34th Annual BGSFN Spring Neuroscience Day

Poster Presentation #123

Abstract Title:	**Serotonin-7 Receptor Binding Sites in the Hippocampus Vary with Time of Day But Not Aging**
Author(s):	M.J. Duncan, Dept. of Neuroscience, U of Kentucky J.T. Smith, Dept. of Neuroscience, U of Kentucky K.M. Franklin, Dept. of Neuroscience, U of Kentucky

Abstract: Activation of serotonin-7 (5-HT7) receptors modulates circadian rhythms, memory, REM sleep, and depression, processes which are deleteriously affected by aging. Endogenous regulation of these receptors is not well understood, although pharmacological regulation has been reported. Chronic treatment with selective serotonin re-uptake inhibitors known to increase extracellular serotonin levels leads to down-regulation of 5-HT7 receptors. Because endogenous serotonin release exhibits a daily rhythm with higher levels at night, we hypothesized that 5-HT7 receptors exhibit 24-h variations characterized by lower nighttime expression. Our previous studies of Syrian hamsters showed that aging decreases 5-HT7 receptors in the dorsal raphe nucleus, a brain region in which these receptors affect circadian rhythms and REM sleep, but not in the several other circadian substrates, such as the suprachiasmatic nucleus. Here we tested whether aging reduces 5-HT7 receptors in the hippocampus, a likely substrate for the effects of 5-HT7 receptor drugs on memory and depression. Male Syrian hamsters (young, 3-5 months; old, 17-21 months) exposed to a daily alternating cycle of 14 h light:10 h dark were euthanized at 4 times of day (zeitgeber times [ZT]1, 6, 13, & 19; ZT12 = time of lights:off; N=8-13/tim/age). Coronal sections through the hippocampus were processed for 5-HT7 receptor autoradiography using [3H]8-OH-DPAT [2 nM] as the radioligand and SB-269970 [1 µM] to define nonspecific binding. Tissue sections and radioactive standards were apposed to X-ray films to generate autoradiograms that were assessed by computer-assisted microdensitometry. Robust specific 5-HT7 receptor binding was observed in the hippocampal dentate gyrus (DG), CA1, and CA2 but not in CA3. In the DG and CA1, specific 5-HT7 receptor binding sites exhibited 24-h rhythms with troughs at night ($P<0.005$; $P<0.05$, respectively), in support of the hypothesis. Specific 5-HT7 receptor binding in the CA1 and DG were not significantly affected by age or by interactions between time and age. In conclusion, these data indicate that 5-HT7 receptors in the hippocampus are influenced by time of day but not by aging. Furthermore, these findings suggest that the therapeutic effectiveness of 5-HT7 drugs may persist in old age but will depend on the daily time of administration.

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13th Annual CCTS Spring Conference
Friday, April 13, 2018 Lexington Convention Center
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Poster Presentation #124

Abstract Title: **Characterization of Sleep and Seizures in a Knockout Mouse Model of Lafora Disease**

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Abstract: Lafora disease (LD) is a highly severe form of progressive myoclonic epilepsy, for which there is no cure. The development of treatments for LD would therefore provide inestimable relief from suffering. Animal models such as the Laforin KO (LKO) mouse have been used to study LD and its response to therapy. A major impediment to investigation is that the spontaneous seizures in LKO mice are subtle and infrequent. Investigators have used chemical convulsants to induce acute seizures in LKO mice and test the therapeutic potential of drugs. However, the targets/pathways impacted acutely by convulsants may be completely unrelated to those involved in seizure generation in LKO mice. We therefore set out to detect and characterize spontaneous seizures and sleep patterns in LKO mice using a noninvasive piezoelectric motion sensor (Signal Solutions, LLC). We have previously used this “piezo” sensor for noninvasive sleep scoring and in vivo detection of spontaneous seizures the pilocarpine mouse, a chronic epilepsy model. Here, we monitored six male LKO mice (6-8 months old) for eight weeks each using the “piezo” sensor. In addition, we implanted three LKO mice (2M/1F; 1-12 months old) with EEG/EMG headmounts and monitored them for several weeks with simultaneous piezo and video. The data thus collected were analyzed using automated algorithms. Using this approach, several brief myoclonic events of varying duration have been detected and verified. This establishes the feasibility of analyzing behavior in LKO mice for weeks at a time, a prerequisite to testing novel therapeutic interventions aiming to reduce seizures.

Supported by: Igniting Research Collaborations seed grant from the University of Kentucky

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13th Annual CCTS Spring Conference
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Poster Presentation #125

Abstract Title: **Signaling and Expression of a Truncated, Constitutively Active Human Insulin Receptor in Hippocampal Neurons**

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Abstract: Insulin signaling is indispensable in the periphery and it is becoming clear that insulin is also important for normal 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. To address alternative strategies for enhancing insulin signaling in the brain, we conducted a series of experiments using a constitutively active human insulin receptor (IR). Primary hippocampal neurons were infected with either a mammalian expression plasmid encoding a red fluorescence protein (dTomato), or a construct containing a truncated human IR? subunit (HA-IR?-dTomato) via a targeted lentiviral system. Immunocytochemistry assays probing for HA-IR? confirmed expression of the plasmid in hippocampal neurons. The expression level and effect of IR? on insulin signaling was confirmed via immunocytochemistry and Western immunoblots. Whole-cell calcium currents were recorded in infected cultures using patch-clamp techniques. Channel subtype specificity of the effect was also evaluated. Other experiments included 2-NDBG glucose imaging and Fura-2 calcium imaging. Lentiviral infection of mixed primary hippocampal cultures was successful for all constructs. Western blots of infected cells provide evidence that the truncated IR? plasmid confers elevated IR signaling. Immunocytochemistry shows IR? expression in 80% of infected cells. Constitutive activity was also detected. Patch-clamp recordings of IR?-expressing neurons show calcium currents are a target of IR activity. Calcium levels were not altered, indicating little impact of insulin signaling on resting conditions. Glucose utilization was altered with expression of IR?. This characterization provides insights into future intervention approaches.

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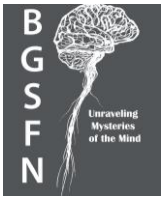

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Poster Presentation #126

Abstract Title: **Vessel Painting Using DiI Following Traumatic Brain Injury in Mice**

Author(s): C. N. Bodnar, Department of Neuroscience, Spinal Cord and Brain Injury Research Center, U of Kentucky
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Abstract: Traumatic brain injury (TBI) is a major health concern in the United States. In 2013, there were a total 2.8 million TBI related ER visits, hospitalizations, and deaths. The mechanical insult and secondary injury cascades can lead to disruption of the vasculature and blood brain barrier (BBB) causing inflammation, metabolic dysfunction, and ischemic injury. We have recently found using (Pseudo-Continuous Arterial Spin Labeling (pCASL) magnetic resonance imaging (MRI) deficits in cerebral blood flow following experimental mild TBI. To explore the cellular underpinnings of the decreased cerebral blood flow seen following an experimental mild TBI, we used the recently described vessel painting approach. This method uses a solution of DiI perfused through the animal. Previous work with DiI has been shown to label large and small vessels and can be used to identify individual endothelial cells. We hypothesized that experimental mild TBI would result in a decrease in perfusion of DiI to injured regions of the brain, caused by loss of perfusion of the small blood vessels and capillaries. To test the hypothesis adult mice were subjected to either sham or mild closed head injury (CHI) and sacrificed 6 hours or 3 days following injury. DiI was perfused through the circulatory system in order to label vessels and microvessels. Whole brain tissue was collected and imaged using a Confocal microscope. Following CHI, mice had a reduction in the amount of microvessels within the cortex surrounding the injury. Loss of microvascular network following mild TBI presents a potential point for therapeutic intervention.

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13th Annual CCTS Spring Conference
Friday, April 13, 2018 Lexington Convention Center
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Poster Presentation #127

Abstract Title: **Long Term Intranasal Administration of Rapid Acting Insulin Aspart in Young and Aged F344 Rats**

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Abstract: The need for novel therapeutics to combat AD progression is indispensable. It has been observed that patients with AD have deficient brain insulin signaling, thus the use of intranasal insulin is gaining strength as a tool to offset cognitive decline. Our group previously reported enhanced brain insulin signaling, memory recall, and increased cerebral blood flow in studies utilizing intranasal delivery of short and long acting insulin formulations, as well as in single or short term (9 doses) conditions. This study addresses the effect of long term (>60 doses) rapid acting insulin aspart on learning and memory in young (5 months) and aged (21 months) F344 rats. Over 3 months, animals received either daily intranasal insulin aspart or saline. Memory recall and spatial mapping were assessed using the Morris water maze. An aging difference was present, including a significant interaction term in the total proximity average to the platform. This indicates intranasal insulin aspart influences memory recall differently in young and aged animals. Left brain hemispheres were sectioned and probed for IHC and percent immunostained areas in different subfields of the hippocampus were quantified, revealing an aging trend and a significant interaction term. Right hippocampi were RNA extracted and analyzed using microarray. Results show long term intranasal insulin altered some aspects of memory recall in aged animals. Further, it appears chronic insulin significantly reduced insulin receptor immunostaining in dorsal hippocampus of aged animals. This result suggests long term intranasal insulin exposure may influence receptor expression differently in young and aged hippocampus.

