributes to chronic pain states (5). Less appreciated, however, are data which suggest that central sensitization outlasts overt signs of hyperalgesia, in a silent form termed “latent central sensitization” (LCS). LCS can be revealed with numerous interventions including stress or drugs that “rekindle” or reinstate hyperalgesia. LCS is thought to prime nociceptive systems such that a chronic pain state develops when inhibitory systems fail. For example, in our mouse model we reported that the opioid receptor blocker naltrexone (NTX) reinstates pain-like behaviors and signs of spinal neuron activation such as glutamate-evoked Ca2+ responses when administered long after the resolution of the initial inflammatory hyperalgesia. Ca2+ signaling is crucial in the central sensitization in the dorsal horn DH that drives chronic pain. However, very little is known about the underlying mechanisms of LCS. The glutamate a-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) are critically involved in the excitatory synaptic transmission, and blocking AMPARs at the spinal level reverses neuropathic pain. Acute inflammatory insult (1-3 days) triggers spinal accumulation of calcium permeable (CP) AMPARs that coincides with inflammatory hyperalgesia. Because AMPARs are critically involved in CS, we evaluated AMPAR-mediated pain behavior, Ca2+ signals and membrane currents in our model of LCS. To test the hypothesis that blockade of opioid receptor activity with naltrexone (NTX) will reinstate increased AMPAR-mediated Ca2+ signaling during LCS, we performed 3 studies. First, we measured the effect of an antagonist of Ca2+ permeable AMPAR, Naspm on NTX-induced behavioral reinstatement 21d after inflammatory injury. Consistent with our previous studies, 1 ug NTX (i.t.) reinstated mechanical hyperalgesia in mice 21d post-CFA. We found that Naspm (1 nmol, i.t.) dose-dependently prevented NTX-induced mechanical hypersensitivity. Second, we measured Ca2+ signals in response to AMPA 21d after CFA injury. AMPA-evoked Ca2+ signaling was greater in the presence of 30 μm NTX. Third, we characterized the electrophysiological characteristics of AMPARs at 3 (coinciding with acute hypersensitivity) and 21 d after CFA injury (during LCS). I-V curves obtained from lamina II 3d after saline-treatment had weak rectification at positive potentials. Consistent with previous studies, CFA-treatment induced marked inward rectification. Similarly, we observed inward rectification 21d after CFA treatment, but not after saline. These data suggest that synaptic Ca2+ permeable AMPARs are increased in soon after injury, and remain at the synapse during LCS. Targeting these Ca2+ permeable AMPARs may provide relief in chronic inflammatory pain states.

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264 Abstract Title:	**Spatial Learning Deficits in a Novel Mouse Model of Fetal Alcohol Spectrum Disorders**
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Abstract: Fetal ethanol (ETOH) exposure is associated with a variety of behavioral and learning deficits in both humans and rodent models. Laboratory models of alcohol abuse frequently use C57BL6J mice, a strain that voluntarily drinks ETOH. Boehm and colleagues (2009) previously used this strain to study the effects of ETOH on the developing brain. However, mice are born prior to the 3rd trimester equivalent "brain growth spurt" when areas related to spatial learning, like the hippocampus, still undergo considerable development. To model “3rd trimester” ETOH exposure, ETOH must be given to the neonatal mouse. This study compared the effects of ETOH exposure on spatial learning in adolescent mice following either prenatal, neonatal or all 3 trimesters ETOH exposure. Pregnant dams received daily access to 20% ETOH or water for 4 hours during their dark cycle to model voluntary ethanol consumption. After birth, control and ETOH-exposed pups received no treatment or daily intubations (3 or 4 g/kg ETOH) on postnatal days (PND) 3-10. Offspring were tested during adolescence in a hippocampal-dependent spatial task using the Morris Water Maze (MWM). Offspring that received postnatal intubations of 4 g/kg, regardless of maternal treatment, performed more poorly during acquisition and retention of the task, and were slower to find the platform in a novel location, relative to controls. These data suggest that learning deficits following fetal ETOH are dose- and timing-dependent, with higher doses in the 3rd trimester representing an increased risk for these problems. This research was supported in part by AA020051 to GC.