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Poster Presentation #128

Abstract Title: **Behavioral Economic Approach to Understanding Co-Use of Alcohol and Nicotine in Female P Rats**

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Abstract: The co-use of alcohol and nicotine is the most prevalent polysubstance use disorder worldwide. Toward understanding the relationship of alcohol and nicotine, the current study applied behavioral economic principles to co-self-administration of alcohol and nicotine in an attempt to describe the relationship between these drug reinforcers when they are concurrently available. Young adult female alcohol-preferring (P) rats were used to examine the changes in consumption of concurrently available oral ethanol (EtOH; 0 vs. 15%, 2-bottle choice) and i.v. nicotine (0.03 mg/kg/infusion, active vs. inactive lever). Across daily 1-hr sessions, the price of nicotine increased (increased FR requirement per infusion), while the price of alcohol remained constant. Results showed a significant interaction ($F(11, 110) = 15.72$, $p < 0.05$), such that as the price of nicotine increased from FR1 to FR135, nicotine intake decreased, whereas EtOH consumption increased. There was no significant change in water consumption as the price of nicotine increased. Results also showed that increases in the relative price of nicotine eventually shifted preference from nicotine to EtOH. When the changes in consumption for nicotine, EtOH and water were quantified via a cross-price elasticity analysis, results indicated that EtOH served as an economic substitute for nicotine, $I = -0.79$ ($p<0.05$), whereas water was economically independent of nicotine, $I = -0.18$ (n.s.). In summary, when EtOH and nicotine are concurrently available, EtOH acts as an economic substitute for nicotine in female P rats, suggesting that common neurobehavioral mechanisms may influence the relationship between EtOH and nicotine co-use.

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Poster Presentation #129

Abstract Title: **Myelin Modulates Macrophage Inflammatory Responses After Spinal Cord Injury**

Author(s):
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Abstract: Spinal cord injury (SCI) produces chronic inflammation largely mediated by resident microglia and infiltrating monocytes (here, collectively referred to as macrophages). These activated SCI macrophages eventually adopt a pro-inflammatory, pathological state that continues long after the initial injury. Pro-inflammatory macrophages potentiate secondary damage and impair SCI recovery, yet the mechanisms driving chronic pathological SCI macrophage activation are poorly understood. After SCI, macrophages clear and accumulate extensive myelin debris. Published data demonstrates that myelin debris can directly stimulate macrophages to adopt different activation states. We hypothesize that myelin, in combination with inflammatory stimuli within the SCI lesion environment, increases pro-inflammatory macrophage activation. To test this hypothesis we stimulated bone marrow derived macrophage with pro-inflammatory stimuli (LPS+INF-gamma) in vitro in the presence or absence of myelin. Myelin co-stimulation significantly increased pro-inflammatory IL-12 cytokine production, decreased anti-inflammatory IL-10 production, and increased reactive oxygen species production relative to unstimulated or LPS+INF-gamma treated controls. One potential mechanism for the myelin-mediated pro-inflammatory potentiation is increased activation of the enzyme cytosolic phospholipase A2 (cPLA2) within macrophages. This enzyme has the potential to modify membrane lipids into direct and indirect pro-inflammatory stimuli. Indeed, through immunohistochemical analyses of spinal cord tissue sections after T9 contusion SCI in female C57BL/6 mice we observed cPLA2 activation in myelin-laden macrophages at both 7 and 28 days post injury. Ongoing studies aim to link this continued cPLA2 activity to potentiated pro-inflammatory macrophage activation and explore potential therapeutics to block these pathways after SCI.

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Poster Presentation #130

Abstract Title:	**Myelin Basic Protein and Degraded Myelin Basic Protein in the Frontal Cortex of Individuals with Down Syndrome and Alzheimer Disease**
Author(s):	F. Triani, Sanders-Brown Center on Aging, U of Kentucky and Department of Biochemical Sciences, Sapienza U of Rome, Rome, IT F. Schmitt, Sanders-Brown Center on Aging and Department of Neurology, U of Kentucky E. Head, Sanders-Brown Center on Aging and Department of Pharmacology & Nutritional Sciences, U of Kentucky

Abstract: Adults with Down syndrome (DS) are at increased risk for cognitive decline, dementia and virtually all have Alzheimer's disease (AD) neuropathology by 40 years. Previous studies showed a loss of white matter (WM) integrity in demented adults with DS by magnetic resonance imaging based measures of fractional anisotropy (FA). Thus, we hypothesized that losses in WM integrity may be attributable to losses in myelin basic protein (MBP) in frontal cortex. MBP is a major component of the myelin sheath and the second most abundant protein in the central nervous system. The major MBP isoform is 18.5 kDa, but numerous post-translational modifications occur. Post-translation modifications can lead to instability of myelin and degradation. Destruction of this myelin sheath in demyelinating disease results in nerve conduction failure and neurodegeneration. To test our hypothesis, we analyzed by western blot the expression level of MBP and degraded MBP (dMBP) in an autopsy series of 39 cases with DS (n=8 with DS and n=31 with DS and AD) and 28 controls without DS, along with 7 sporadic AD cases. Our results, suggest that total MBP remained unchanged although there was a trend towards increased protein in sporadic AD. In contrast, dMBP increased with AD both in DS and in sporadic AD cases. This result suggest that white matter integrity is compromised by an accumulation of degraded MBP in DS with AD. WM degeneration may be attributable to the accumulation of degraded myelin sheaths.

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13th Annual CCTS Spring Conference
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Poster Presentation #131

Abstract Title: **A Mouse Glioblastoma Model to Study New Therapeutic Strategies**

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Abstract: Glioblastoma is one of the deadliest human cancers. Even aggressive treatment regimens improve patient survival only by months. Chemotherapy fails because anticancer drugs are substrates for the blood-brain barrier efflux transporters P-glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP) and do not reach the tumor at therapeutic concentrations. Thus, novel treatment strategies are necessary to improve patient survival. For decades, researchers used U87MG glioblastoma cells to test new approaches. In 2016, researchers at Uppsala University using Small Tandem Repeat and mitochondrial DNA analysis found that the origin of these cells is unknown{Allen, 2016 #433}{Allen, 2016 #433}{Allen, 2016 #433}{Allen, 2016 #433}. Thus, there is a need for new glioblastoma models. Here, we compare the U87MG and U251MG models. U251MG cells were transfected with the reporter gene luciferase. U87-luc2 (n=6) and U251-luc2 (n=12) cells were implanted into the brains of immunocompromised mice. Tumor take was verified and monitored weekly with in vivo bioluminescence imaging. MRI was used for additional analysis. Survival was analyzed with the Kaplan-Meier method. Mice implanted with 150,000 U87-luc2 cells had a median survival of 31 days. Tumors grew exponentially as shown by an increasing bioluminescence signal. While mice implanted with the same number of U251-luc2 cells had exponentially growing tumors as shown by bioluminescence imaging, they did not succumb to their tumors within the 120-day study. Increasing the number of implanted U251-luc2 cells to 500,000 decreased survival time to 82 days. The U251-luc2 model requires further characterization, but our current data suggest that it can be used for our glioblastoma research.

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Poster Presentation #132

Abstract Title:	**Characterizing the Endogenous Nrf2-ARE Time Course after Controlled Cortical Impact Injury in Male and Female Mice**
Author(s):	J. Dunkerson, Department of Neuroscience, U of Kentucky E. D. Hall, Spinal Cord and Brain Injury Research Center, U of Kentucky

Abstract: Traumatic brain injury is a complex and chronic disease affecting nearly 2.8 million individuals in the U.S. each year. Free radical induced oxidative damage, arguably one of the most validated secondary injuries, remains a sensible target for acute neuroprotective interventions. In that regard, understanding the endogenous antioxidant response, specifically the mechanisms of the redox sensitive transcription factor Nrf2, has become of interest from a pharmacological standpoint. This study aimed to establish the time course of Nrf2 activity and a selection of its detoxifying enzymes. CF-1 mice (n=30 males, n=30 females) received a unilateral controlled cortical impact (CCI) injury centered over the left parietal cortex. Cortical tissue samples of the contusion site and the ipsilateral hippocampus were harvested at varying time points (24hr, 48hr, 72hr, 7 days) post injury. Western blot analysis was performed using whole cell lysates on the following proteins: Nrf2, HO-1, and NQO1. Regarding cortical protein quantities, Nrf2 steadily decreased in females over 7 days, whereas males experienced a sudden and sustained drop until day 7. HO-1 spiked at 72hrs in both males and females, and remained elevated by day 7 only in females. There were no significant differences in NQO1 in either sex. Regarding hippocampal protein quantities, Nrf2 fluctuations did not reach significance at any of the recorded time points in either sex. HO-1 increased at 48hrs and 72hrs in males. NQO1 increased at 72hrs in females. The time course of Nrf2 activity appears to be different in male and female mice.

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Poster Presentation #133

Abstract Title:	**Reduced Voltage-Gated K+ Channel Function in GABAergic NTS Neurons in a Murine Model of Acquired TLE and SUDEP**
Author(s):	I.D. Derera, Department of Physiology, U of Kentucky B.N. Smith, Department of Physiology, Epilepsy Center (EpIC), U of Kentucky

Abstract: Sudden unexpected death in epilepsy (SUDEP) accounts for approximately 17% of epilepsy-related deaths. Altered voltage-gated K+ current in neurons of the nucleus tractus solitarius (NTS) may contribute to SUDEP in genetic epilepsy models, however, little is known regarding possible changes in voltage-gated K+ channels in NTS neurons during development of TLE. GABAergic NTS neurons receive information regarding cardiac and respiratory function and serve to modulate this information to regulate cardiorespiratory output. In K+ channelopathies, altered NTS neuron function contributed to cardiorespiratory collapse and sudden death after seizures. We hypothesized that voltage-gated K+ channel function in GABAergic NTS neurons is altered in the pilocarpine-induced SE model of TLE. Pilocarpine (282 mg/kg) was administered to 4 week old male FVB-Tg(GADGFP)4570Swn/J (i.e. GIN mice) to induce SE and eventual development of TLE. Electrophysiological results show an increase in action frequency and half-width in GABAergic NTS neurons from TLE mice compared to age-matched controls. Upon application of 4-AP (5mM), action potential firing rate and half-width in GABAergic NTS neurons from control mice was increased to levels similar to that in neurons from TLE mice, suggesting that A-type K+ current function may be suppressed following TLE. Peak A-type K+ current is reduced in GABAergic NTS neurons from TLE mice compared to controls, consistent with the increase in action potential firing and half-width in TLE mice. These results suggest voltage-gated K+ channel function is reduced in the NTS of mice with acquired TLE, which contributes to increased neuronal activity and may increase SUDEP risk.

Supported by: NIH Award R21 NS 088608 NIH Award R01 DK056132 UK Epilepsy Center

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Poster Presentation #134

Abstract Title:	**Estradiol Effects on Excitatory and Inhibitory Neurons Controlling Fluid Intake in the Subfornical Organ**
Author(s):	J. A. Howell, Department of Biology, U of Kentucky J. Santollo, Department of Biology, U of Kentucky

Abstract: Estradiol decreases fluid intake in females, but the mechanism(s) underlying this effect is unclear. The subfornical organ (SFO) is a key brain region that regulates fluid intake. It contains excitatory neurons that stimulate drinking, inhibitory neurons that suppress drinking, and estrogen receptors. We, therefore, tested the hypothesis that estradiol decreases water intake by inhibiting excitatory signals and increasing inhibitory signals in the SFO that controls fluid intake. First, we replicated previous reports showing that estradiol treatment in ovariectomized (OVX) rats reduces fluid intake stimulated by 24 h water deprivation. Using a repeated-measures design, animals were injected with estradiol benzoate (EB, 10 µg) or vehicle for two consecutive days. Twenty-four hours later rats were water deprived or retained fluid access as a control. The following day, rats were given water and intake and licks were measured for 1 h. As expected, after water deprivation EB-treated rats drank significantly less than oil-treated rats ($p < 0.05$). Furthermore, we extended previous research by analyzing drinking microstructure during the test period. There was no difference in average burst size but after water deprivation oil-treated rats had significantly more bursts than EB-treated rats ($p < 0.05$) suggesting that estradiol reduces intake by increasing post-ingestive feedback signals. Ongoing studies are elucidating the neural mechanisms involved by using immunohistochemistry to measure neuronal activation in excitatory and inhibitory neurons in the SFO after fluid deprivation or rehydration in OVX rats treated with EB or vehicle. This work will provide insight on how estradiol influences the neural thirst circuit.

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Poster Presentation #135

Abstract Title: **An Emerging Role for Hsp27 in VCID**

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Abstract: Hyperhomocysteinemia (HHcy) is a risk factor for vascular cognitive impairment and dementia (VCID), as well as Alzheimer's disease. The mechanism by which HHcy promotes VCID or AD remains unknown. Using the HHcy mouse model of VCID, we found the earliest detectable event in the brain is a robust neuroinflammatory response. This is followed by neurovascular astrocyte disruptions, cerebral hypoperfusion, microhemorrhages, white matter degeneration, and cognitive impairment. To gain mechanistic insights into the signaling pathways by which HHcy induces these, we focused on heat shock protein 27 (Hsp27). Hsp27 binds protein-folding intermediates and prevents their aggregation. Given that Hsp27 is shown to be involved in cerebrovascular dysfunction in stroke models, and is known to signal through the p38 MAPK signaling pathway, a critical driver of the proinflammatory response, we hypothesized Hsp27 is an early mediator of HHcy-induced neuroinflammation, and therefore, the aforementioned downstream events. Our wildtype-HHcy model displayed significant pro-inflammatory responses and astrocytic end-foot disruptions, as well as significant microhemorrhage induction. We found that there was no induction of the pro-inflammatory phenotype in the Hsp27-/- mice subjected to the HHcy-inducing diet for 14 weeks. We also found a reduction in the microhemorrhage incidence in the Hsp27-/- mice, as well as improved survival of the mice, indicating that they were resistant to the HHcy diet. Hsp27 appears to be an early essential mediator of HHcy-induced pathology. Deletion of Hsp27 provides protection from diet-induced attrition, neuroinflammation, and cerebrovascular events. This suggests that Hsp27 may be an attractive therapeutic target for treatment of VCID.

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Poster Presentation #136

Abstract Title:	**Kappa opioid receptors provide endogenous analgesia and prevent the transition from acute to chronic pain via an adenylyl cyclase-1 dependent mechanism**
Author(s):	L. Custodio-Patsey, Department of Physiology, U of Kentucky B. Smith, Department of Physiology, U of Kentucky B.K. Taylor, Department of Physiology, U of Kentucky; Department of Anesthesiology, U of Pittsburgh, Pittsburgh, PA

Abstract: Tissue injury elicits latent sensitization (LS), a prolonged period of pain vulnerability. LS is masked by compensatory activity of endogenous inhibitory systems in the dorsal horn. We previously reported that intrathecal administration of mu opioid receptor (MOR) antagonists reinstates hyperalgesia for several months after cutaneous inflammation. However, whether other opioid receptors contribute to LS inhibition is unclear. To address the contribution of the kappa opioid receptor (KOR), we performed intrathecal injection of KOR selective antagonists (nor-BNI or LY2456302) at 1 or 13months after surgical incision of the hindpaw. The initial hyperalgesia resolved within 1-3weeks, and we hypothesized that KOR analgesia contributed to this resolution. Indeed, when given four weeks after injury, we found that nor-BNI or LY2456302 reinstated hyperalgesia in a dose-dependent manner (0.1ug-10ug,i.t.). Remarkably, reinstatement to LY 10ug was also observed 13 months after surgery, indicating that LS and compensatory KOR analgesia is very long-lasting. Next, we found that LY2456302(10ug,i.t.) increased the expression of touch–induced phosphorylated signal-regulated kinase (pERK)-positive profiles in the dorsal horn 3 weeks after surgery, consistent with LS-associated sensitization of spinal neurons. Since adenylyl cyclase-1 (AC1) mediates the LS masked by MOR, we tested the hypothesis that AC1 also mediates the LS masked by KOR analgesia using the AC1 inhibitor, NB001. We found that pre-administration of NB001 blocked pain reinstatement by LY. Ongoing studies are examining the hypothesis that KOR signaling opposes LS through a signaling pathway that includes one or both of the downstream cAMP signaling receptors, protein kinase A (PKA) or exchange protein activated by cAMP (Epac).

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Poster Presentation #137

Abstract Title:	**Insulin-like growth factor-1 overexpression enhances neurogenesis and activates the mTOR pathway after moderate TBI**
Author(s):	E.L. Littlejohn, Spinal Cord and Brain Injury Research Center, Dept of Physiology, U of Kentucky D. A. Scott, Spinal Cord and Brain Injury Research Center, Dept of Physiology, U of Kentucky A. J. DeSana, Spinal Cord and Brain Injury Research Center, Dept of Physiology, U of Kentucky J. A. Juras, Spinal Cord and Brain Injury Research Center, Dept of Physiology, U of Kentucky K. E. Saatman, Spinal Cord and Brain Injury Research Center, Dept of Physiology, U of Kentucky

Abstract: Nearly 5 million people in the United States are living with TBI related disabilities, in part because of the brain's limited capacity to replace lost and damaged neurons. Immature neurons in the hippocampus are highly vulnerable to trauma, but can be replaced through proliferation and differentiation of neural stem cells in the subgranular zone. Insulin-like Growth Factor 1 (IGF1) modulates basal and injury-induced hippocampal neurogenesis. Mammalian target of rapamycin (mTOR), a signaling molecule downstream of IGF1, has been identified as a potential target for TBI interventions because of its regulatory role in plasticity and cell survival. We hypothesized that increased IGF1 would stimulate mTOR activity following injury, resulting in improved neurogenesis. We utilized a transgenic mouse model with IGF1 overexpression restricted to astrocytes (IGF Tg) to raise brain levels of IGF1 by means of injury-induced astrogliosis. To this end IGF Tg and wild-type (WT) mice received moderate controlled cortical impact injury or received sham injury and survived 1, 3 or 10d. At 1 and 3d following moderate 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 suggests that IGF1 stimulates activity of the mTOR pathway following moderate TBI in a region-specific manner. At 10d after moderate injury, IGF1 overexpression enhances recovery of immature neurons.

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Poster Presentation #138

Abstract Title:	**Lafora disease premature termination condons (PTCs) are likely candidates for suppression by aminoglycosides**
Author(s):	Z. Simmons, Dept of Molecular Medicine and Biochemistry, U of Kentucky A. Sherwood, Dept of Molecular Medicine and Biochemistry, U of Kentucky S. Li, Dept of Pharmacology and Nutritional Sciences, U of Kentucky S. Garneau-Tsodikova, Dept of Pharmacology and Nutritional Sciences, U of Kentucky M. Gentry, Dept of Molecular Medicine and Biochemistry, U of Kentucky

Abstract: Personalized medicine-based treatments are providing patient specific therapeutics for patients with single nucleotide changes resulting in a premature termination codon (PTC). These PTC mutations often result in truncated protein products. Typically, these truncated proteins provide little to no proper function or activity, and thus disease is imminent. Suppression of PTC mutations with novel compounds, including aminoglycosides, has been an effective therapeutic in Cystic Fibrosis and Duchenne Muscular Dystrophy. Lafora disease (LD) is another disease that can result from PTC mutations. In LD, autosomal recessive PTC mutations within the gene EPM2A cause dysregulation of glycogen metabolism. This glycogen storage disorder results in neurodegeneration, myoclonus, and epilepsy in the patient's second life, followed by death in the third decade. We have developed multiple in vitro models to test LD PTC suppression. Our data demonstrate that PTCs in Lafora disease are likely candidates for suppression with novel compounds. Suppression of PTCs in LD will provide a mutation-specific genetic-medicine that will cure patients of the disease as well as corroborate the evidence that PTC-therapy may be an effective therapeutic across a vast range of diseases.