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265 Abstract Title:	**Rapamycin: A Preventative Therapeutic for Alzheimer's Disease**
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Abstract: The ε4 allele of apolipoprotein E gene (APOE4) is the strongest genetic risk factor for Alzheimers disease (AD). AD patients typically exhibit high concentration of Amyloid beta (Aβ). Yet Aβ is detected too late in the aging process. Human neuroimaging studies have indicated that APOE4 carriers develop vascular deficiency several decades prior to Aβ deposition, which further leads to neurometabolic deficits, neuronal loss, and dementia. This research aims to test Rapamycin (Rapa), an FDA approved drug, as a preventative therapeutic for AD. Rapa has been shown to restore brain vascular functions. Using neuroimaging and behavior testing, we assessed the efficacy of Rapa in E4FAD mice model. E4FAD mice, prior to being fed Rapa, showed significantly impaired CBF. After 16 weeks, E4FAD-Rapa showed significant CBF restoration when compared to E4FAD control mice. E4FAD-Rapa mice had significantly enhanced recognition memory, as well as physical activity and responsiveness. In conclusion,Rapa restored brain vascular, metabolic and cognitive functions in pre-symptomatic AD mice. These results show the potential of Rapa as an effective intervention to prevent AD for pre-symptomatic APOE4 carriers.

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266 Abstract Title:	**Pharmacological Modulation of the Mammalian Target of Rapamycin to Alter Maladaptive Plasticity Associated with Autonomic Dysreflexia**
Author(s):	K.C. Eldahan, Dept. of Physiology and Spinal Cord & Brain Injury Research Center, U of Kentucky J.L. VanRooyen, Dept. of Physiology and Spinal Cord & Brain Injury Research Center, U of Kentucky S.P. Patel, Dept. of Physiology and Spinal Cord & Brain Injury Research Center, U of Kentucky A.G. Rabchevsky, Dept. of Physiology and Spinal Cord & Brain Injury Research Center, U of Kentucky

Abstract: The mammalian target of rapamycin (mTOR) is an important mediator of neuronal growth and differentiation in the central nervous system. Mounting evidence shows that genetic enhancement of cortical mTOR activity after spinal cord injury (SCI) increases corticospinal tract sprouting correlated with improved locomotor recovery. Alternatively, we have shown that the development of autonomic dysreflexia (AD) after complete high thoracic spinal cord transection correlates with maladaptive plasticity of both primary afferent fibers and ascending propriospinal pathways. Therefore, while others have used genetic models to increase mTOR activity, we are testing the hypothesis that inhibiting mTOR with the FDA-approved drug rapamycin (RAP) can mitigate aberrant plasticity associated with AD. Preliminary data from naïve, 3, 10 and 21 days post-injury (DPI) spinal cord tissue showed an approximately two-fold increase in phosphorylated mTOR at 10 DPI, with further expression induced by prolonged, intermittent colorectal distension (CRD) at 21 DPI. Similarly, there was increased phosphorylation of the downstream ribosomal protein S6 at 10 and 21 DPI, with further expression induced by CRD. Critically, RAP treatment (6 mg/kg i.p., every other day) significantly reduced pS6 at 10 and 21 DPI, and also prevented CRD-induced increases in pS6. Furthermore, while CRD elicited a five-fold increase in c-FOS expression compared to naive, RAP treatment significantly abated this effect. Preliminary immunohistochemical and behavioral analyses revealed that RAP treatment for 3 weeks decreased CGRP+ c-fiber sprouting into the lumbosacral dorsal horns and reduced hindlimb spasticity during CRD, the latter a surrogate indicator of AD severity. To further establish whether mTOR inhibition can prevent aberrant reorganization of spinal circuitry underlying AD, we are currently employing hemodynamic monitoring to assess the effects of chronic RAP administration on the incidence and severity of AD after SCI in an attempt to develop a prophylactic intervention to prevent the development of AD altogether.