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Poster Presentation #139

Abstract Title:	**Determining the Utility of the African Spiny Mouse as a Novel Mammalian Model of Spinal Cord Injury**
Author(s):	M. B. Orr, Department of Physiology, Spinal Cord and Brain Injury Research Center, U of Kentucky C. M. Kloske, Integrated Biomedical Sciences, U of Kentucky K. R. Richards, College of Health Sciences, Spinal Cord and Brain Injury Research Center, U of Kentucky A. W. Seifert, Department of Biology, U of Kentucky J. C. Gensel, Department of Physiology, Spinal Cord and Brain Injury Research Center, U of Kentucky

Abstract: Less than 1% of hospitalized victims of spinal cord injury (SCI) experience full recovery by the time of discharge, which reflects an inability to regenerate injured spinal tissues and an absence of SCI therapies. Researchers commonly use regenerating, non-mammalian models to identify targets for inducing regeneration, but test therapies on mammals that recapitulate human SCI. Unfortunately, the phylogenetic gap between animal models creates a barrier to translation. A mammalian model with enhanced regenerative capabilities would serve as a powerful tool for identifying translatable therapeutic targets for inducing regeneration after SCI. Spiny mice, mammals closely related to mice, exhibit scar-free regeneration from peripheral injuries, which coincides with a suite of pro-regenerative inflammatory and extracellular matrix (ECM) responses. The inflammatory and ECM response are key regulators of SCI progression, but spiny mouse SCI responses and regenerative capacity remain uninvestigated. We hypothesize that spiny mice will exhibit pro-regenerative inflammatory and ECM responses to SCI, which will lead to enhanced axonal regeneration compared to lab mice. Histological and in vitro techniques have been established for comparative SCI studies in spiny mouse and mouse. Initial results indicate spiny mice and mice have comparable gross spinal neuroanatomy and similar dorsal root ganglion neurite outgrowth inhibition by chondroitin sulfate proteoglycans; preliminary data also indicate potential differences in SCI responses. Future studies will more closely analyze SCI responses and the subsequent effects on axon regeneration following SCI. This study will determine the utility of the spiny mouse as a novel mammalian model of SCI and spinal regeneration.

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Poster Presentation #140

Abstract Title: **Comparative study of sleep and eye closure between A. cahirinus and M. musculus**

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Abstract: To understand the function and origins of sleep, sleep needs to be studied across many different species. Although sleep is well conserved throughout mammals, 95% of papers are on three species: Homo sapiens, Mus musculus, and Rattus norvegicus. We aimed to characterize sleep in a Murid rodent, Acomys cahirinus. Previous research, using a well validated, non-invasive, piezoelectric system, have shown that A. cahirinus and M. musculus have relatively similar sleep and wake profiles, with a few interesting differences. In order to further understand these differences in sleep architecture, electroencephalogram (EEG) recordings were performed. Our data show that A. cahirinus have significantly longer sleep periods and exhibit a higher amount of REM sleep. Most strikingly, A. cahirinus do not close their eyes while sleeping, day or night. This allows for easy examination of pupil size dynamics during sleep. In order to test whether the sleep patterns of A. cahirinus are affected by external light stimulation, we designed a light flashing experiment. A. cahirinus spend significantly less time in REM during light flashing compared to baseline data, but M. musculus have no difference in REM sleep percentage. Interestingly, histological data show that A. cahirinus have much larger eyes, thinner retinas, and thicker corneas than M. musculus. Electroretinography (ERG) results, specifically b-wave amplitudes, are significantly different. While some other mammals can sleep with eyes half open, or short periods fully open, this is the first report of eyes open 100% of the time, raising questions regarding the adaptive value of this unusual behavior.

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13th Annual CCTS Spring Conference
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Poster Presentation #141

Abstract Title:	**Elucidating Periocular Mesenchyme Migratory Behaviors during Ocular Anterior Segment Development in Zebrafish**
Author(s):	K. L. Van Der Meulen, Department of Biology, U of Kentucky J. K. Famulski, Department of Biology, U of Kentucky

Abstract: The anterior segment (AS) is critical for directing light onto the vertebrate retina and maintaining intraocular pressure. Anterior Segment Dysgenesis (ASD) is a spectrum of developmental disorders effecting the AS and resulting in visual impairment. The neural ectoderm, surface ectoderm, and neural crest-derived Periocular Mesenchyme (POM) cell lineages come together to assist in assembling these structures. Missteps in the processes incorporating the POM into AS tissues may predispose individuals to ASD. I hypothesize that the AS-associated POM population is comprised of several subpopulations, each with unique population sizes and migratory behaviors. Transgenic embryos of four POM genes (FoxC1b:GFP, FoxD3:GFP, Pitx2:GFP, and Lmx1b:GFP) were imaged using 3D confocal and Lightsheet microscopy. POM cells in fixed samples (22-72hpf) were quantified based on total population size and quadrant of origin. Population size was variable as development progressed with significantly more cells expressing FoxC1b. FoxC1b and FoxD3 cells distribute throughout the AS, while Pitx2 cells remain in temporal regions and Lmx1b cells restrict to nasal regions. AS POM cells were imaged for 24 hours and analyzed for behavior, trajectory, average velocity, and total distance traveled using Fiji and Arivis 4D software. FoxC1b expressing cells migrate farther and faster than cells within the other subpopulations. However, all POM cells on the AS exhibited the same stochastic migratory behaviors. Results thus far indicate the presence of at least four distinct subpopulations within the AS-POM population. Future directions will look into the possibility that each subpopulation contributes to a unique cell type or structure within the AS.

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Poster Presentation #142

Abstract Title:	**Siah E3 ubiquitin ligase indirectly regulates Pax2 gene expression by targeting Nlz2 for proteosomal degradation during retinal morphogenesis.**
Author(s):	W. P. Piedade, Department of Biology, U of Kentucky J. K. Famulski, Department of Biology, U of Kentucky

Abstract: We screened the zebrafish proteome for the Siah “degron” motif and identified a potential target, Nlz2, a repressor of Pax2 expression known to be involved in proper choroid fissure fusion. Using whole mount in situ hybridization (WISH) and Immunohistochemistry (IHC), we found Siah1 and Siah2l genes and proteins expressed in the nervous system and eyes during early embryonic development. The expression of Nlz2, overlaps with the expression of both Siah genes. In order to analyze endogenous Siah activity, we constructed a GFP reporter construct containing the Siah “degron” motif found in Nlz2 thus enabling real time readout of Siah activity. Our GFP-degron reporter assay confirmed endogenous Siah activity in the developing eye between 20-48hpf. To study Siah ubiquitin ligase function during eye morphogenesis, we employed gain and loss-of-function approaches by injecting Siah or dominant negative, Siah?RING, mRNA. Injected embryos were analyzed using IHC for laminin, as a readout of basement membrane integrity and fissure fusion while WISH for Pax2, served as an indicator of Nlz2 activity. Siah and Siah?RING mRNA injections both inhibited disassembly of the basement membrane and ultimately optic fissure fusion up to 72hpf. Siah gain-of-function resulted in an increase in Pax2 expression, while the dominant negative ?RING construct resulted in decreased Pax2 expression. Taken together, our results suggest that Siah ubiquitin ligase controls Nlz2 protein stability and therefore indirectly regulates Pax2 gene expression in order to modulate timing of choroid fissure closure.

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Poster Presentation #143

Abstract Title:	**Fibroblast Growth Factor 19 Alters Parasympathetic Output of the Dorsal Motor Nucleus of the Vagus**
Author(s):	J.B. Wean, Department of Physiology, U of Kentucky B.N. Smith, Departments of Neuroscience and Physiology, U of Kentucky

Abstract: According to the CDC, there are more than 30 million Americans living with diabetes. Although most diabetes research focuses on defects in insulin and glucose metabolism, emerging evidence suggests that the brain plays an underappreciated role in systemic glucose regulation. One such homeostatic regulatory center is the brainstem dorsal vagal complex (DVC) which monitors metabolic status through both vagal afferent neural and humoral signals including glucose, insulin, and leptin. Parasympathetic motor neurons in the DVC respond to this information by altering vagal output to regulate pancreatic hormone release and hepatic glucose production. Fibroblast growth factor 19 (FGF19) has potent, insulin-independent antidiabetic effects when injected intracerebroventricularly, though the mechanisms of action are unknown. This information, together with the fact that FGF19's receptor/co-receptor combination is present in the DVC, suggests that this area is a prime candidate for the observed antidiabetic effects. Here, patch-clamp electrophysiology was used to measure the effects of FGF19 on action potential (AP) frequency and synaptic currents in vagal motor (i.e., DMV) neurons in brainstem slices. Application of FGF19 (230 pM) either increased (33%), decreased (44%) or caused no change in AP firing in DMV neurons. The frequency of spontaneous synaptic currents was also altered, and FGF19 also caused significant outward whole-cell currents in most DMV neurons. These cellular effects are consistent with the hypothesis that FGF19 modifies parasympathetic output to the viscera and could contribute to the peptide's effects on metabolism. Studies aimed at understanding anti-diabetic effects of FGF19 in the DVC are underway.

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Poster Presentation #144

Abstract Title:	**Hyperalgesia in experimental autoimmune encephalomyelitis: mediation by astrocytes in the dorsal horn?**
Author(s):	B. C. Shaw, Department of Physiology, U of Kentucky S. Doolen, Department of Physiology, U of Kentucky R. R. Donahue, Department of Physiology, U of Kentucky C. M. Grachen, Department of Physiology, U of Kentucky B. K. Taylor, Department of Physiology, U of Kentucky and Pittsburgh Center for Pain Research, U of Pittsburgh, Pittsburgh, PA

Abstract: Multiple sclerosis (MS) is a human neuroinflammatory disease, affecting millions of individuals worldwide. About half of all MS patients experience chronic pain, usually refractory to pharmacotherapy. Despite this, we know little of the mechanisms underlying MS pain. Our recent publication in PAIN established a dose-dependent correlation between fingolimod, an S1PR agonist, and reduction of experimental autoimmune encephalomyelitis (EAE)-induced hyperalgesia. Here we extend this correlative data into a more causative description of the spinal mechanisms of EAE-induced hyperalgesia with a focus on a key cellular mediator of chronic pain in many animal models: astrocytes. We induced a mild form of EAE using a female C57BL/6, MOG35-55 model. We found that dorsal horn astrocytes robustly express plasmalemma S1PR1, a key target for the treatment of the pain of EAE. We injected L-alpha-aminoadipate (LAA, 100 nmol, intrathecal) on Day 11 at peak hyperalgesia to selectively ablate astrocytes and then tested mechanical and cold hyperalgesia over 24 hours. Preliminary qualitative analysis of GFAP immunohistochemistry strongly suggests that LAA robustly decreased GFAP immunoreactivity at 24 hours. LAA reduced mechanical (two-way ANOVA $F_{1, 10} = 574$, $p < 0.0001$) and cold (two-way ANOVA $F_{1, 10} = 21$) hyperalgesia in EAE mice. We are the first to show that LAA reduces mechanical hyperalgesia in EAE mice and that LAA reduces cold hyperalgesia in any model of neuropathic pain. These data suggest that astrocytes are key mediators of EAE-induced hyperalgesia. Current studies are in progress to quantify LAA-induced astrocyte ablation, and to determine astrocytic signaling pathways in EAE.

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Poster Presentation #145

Abstract Title:	**RNA integrity is associated with weakened expression of genes in the lysosomal and mitochondrial pathways in human cadaver brain tissue samples.**
Author(s):	E.S. Johnson, Department of Pharmacology and Nutritional Sciences, U of Kentucky K.E. Hargis-Staggs, Department of Pharmacology and Nutritional Sciences, U of Kentucky E.M. Blalock, Department of Pharmacology and Nutritional Sciences, U of Kentucky

Abstract: RNA degradation can be influenced by many factors (e.g., post-mortem interval, sample preparation, storage duration, etc). The degree to which RNA is degraded prior to quantification affects downstream measurements (e.g. situ hybridization, RT-PCR, transcriptional profiling). In fact, Agilent Technologies introduced the RNA integrity number (RIN; 1 being the worst to 10 being the best) to help quantify and standardize degradation across samples and labs. Recent studies have shown RIN influences mRNA expression levels, though relatively little work has been done to determine whether RNA damage is random or more prevalent in certain biological pathways. In previous work, we compared RIN values and gene expression from two transcriptional profile studies of brain tissue to determine whether RNA degradation was consistent across different laboratories and samples, and targeted towards specific categories of genes. However, one of the studies in that prior comparison was statistically underpowered and more statistically powerful and balanced studies have been reported. Therefore, we modified our approach and exchanged the statistically weaker study for a stronger one. We tested to see if the two studies with strong and balanced statistical discovery power would show strong agreement with regard to which genes were targeted by poor RNA quality, and whether that set of genes revealed consistent pathways of effect. We report a consistent influence of RIN on genes associated with mitochondrial and protein-degrading processes, suggesting that pockets of subcellular RNA close to mitochondria and lysosomes may be more adversely affected during the course of RNA degradation in human brain tissue.

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Poster Presentation #146

Abstract Title: **Treatment of Lafora Epilepsy by a Therapeutic Enzyme that Degrades Lafora Bodies**

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Abstract: Lafora disease (LD) is one of the most severe forms of progressive myoclonic epilepsy (PME). LD typically manifests with seizures in adolescence, followed by rapid neurological deterioration, increasingly frequent epileptic episodes, and dementia. Death typically occurs ten years after onset. LD is caused by mutations in the EPM2A and EPM2B genes, encoding the glycogen phosphatase laforin and the E3 ubiquitin ligase malin, respectively. LD is distinguishable from other PMEs by cytosolic polyglucosan inclusions known as Lafora bodies (LBs) in neurons, heart, skeletal muscle, and other tissues. Among the PMEs, LD is uniquely considered a disorder of glycogen metabolism. Since eliminating cerebral glycogen synthesis rescues LD in mouse models, therapies are being developed to target LBs and glycogen. We took an enzymatic approach to degrade LBs in vivo, however, cell penetration remains a significant hurdle in the field of enzyme therapy. One strategy is to utilize antibody fragments to enable cellular uptake of the target enzyme. We fused a humanized Fab fragment from systemic lupus erythematosus antibody 3E10 to pancreatic ?-amylase, an enzyme that naturally degrades glycogen, to generate a fusion that both enters cells and degrades LBs (VAL-0417). We show that VAL-0417 reduces glycogen load in cell culture. We developed a novel protocol for purifying LBs from EPM2A-/- and EPM2B-/- mice and show that VAL-0417 degrades isolated LBs in vitro. Most importantly, we show that VAL-0417 injections reduce glycogen load in vivo. VAL-0417 is a promising therapeutic for Lafora Epilepsy, potentially the first drug to provide a significant clinical benefit.

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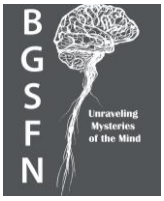

Poster Presentation #147

Abstract Title: **Cerebrovascular Pathology in Horse Brains**

Author(s): T. Sudhakar, Paul Laurence Dunbar High School, Lexington, KY
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Abstract: Background: As the most common form of dementia, Alzheimer's disease (AD) affects over 5.5 million Americans, and 40 million patients worldwide (Selkoe and Hardy, 2016). Though AD has been widely studied, there are still no preventative therapeutic targets in its earlier stages. Recent research has shown that cerebrovascular dysfunction may play a large role in the early pathogenesis of Alzheimer's disease, appearing in 60% of AD patients in addition to the appearance of plaques and tangles (Pimentel-Coelho and Rivest, 2012). This study used a horse brain model to explore cerebrovascular pathology, as horses have not been explored in past research, but may exhibit AD pathology such as amyloid-beta plaques (Youssef et al., 2016). It was hypothesized that the development and significance of cerebrovascular alterations would positively correlate with the age of the animal. Methods: Eight horses ranging in age from 16-32 years were studied. Tissue from the prefrontal cortex and hippocampus was collected in 50 ?m slices using a Vibratome. The tissue was run in a Prussian Blue study, along with human AD+ tissue, to identify and quantify microbleeds. Results & Conclusions: The hypothesis was disproved in the prefrontal-cortex tissue of the horse brains (r= -0.049; p=0.909), but upheld in the hippocampus tissue (r=0.654; p=0.079), indicating that age is a factor in the quantity of microbleeds in the hippocampus. Exploring this relationship between cerebrovascular pathology and age-related cognitive impairment could lead to a preventative therapeutic target for AD.

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Poster Presentation #148

Abstract Title: **Activation of the Reward Circuit by Social Play in Adolescent Rats**

Author(s): M. Z. Slusarewicz, L. R. Hammerslag, E. D. Denehy, D. Vazquez-Sanroman, M. T. Bardo

Abstract: Social interaction, particularly play behavior, in adolescents is widely regarded as critical for the proper development of social and cognitive function. In animal models, isolation during adolescence leads to elevated drug seeking behavior. This may be due to the connection between play and the reward system. Regions of the brain involved in the control and direction of reward behavior, like the prefrontal cortex (PFC) and nucleus accumbens (NAc), are necessary for the execution of play behavior. Thus, the activation of these regions may mediate the effects of play. In order to investigate play-induced activation in these regions, we used cFos, a gene expressed following activation, to label recently activated neurons. Briefly, we placed adolescent male rats (postnatal day 38) into a chamber, either alone or with a partner, and recorded their behavior. After 15 minutes of play or isolation the trial ended and rats were returned to their home cages for 40 minutes before they were anesthetized and perfused transcardially with paraformaldehyde. We then used immunohistochemistry to analyze cFos-positive cells in the PFC (ventromedial and dorsomedial) and NAc (core and shell). Play increased activation within the NAc (core and shell) and dorsomedial PFC, but not in the ventromedial PFC. These results add to the growing body of work suggesting that the reward circuit serves a critical role in social play. Further studies will examine whether there are long-term changes in these regions following adolescent play that could be protective against drug use.

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Poster Presentation #149

Abstract Title:	**Acute Mitochondrial Dysfunction after Mild Traumatic Brain Injury (mTBI) and Implications for Repeated mTBIs**
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Abstract: Mild traumatic brain injuries (mTBIs), accounting for over 80% of TBIs, can cause cognitive and behavioral impairment. While it is known that mTBI does not cause widespread neuronal death, the mechanisms underlying neurological impairment and increased cellular susceptibility to subsequent head impacts are unknown. To examine mitochondrial bioenergetics following mTBI, we employed a mouse model of closed head injury (CHI) to examine mitochondrial respiration in isolated mitochondria after mTBI. A single CHI was produced by a pneumatically controlled impact device with a silicone tip at midline to model a bilateral diffuse injury. Mitochondrial function was assayed from ventral (including entorhinal) cortex and hippocampus homogenates collected at 6, 24, 48, and 96 hours post-injury (n=6/group). Oxygen consumption rates (OCRs) were measured from isolated mitochondria using a Seahorse XF24 Flux Analyzer. Ventral cortex-derived mitochondria after CHI exhibited a decrease in State III (ADP-mediated) OCRs at 24 and 48 hours post-injury ($p<0.01$). Conversely, State III respiration OCRs were significantly decreased in hippocampal mitochondria of the CHI group compared to sham at 48 hours ($p < 0.01$) but not 24 hours post-injury. No significant differences were observed at 6h or 96h post-injury. In addition, we looked at the influence of repeated CHI on mitochondrial bioenergetics and observe that repeated mTBI prolongs mitochondrial dysfunction and produces mitochondria-derived oxidative stress compared to single CHI. This study establishes that mTBI results in early mitochondrial dysfunction which has region-specific temporal characteristics. Future directions will include targeting this dysfunction with novel therapeutics after mild TBI.