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267 Abstract Title:	**Effects of r-modafinil on Concomitant Oral Ethanol Consumption and Nicotine Self-Administration in P Rats**
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Abstract: Tobacco ranks as the leading cause of preventable deaths in the United States, while alcohol-related deaths follow closely behind as the third leading cause of preventable deaths. Abuses of alcohol and nicotine have generally been treated as separate addictions, despite an estimated 80% of alcohol abusers being regular smokers. This highlights the need for discovery of a single therapeutic treatment for alcohol and nicotine co-dependence. The current study designed a paradigm for optimal alcohol and nicotine intake using an animal model to test the novel therapeutic treatment r-modafinil, a unique dopamine uptake inhibitor which has demonstrated effectiveness in attenuating nicotine taking and seeking in alcohol-preferring P rats (Wang et al., 2015). In the current study, 4 female alcohol-preferring rats (P rats), a pharmacogenetic model of alcoholism, were given concomitant access to alcohol and nicotine in 1-hr daily sessions in a standard 2-lever operant conditioning chamber. For alcohol, a two-bottle choice paradigm (water vs. 15% ethanol) was used; for nicotine, a two-lever choice paradigm (i.v. nicotine vs. no programmed consequence) was used. Total intake of both substances, as well as effects of nicotine on alcohol intake and effects of alcohol on nicotine intake, were measured. Once stable rates of alcohol drinking and nicotine self-administration were achieved, r-modafinil (0, 56, and 100 mg/kg) was given via ip injection immediately prior to concurrent access session. Results showed that access to 15% ethanol and water alone (no nicotine) produced pharmacologically relevant rates of alcohol consumption, confirmed by blood ethanol concentrations. Reliably high rates of operant nicotine self-administration were also observed in the absence of alcohol. When concurrent access to alcohol and nicotine was implemented, a significant decrease in alcohol drinking and a non-significant increase in nicotine self-administration were observed. More important, r-modafinil produced significant decreases in concomitant alcohol drinking and nicotine self-administration.

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Poster Presentation Abstracts

268 Abstract Title:	**Neuropeptide Y Y1 receptor neurons in the substantia gelatinosa of the spinal cord exhibit tonic firing upon current injection**
Author(s):	G.P. Sinha, Department of Physiology, U of Kentucky W. Fu, Department of Physiology, U. of Kentucky B.N. Smith, Department of Physiology, U of Kentucky B.K. Taylor, Department of Physiology, U of Kentucky

Abstract: Neuropeptide Y (NPY) receptors are expressed in substantia gelatinosa (lamina II) neurons of the spinal cord. NPY reduces behavioral signs of acute and chronic pain, in part through activation of the NPY Y1 receptor (Y1R). However, the cellular mechanism of Y1R-mediated analgesia remains unclear. One outstanding question is whether they are expressed in inhibitory and/or excitatory neurons, and where they fit within the dorsal horn microcircuity of pain transmission and pain control, especially in the setting of chronic pain arising from tissue or nerve injury. Behavioral pharmacology and targeted neurotoxin results from our laboratory support the hypothesis that Y1R-expressing neurons are excitatory. Furthermore, we now report that Y1Rs co-exist with multiple markers of excitatory neurons such as calbindin, calretinin, and somatostatin, but not PAX2, a widely accepted marker of spinal inhibitory interneurons. To further test the hypothesis that Y1R neurons are largely excitatory, we performed patch-clamp electrophysiology in dorsal horn neurons from adult mouse spinal cord slices. In current clamp mode, we recorded from Y1R neurons identified either in Y1-eGFP mice or after Rhodamine-NPY labeling. The GFP or internalized NPY-Rhodamine labelled cells were identified for recording using epifluorescence microscopy. In randomly recorded neurons, we observed firing patterns in the following ratios: tonic (30 %), initial burst (18%), gap (11%) and delayed (8%). In Y1R identified cells by NPY-Rhodamine internalization assay the majority of the cells showed tonic (60%) or initial burst (25%) firing pattern. We did not see evidence for gap or delayed firing in Y1-eGFP neurons. These results were unexpected, as it is widely assumed that tonic and initial burst firing represent inhibitory, GABAergic neurons, whereas delayed and gap firing properties are associated with glutamatergic neurons and thus represent an excitatory population. Single-cell PCR experiments are underway to clarify whether a large subpopulation of Y1R neurons s are actually GABAergic, and if so, what proportion is glutamatergic.

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269 Abstract Title:	**Investigating the Behaviors of Positive and Negative Urgency in Rodent Models**
Author(s):	J.A. Batuhan, Department of Psychology, U of Kentucky A.G. Wilson, Department of Psychology, U of Kentucky M.T. Bardo, Department of Psychology, U of Kentucky

Abstract: Positive urgency refers to the tendency to engage in rash behavior following a highly positive event, whereas negative urgency refers to the tendency to engage in rash behavior following a highly negative event. A rodent model of negative urgency has been previously developed, thus the current study attempts to extend these findings via investigation of both positive and negative urgency in a single cohort of rodent subjects. Our experiment utilized 4 different testing conditions (two specific to positive urgency and two to negative urgency) to determine whether rates of responding for a costly food reward would increase after positive or negative events occurred within a trial. Subjects initially learned a light-sucrose Pavlovian association. Next, subjects learned that sucrose could be earned by meeting a fixed ratio (FR) operant response (i.e., lever pressing), the response requirement iteratively increased (FR1, FR3, FR5, and FR10) over successive sessions. Subjects then transitioned to the final training phase where the Pavlovian and Operant components were combined. Test sessions consisted of both training (24) and test (8) trials, randomly intermixed. During test trials a negative or positive event occurred, and increased operant responding following these events was operationally defined as negative or positive urgency, respectively. Subjects displayed evidence of both positive and negative urgency during test trials of all four conditions. Combined these results suggest that rodents, similar to humans, engage in rash behaviors following positive and negative events.