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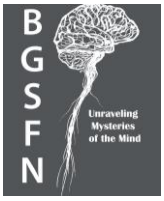

13th Annual CCTS Spring Conference
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Poster Presentation #150

Abstract Title: **Nanoparticle Delivery of microRNAs Targeting Inflammation in Traumatic Brain Injury**

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Abstract: Traumatic brain injury (TBI) is a leading cause of long-term impairments in higher cognitive function. At acute post-injury time points, functionally diverse subsets of pro-inflammatory M1 and reparative anti-inflammatory M2 microglia and macrophages contribute to secondary injury pathology and repair, respectively. It has been documented that M2-like macrophages/microglia peak at 5-7 days and then decline, while M1-like macrophages/microglia persist. Therefore, modulating the inflammatory environment to favor expression of the reparative M2 phenotype has potential to limit secondary injury. One approach is the use of specific microRNAs (miRNAs) that inhibit the pro-inflammatory M1 phenotype and/or promote anti-inflammatory M2 expression/activity. We recently found that miR-146a and miR-223 levels in the rat hippocampus are altered in TBI. Both miRNAs play a significant role in regulating microglia/macrophage polarization and/or expression of inflammatory cytokines. MiR-146a down-regulates pro-inflammatory NF-kB signaling by inhibiting IRAK1 and TRAF6 expression. We now report that peptide-based nanoparticle delivery of miR-146a inhibits IRAK1 and TRAF6 expression in LPS treated BV-2 microglia cells and in rat hippocampus 48 hr following TBI. TaqMan low-density array analysis revealed that miR-146a delivery resulted in significant down-regulation of the M1 genes IL-6 and NOS2 and up-regulation of the M2 genes IL-4 and Arg1. These initial experiments examining miR-146a are promising, however miR-223 may prove more effective as it targets a broader scope of pro-inflammatory signaling. Regardless, these results demonstrate that nanoparticle delivery of miRNAs targeting inflammatory signaling pathways may direct phenotypic expression of M1 and M2 microglia/macrophage states and limit pro-inflammatory signaling after TBI.

Supported by: KSCHIRT Trainee Award

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Poster Presentation #151

Abstract Title:	**Targeting neuroinflammation in the context of Alzheimer's disease with comorbid vascular pathology**
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Abstract: Background: Estimates of “pure” Alzheimer’s disease (AD) indicate that such patients make up less than 20% of cases. Of the comorbidities present in most patients, vascular pathology is the most common. One major targetable point of intersection between vascular and amyloid pathology is neuroinflammation, and we therefore tested our brain-penetrant anti-inflammatory small molecule, MW151, in a mouse model of AD with comorbid vascular pathology. Methods: AD mice (B6.Cg-TgAPPswe/PS1dE9) and wildtype littermates were placed on vitamin B and folate-deficient diet for 8 weeks to induce hyperhomocysteinemia (HHcy) and associated vascular dysfunction. Mice were then recovered on normal chow for 2 weeks, before beginning 2 weeks of treatment with MW151 (5 mg/kg, I.P., daily). In the final week of treatment, mice underwent behavioral testing for cognitive and non-cognitive deficits. Results: Treatment with MW151 normalized hippocampal-dependent spatial learning in the radial arm water maze, associated primarily with a reduction of macrophage inflammatory protein 1 alpha, and interleukin-33 levels in the hippocampus. Conclusions: Methods of amyloid reduction alone may have limited utility in the common clinical condition of an individual presenting with multiple dementia-inducing pathologies. The present study highlights the potential of targeting dysregulated neuroinflammation in the context of comorbid AD-type and vascular injury.

Supported by: Weston Brain Institute Fellowship

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13th Annual CCTS Spring Conference
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Poster Presentation #152

Abstract Title: **Influence of drug intervention on acute PS in young and aged rats**

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Abstract: Psychosocial stress (PS) occurs when a non-noxious stimulus (e.g. loss of a loved one or solitary confinement) provokes a physiological response and has the potential to negatively affect numerous systems (e.g., corticosterone level, sleep, cognition). Prior studies from our lab have investigated the consequences of PS on deep sleep and cognition through aging. Young animals have demonstrated a sensitivity to stress, in particular having a poor probe trial performance. Compared to young, aged animals demonstrated cognitive deficits, but were interestingly hyporesponsive to acute stress. Because deep sleep is important for cognition and decreases relative to age, our lab chose to investigate the influence of a pharmacological intervention on the stress response in young and aged animals. We hypothesized that a deep sleep promoting drug (e.g., Gaboxadol) would improve cognition. To test this, young (3 mos) and aged (19 mos) male Fischer 344 rats were divided into four different groups: control (vehicle and drug) and stress (vehicle and drug). Half of the animals underwent acute restraint stress (3h/ day, 4 days) prior to all animals being trained in the MWM. Behavior, activity, and plasma hormone levels were used to determine Gaboxadol's influence on the stress response. In line with our lab's previous work, young animals suffered stress-induced cognitive deficits. The drug improved cognition in these animals, while maintaining no effect on cognition in the absence of stress. Aged animals were hyporesponsive to stress, even in the presence of Gaboxadol. Taken together, Gaboxadol could be used to improve stress resiliency in young.

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13th Annual CCTS Spring Conference
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Poster Presentation #153

Abstract Title: **The role of arginase in immunomodulatory neurotherapies**

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Abstract: Spinal cord injuries cause CNS macrophage activation that has two prominent subsets. There are M1, pro-inflammatory macrophages, and M2, anti-inflammatory macrophages. M1 macrophages are predominantly in the early inflammation response and typically cause neurotoxicity. M2 macrophages are in the second phase and promote axon growth and remyelination. After both human and rodent spinal cord injuries, M1 predominate. Research has shown that driving the inflammatory phase towards M2 activation enhances recovery but the underlying basis of the improvement is not yet fully comprehended. Discerning the mechanism of M2-mediated repair is vital since macrophages express great plasticity and adapt their phenotype in accordance with their microenvironment. Arginase-1 (Arg1) is a hallmark of M2 phenotypic expression and has a distinct modulating characteristic in tissue repair. We therefore hypothesize that the reparative effects of M2 macrophages are dependent on the production of Arg1. To test our hypothesis we generated macrophage-specific arginase knockout animals. We demonstrate selective knock-down of Arg1 in infiltrating macrophages after SCI in our transgenic model using immunohistochemical and functional assays. We confirmed these results with in vitro analyses. Further, we observed that the efficacy of an immunomodulatory therapy that reduced M1-mediated neurotoxicity is dependent upon arginase expression. Understanding the anti-inflammatory mechanism of macrophage activation can offer novel therapeutic strategies for patients suffering from spinal cord injuries.

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Poster Presentation #154

Abstract Title: **The Effect of Sex on Spinal Cord Injury Recovery and Pain**

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Abstract: The impact of physiological factors, including sex, on the immune response after spinal cord injury (SCI) is not well understood. With recent demographic shifts towards a more equal proportion of male and female SCI patients it becomes increasingly important to identify these differences. Emerging evidence has identified potentially sexually dimorphic underlying mechanisms of behavioral recovery and pain sensitization after SCI. In models of neuropathic pain, pain development is predominantly driven by peripheral inflammation in females and intraspinal inflammation in male. We are characterizing differences in peripheral and intraspinal immune cells in males and female mice after SCI. In order to (further) probe these differences after SCI we tested the therapeutic efficacy of the targeted pharmacological agents pioglitazone (PIO) for peripheral and Azithromycin (AZM) for intraspinal immunomodulation. While there was a trend towards greater behavioral recovery in females there was no difference in overall anatomical recovery or pain development between males and females after SCI. Further, AZM reduced pain sensitization equally in males and females. However, PIO had a significantly greater antiallodynia effect in females compared to males after SCI. This supports our hypothesis that underlying mechanisms of SCI related pain is different between sexes. Moving forward, these results emphasize the importance of considering physiological factors when identifying clinically relevant treatments after SCI.

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13th Annual CCTS Spring Conference
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Poster Presentation #155

Abstract Title: **Promoting a targeted Neuroprotective Immune Response**

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Abstract: Macrophages, derived from resident microglia and blood monocytes, persist indefinitely at sites of spinal cord injury (SCI) and contribute to both pathological and reparative processes. More specifically, the classically activated macrophage phenotype (M1) is associated with cell loss and pathology whereas the alternatively activated phenotype (M2) is believed to promote cell protection, regeneration, and plasticity. Unfortunately, the post-injury environment drives macrophages toward an M1 phenotype. The goal of our work is to utilize gene therapy to stimulate and sustain the restorative M2 phenotype by altering the mechanism by which cells respond to injury. We are optimizing a method to directly target microglia / macrophages in vivo using a cell-specific promoter delivered in an optimized viral vector. Transduction efficiency and specificity of candidate promoter regions has been characterized in vitro using qPCR and flow cytometry. Identifying the appropriate viral serotype to utilize these constructs in vivo remains a challenge. Once developed, this targeted approach will be a powerful tool to analyze the role of M2-phenotype macrophages in the dynamics of progression and repair in spinal cord injury. Further, this work could inform clinical therapy approaches to the broad spectrum of injuries in which microglia / macrophages are involved.

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Poster Presentation #156

Abstract Title:	**Therapeutic Window of Intervention for Pioglitazone Following Traumatic Brain Injury**
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Abstract: Traumatic brain injury, (TBI) is a serious health concern for which no pharmacological treatment is approved. Our group has demonstrated that Pioglitazone, an FDA approved compound used to treat diabetes, has neuroprotective properties following TBI and spinal cord injuries via interaction with the mitochondrial protein, mitoNEET. Recently, we determined the optimal dosing (20 mg/kg) of Pioglitazone and in this study we examine the therapeutic window of opportunity for Pioglitazone administration following TBI. Adult C57B/6 male mice received a severe TBI followed by initiation of Pioglitazone treatment at 1, 3, 6, 12, 18 or 24 hours post-injury. At 48 hours post-injury, animals were euthanized and mitochondria was isolated from the cortex, hippocampus and septal region and oxygen consumption rates (OCRs) were assessed. Results showed that initiating pioglitazone at 1 and 3 hours post-injury did not produce an increase in mitochondrial bioenergetics compared to vehicle treated animals. However, there was a significant increase in OCR in mitochondria extracted from ipsilateral cortex during State III and State V respiration when treatment was initiated at 6, 12, 18 and 24 hours post-injury. In both the Hippocampus and Septal regions there was an increase in respiration when the treatment was initiated at 12 hours that varied across respiration states. These results in conjunction with previous work in our lab where mitochondrial respiration was rescued when treatment was initiated 15 minutes post injury indicate that there is potentially a biphasic, extended treatment window in which pioglitazone can be administered to maintain mitochondrial homeostasis after TBI.

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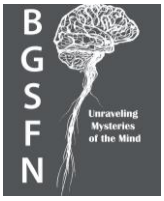

Poster Presentation #157

Abstract Title:	**The role of non-coding RNA in Corneal Nerve Regeneration Following Ocular Surface Injury**
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Abstract: Primary sensory neurons innervating the cornea reside in the ophthalmic branch (V1) of the trigeminal nerve (V) and their cell bodies are surrounded by satellite glia cells (SGCs) in the trigeminal ganglion (TG). While the capability of corneal sensory nerves to regenerate following peripheral injury has been well demonstrated, the underlying mechanism of this process remains to be elucidated. In this study, corneal nerve injury was induced in mice and the role of Alu RNA, non-coding RNA, in axonal regeneration was investigated. First, we found that nerve injury reduced production of Dicer1, a microRNA (miRNA)-processing enzyme, but elevated the levels of Alu-like B2 RNA in TG of mice. In addition, an in vitro study showed that B2 RNA stimulates the SGCs to produce multiple neurotrophic factor genes. Taking advantage of Dicer1 dysmorphic (Dicer1 dys) mice in which B2 RNA accumulates due to inactivated Dicer1, we tested whether abundant B2 RNA levels could enhance the axonal regeneration. Interestingly, even in the absence of injury, there was a difference in the density of corneal nerves between Dicer1 dys mice and their littermates. Dicer1 dys mice had greater nerve density in the cornea, indicating the involvement of excess B2 RNAs in neural growth. Moreover, in terms of regeneration, Dicer1 dys mice showed faster axonal regeneration compared to their littermates. These findings suggest that injury-induced B2 RNA upregulation is associated with peripheral nerve regeneration, which could be a potential therapeutic agent to enhance the axonal regeneration.

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Poster Presentation #158

Abstract Title:	**Considerations in repetitive activation of light sensitive ion channels for long-term studies: channel rhodopsin in the drosophila model**
Author(s):	C. Hermanns, Department of Biology, U of Kentucky M. Mattingly, Department of Biology, U of Kentucky J. Higgins, Department of Biology and College of Nursing, U of Kentucky C. Malloy, Department of Biology, U of Kentucky R. L. Cooper, Department of Biology, U of Kentucky

Abstract: For nervous systems to function properly, the efficacy of such synapses should be finely regulated and adjustable to respond to changing circumstances and requirements. Too high or too low a synaptic output results in inappropriate communication to target cells. This is most apparent during development and maturation. As the size of postsynaptic cells increases dramatically, a matched increase of neurotransmitter release is required and/or sensitivity of postsynaptic cell to the transmission. On the other hand, the nerve terminals also grow rapidly, both in size and output, and continuously show different types of remodeling to maintain proper synaptic output throughout the life of the animal. We are addressing homeostatic regulation in synaptic function at the larval Drosophila NMJ by over and under excitation of the motor nerve terminal and muscle by the use of optogenetics throughout larval development. The biological significance and aim of this study is to demonstrate that in controlling particular neurons or targets of neurons, over time, and throughout development, one will have a better understand the dynamic nature of forward and retrograde communication in regulating synaptic formation and maintenance. Optogenetics has provided a tool to use but there are limitation in the extent of activation and inhibition which needs careful consideration. We have noted long term (minutes) unexpected effects (i.e., neuron refractory in electrical excitability) from only 10 sec activation of channel rhodopsin (ChR-XXL) targeting motor neurons (D42 expression). Paralysis and inability to eat are considerations for long term neural developmental studies when manipulating neurons and muscles.

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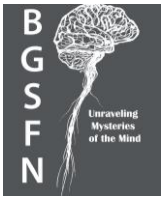

Poster Presentation #159

Abstract Title:	**Examining Temporary Loss of Sensory Perception Over Development in Altering Long-Term Function and Neural Circuitry Effects Behavioral Responses**
Author(s):	T. Donovan, C. Ballinger Boone, E. Somasundaram, C. Malloy, R. L. Cooper

Abstract: Since Hubel and Wiesel (1963) the effects of sensory deprivation in a developing CNS has been a focus in determining critical periods and the effects on neural circuitry. The ability to temporarily enhance or depress electrical activity in sensory neurons at various stages in development provides cues in understanding the plasticity of the nervous system. Temporarily altering activity of presynaptic neurons can have effects on morphology and function of target cells subsequent to the experimental manipulations. Thus, altered neural circuits may manifest themselves in asymptomatic behaviors to standard sensory cues. We are addressing these topics in the larval Drosophila model over embryonic and larval development. In using genetic approaches, we are controlling activity in sensory systems and examining eating and locomotive behaviors as well as tactile sensory assays. In addressing the effects on neural architecture to correlate with the neural activity conditioning paradigms, sensory endings as well as projections into the CNS are being investigated. We will report on the behavioral responses to tactile stimuli throughout larval develop during various experimental manipulations. We also investigating how a previously deprived neural circuit can regain the ability for normal behavior, anatomical structure and function, providing a novel understanding of the understanding synaptic plasticity within defined neural circuits. This relates to various disease states as well as to exomedicine in the terms of development within weightless of space and as well as re-exposure to gravity.

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Poster Presentation #160

Abstract Title: **The Effects of Bacterial Endotoxin on Neural Circuits in a Drosophila Model**

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Abstract: The bacterial endotoxins lipopolysaccharides (LPS) are known to have direct effects on synaptic transmission at neuromuscular junctions in some invertebrates and mammals. Generally, LPS increases Ca2+ loading and random fusion of synaptic vesicles resulting in enhanced transmitter release in a sporadic nature. In some cases, enhanced evoked release, but the effects have been known to vary depending on the synaptic preparations. The effects of sepsis are complex from immunological responses to the direct actions of LPS on cells. In humans, the effects of having bacterial sepsis and being treated can have long term effects in neural function and mobility. We tested the effect of LPS endotoxin on two different neural circuits in larval Drosophila as a model organism. One involved with locomotion and one with an eating assay. Larvae of blow flies, which are used as therapy for debriding dead tissue in wound care, are exposed to bacterial endotoxins and few studies have investigated the actions of forms of LPS endotoxins on therapeutic blowflies to assess survival and physiological function. Larvae of Drosophila melanogaster (24 to 48 hours) were investigated in their locomotion and eating function. Food tainted with 100 µg/ml and 500 µg/ml of LPS from two common strains (Pseudomonas aeruginosa and Serratia marcescens) were used. 24-hour exposure with LPS did not show an altered function with either assay for Drosophila. We are now examining longer exposure times. The results of these studies will be presented.

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Poster Presentation #161

Abstract Title:	**Pharmacological Identification of Muscarinic Receptor Subtypes in Drosophila melanogaster**
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Abstract: Acetylcholine is the excitatory transmitter in sensory neurons as well as among neurons in the CNS of Drosophila melanogaster larvae. Activity of neurons and communicating with target neurons are important in sculpting the developing neural circuitry as well as maintaining established connections. We are interested in investigating the role of muscarinic subtypes in regulating sensory-motor circuits. We will report on the effect of muscarine, an acetylcholine agonist, on the sensory-CNS-motor circuit. Genetically modified lines will be used to knock down type A and type C muscarinic receptors in different sets of neurons: cholinergic neurons, motor neurons, and all neurons. A pharmacological approach will be taken in order to assess behavioral and sensory motor circuit physiology changes in the experimental groups. For behavior, locomotion and feeding will be assessed. For sensory-motor circuit physiology, we will test the modulation of neural circuits in an open preparation. Isolating the CNS in this preparation allows for examination of modulation of motor activity without the influence of confounding variables. A stimulating electrode will be used to activate a sensory neuron, and EPSP activity will be recorded by an electrode inserted in muscle 6. Information regarding the expression of specific receptor subunits within the larval CNS is limited. This research will aid in identifying which muscarinic receptor subtypes are important in modulating sensory-motor circuits.

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13th Annual CCTS Spring Conference
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Poster Presentation #162

Abstract Title: **Activity Dependent Formation of a Somatosensory Circuit in Drosophila Melanogaster**

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Abstract: Activity of developing neurons that make up neural networks impacts their formation and function. Plasticity arises as a result of activity-dependent competition between neurons in a developing neural circuit. The mechanisms that drive this plasticity are poorly understood and it is important to continue to address uncertainties that remain as inappropriate activity of a developing nervous system may lead to defects common in a variety of neurological disorders. Here, we utilize the amenable model organism, Drosophila melanogaster, to address the effects of altering activity of a developing somatosensory neural circuit on the formation and performance of the circuit. Specifically, we utilized an optogenetic approach whereby UAS-Chr2-XXL (Channelrhodopsin) flies or UAS-eNpHR (Halorhodopsin) flies were crossed with PPK-Gal4 (md IV neurons) flies in order to drive expression of light-sensitive opsins in this subset of sensory neurons in order to alter the activity of these neurons throughout larval development. Following chronic manipulation of sensory neuronal activity, circuit performance was analyzed using behavioral, physiological, and imaging approaches. Preliminary results suggest that inappropriate excitation of class IV md neurons, throughout larval development, significantly alters larval locomotion at 3rd instar stage and changes their response to tactile touch. Future analysis will center on morphological assessment and electrophysiological recordings to evaluate alterations in circuit function following manipulation of activity of an entire sensory-CNS-motor somatosensory circuit.