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270 Abstract Title:	**NPY Y1 Receptor Signaling Masks Chronic Pain by Inhibiting PKA and Epac in the Spinal Cord**
Author(s):	W. Fu, Department of Physiology, U of Kentucky B.K. Taylor, Department of Physiology, U of Kentucky

Abstract: Our new models of chronic pain in rodents (Solway et al, PNAS, 2011 and Corder et al, SCIENCE, 2013) and humans (Pereira et al, PLoS ONE, 2015) indicate that inflammation produces a latent, or silent, sensitization of nociceptive neurons in the dorsal horn, which lasts at least several months. For example, intrathecal (spinal) administration of BIBO3304 (a selective antagonist of the Y1 receptor of neuropeptide Y, NPY) reinstated pain-like behavior in a dose-dependent manner when injected during the remission phase of latent sensitization. Since these publications, we found that BIBO reinstatement was prevented by intrathecal administration of an activity-dependent NMDAR blocker (MK801), an AC1 specific inhibitor (NB001), or AC1 gene deletion. To test the hypothesis that endogenous NPY/Y1 signaling silences the spinal sensitization driven by one of its two target proteins protein kinase A (PKA) or exchange protein activated by cAMP (Epac), we intrathecally administered a PKA activator (6Bnz), a PKA inhibitor (H89), or one of several Epac inhibitors, including the novel selective compound HJC0197 (kindly provided by Jia Zhou, UTMB). When administered in mice during remission, we found that 6Bnz reinstated pain-like behavior, while H89 or HJC0197 attenuated BIBO3304-induced behavioral reinstatement. We conclude that injury sensitizes NMDAR-AC1-PKA and NMDAR-AC1-Epac pain signaling pathways, but that Y1 signaling silences the behavioral manifestations of chronic pain. We conclude that Y1R signaling is part of an endogenous braking mechanism whereby mammals naturally recover from both PKA and Epac -mediated hyperalgesia associated with inflammation or nerve injury.

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270 B Abstract Title:	**Is Chronic Pain an Initiator of Tauopathy?**
Author(s):	L.P. Zhang, Department of Physology, U of Kentucky D.N. Lyons, Department of Physology, U of Kentucky K.N. Westlund High, Department of Physology, U of Kentucky

Abstract: Although progressive spread of tau neuropathy from frontal and hippocampal cortex is mapped in humans, animal models have not yet been reported that appropriately replicate tauopathy seen in humans. We are addressing the question of how chronic pain produces vulnerability for tauopathy by investigating the link between neuronal overactivation of the pain circuitry and intracellular stress responses that cause expression of dysfunctional tau proteins and result in neurotoxicity. Utilize animal models that allow study of the transition from persistent to chronic pain. Chronic pain models have early features of tauopathy which over time promote hippocampal stem cell and medial prefrontal cortical neuron loss. The endoplasmic reticulum (ER) stress sensor protein, pPERK (phosphorylated promoter protein kinase R (PKR)-like ER kinase) is a suppressor of protein translation linked to tau pathogenesis. Hyperphosphorylated PHF-1 tau and pPERK signaling pathway proteins are indicative of cellular oxidative stress, neuronal damage and potential neurotoxicity. Chronic neuropathic pain induces expression of dysregulated pPERK and hyperphosphorylated PHF-1 tau protein within 3 weeks that accumulates over 6 months in mice and rats. Treatment with a pPERK inhibitor reduces expression of PHF-1 tau and pPERK proteins, identifying pPERK pathway signaling as a potential therapeutic target for prevention of the long-term neuropathological consequences of chronic pain. Our goal is comprehensive understanding of molecular and cellular mechanisms during the transition of chronic pain. Relevance of this project is underscored by the fact that at present there are 100 million patients with chronic pain in the US at risk for tauopathy and dementia.

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