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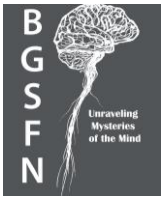

13th Annual CCTS Spring Conference
Friday, April 13, 2018 Lexington Convention Center
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Poster Presentation #163

Abstract Title: **Investigating The Effects Of Homocysteine As An Agonist On Invertebrate Glutamatergic Synapses**

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Abstract: Homocysteine (HCY) is produced in the central nervous system and can act as an excitatory transmitter activating both NMDA and non-NMDA glutamate receptors in mammalian models. Hyperhomocysteinemia (HHcy) in mammals can produce neurological deficits. Thus, understanding the details of the mechanisms of action of HCY in model preparations could help in potential treatments. The glutamatergic synapses of the larval Drosophila and crayfish neuromuscular junctions (NMJs) are common model synaptic preparations to assay pharmacological agents. HCY at a 100 mM did not have any consistent effect on altering evoked synaptic transmission on either preparation. The expectations were that this high concentration would have competed for the endogenous evoked release of glutamate at the NMJ and desensitized the glutamate receptors after an initial rapid depolarization and repolarization. HCY does not have any acute action on the glutamatergic synapses of the larval Drosophila and crayfish neuromuscular junctions. The pharmacology receptor profile of these NMJ receptors are of a quisqualate subtype and not a kainite, AMPA or NMDA subtype. Thus, HCY may not have any action on quisqualate glutamate receptor subtypes.

Supported by: Bio 446 class Dept. of Biology, Univ. of Kentucky

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Poster Presentation #164

Abstract Title: **Modulation of Habituation in the Heart rate Response in Crayfish**

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Abstract: Habituation is an evolutionary adaptation where an organism learns to ignore a repeated stimulus that provides no new information. It is considered to be the simplest form of learning, and therefore key to understanding the more complex forms of mental association. This project investigates several possible modulators for the process of habituation: nicotine, serotonin (5-Ht), and low ambient temperatures. All three of these modulators were predicted to decrease the rate of habituation. These effectors were tested by repeatedly stimulating the crayfish in two ways to observe habituation in tail flips and heart rate (HR). The first was by tapping the crayfish on their tails with a glass stirring rod, then recording whether or not the crayfish tail flipped (Figure 1). The second method was exposing the crayfish to constant light for four hours, then cutting the lights for one second, but not observing tail flip behavior. Both procedures also employed impedance detectors to record the crayfish HRs during the experiment. Any change in HR in response to the trials was calculated. The first treatment had the following groups of subjects: control, low dose of nicotine, and high dose of nicotine. The second used control, saline injected, serotonin injected, and reduced temperature crayfish. The statistical analysis for this project is still underway, and therefore no conclusions can be drawn yet. However, preliminary evidence suggests that 5-HT is slowing down the rate of habituation, while nicotine increases the rate of habituation.

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Poster Presentation #165

Abstract Title: **Investigating Potential Mechanisms of Clove Oil (Eugenol) in Model Crustaceans**

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Abstract: Clove oil contains eugenol as an active ingredient and is used a topical anesthetic in mammals to remedy pain and to anesthetize fish for short periods. The exact mechanisms in the effects are still not fully understood. We examined the resulting activity of eugenol on neuronal activity in sensory and motor neurons in the Red Swamp crayfish (Procambarus clarkii), Blue crab (Callinectes sapidus) and Whiteleg shrimp (Litopenaeus vannamei) with electrophysiological recordings. The neurogenic heart rate in the 3 species was also monitored along with behaviors and responsiveness to sensory stimuli while exposed to eugenol. The activity of the primary proprioceptive neurons was reduced at 200ppm and ceased at 400ppm for both crayfish and crab preparations when saline containing eugenol was directly applied to exposed sensory organs. Flushing out eugenol resulted in recovery in the majority of the preparations within 5 to 10 minutes. Administering eugenol to crayfish and crabs resulted in the animals becoming lethargic. Direct injection into the hemolymph was quicker to decrease reflexes and sensory perception but heart rate was still maintained. Eugenol at a circulating level of 400ppm decreased electromyogram activity in the claw muscle of crabs. Surprisingly, this study found no change in heart rate despite administering eugenol into the hemolymph to reach 400ppm in crabs or crayfish but shrimp preparations decreased. Our next focus is to determine the mechanism of action by intracellular recordings from neurons to support scant evidence of blocking voltage gated-sodium channels and thus decrease neuronal excitability.

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13th Annual CCTS Spring Conference
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Poster Presentation #166

Abstract Title: **Effects of Naltrexone on Alcohol and Nicotine Use in Female P Rats**

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Abstract: Alcohol is the most commonly abused substance worldwide. It is often co-abused with nicotine, which increases the difficulty of cessation of both substances. Despite having similar mechanisms of action, there is no single medication to treat the co-abuse. The objective of the current study is to analyze the effects of the opiate antagonist naltrexone on alcohol consumption and the co-use of alcohol and nicotine in female alcohol-preferring (P) rats. Six female P rats were trained in two phases. During Phase 1 (ethanol access), subjects had 2-bottle choice sessions with 0% (water) and 15% ethanol. In Phase 2 (concurrent access), rats still had access to ethanol bottles, but were also given access to nicotine (0.3 mg/kg/infusion, i.v.) using a standard 2-lever procedure (active vs. inactive levers). Naltrexone (0.15, 0.3, or 0.6 mg/kg s.c.) treatments were administered to determine its effects on alcohol and nicotine consumption. Half the animals received naltrexone treatments during Phase 1, and half received treatments during Phase 2. During Phase 1 (ethanol access), naltrexone had no significant effect on ethanol or water consumption. Results from Phase 2 (concurrent access) showed that naltrexone dose-dependently reduced ethanol consumption, and reduced water consumption at the highest dose (0.6 mg/kg). Naltrexone did not have any significant effects on active lever presses for nicotine, but reduced inactive lever presses only at the lowest dose (0.15 mg/kg). Naltrexone is more effective in treating alcohol use when tested in combination with nicotine rather than when tested alone.

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Poster Presentation #167

Abstract Title: **The Effects of a Bacterial Endotoxin on Synaptic Transmission at the Neuromuscular Junction: Drosophila and Blowfly Models**

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Abstract: The bacterial endotoxins lipopolysaccharides (LPS) are known to have direct effects on synaptic transmission at neuromuscular junctions (NMJ) in some invertebrates and mammals. Generally, LPS increases Ca2+ loading and random fusion of synaptic vesicles resulting in enhanced transmitter release in a sporadic nature. The nature of the effect, and its reversibility, varies depending on the synaptic preparation examined. The effects of LPS toxin has not been investigated in larval Drosophila NMJs to determine the effects. This model offers the ability to potentially address the mechanism of action of LPS on voltage gated Ca2+ channels or other channels with various genetic alterations. In addition, the effect of Ca2+ obtained from extracellular and intracellular organelles in influencing synaptic vesicle fusion can be addressed in this synaptic model. Larvae of blow flies, which are used as therapy for debriding dead tissue in wound care, are exposed to bacterial endotoxins. Few studies have investigated the actions of different forms of LPS endotoxins on therapeutic blowflies to assess survival and physiological function. At 100 µg/ml of LPS from two common strains (Pseudomonas aeruginosa and Serratia marcescens), no effects were observed on evoked transmission or spontaneous vesicle fusion within 2 minutes for larvae of blow flies or Drosophila. At 500 µg/ml of Serratia marcescens, both blowfly and drosophila larvae NMJs displayed a decreased evoked synaptic response amplitude. We are now running 500 µg/ml Pseudomonas aeruginosa trials to determine that strains synaptic transmission. This is an authentic course-based undergraduate research experience (ACURE).

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Poster Presentation #168

Abstract Title: **The Dependence on Nerve Evoked Conditions in Relation to the Occurrence of Spontaneous Quantal Events at Drosophila Neuromuscular Junctions**

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Abstract: Synaptic vesicles will spontaneously fuse at synaptic sites with this mechanism related to the Ca2+ concentration within the presynaptic nerve terminal. We set out to examine if the occurrence of spontaneous events (minis) after a series of evoked stimulations is correlated to the frequency and duration of a stimulus train. Short-term facilitation at the neuromuscular junctions is due in part to residual Ca2+ in the nerve terminal. However, if evoked release from high efficacy synapses result in evoked depression then the limiting factor may be the number of readily release vesicles to sense residual Ca2+. Thus, a lower frequency in occurrence of these minis may depend on the degree of the evoked synaptic depression. In addition, the frequency in occurrence of minis may also be independent of evoked events if the vesicles that give rise to the events are independent of each other. We hypothesize that the residual Ca2+ should affect the frequency of mini occurrence. We analyzed the frequency in occurrences of minis with differing stimulating conditions using the Drosophila NMJ. Preliminary data with 20, 40 and 60Hz stimulation of 30 pulses indicates that the nerve terminal is able buffer the internal Ca2+ level quickly and not impact the frequency of minis under these conditions. A better understanding of these events would help to address the residue effect of nerve stimulation on synaptic transmission in various physiological conditions. This is an authentic course-based undergraduate research experience (ACURE).

Supported by: KY Sci. and Eng. FDN, KSEF-3712- RDE-019 (RLC)

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Poster Presentation #169

Abstract Title:	**Loss of myelin integrity causes marked astrogliosis in an Alzheimer's disease-relevant mouse model.**
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Abstract: Loss of myelin integrity may either be a primary or secondary phenomenon in Alzheimer's disease (AD). There is strong clinical support for progressive and AD-specific white matter degeneration in the medial temporal lobe and frontal cortex. Proteolipid protein (PLP), which is the principal protein of CNS myelin, is critical for maintenance of the compact myelin of the internode; yet, PLP deficient mice have essentially normal myelination. We hypothesize myelin disruptions, in the form of decreased myelin proteolipid protein, will induce a white matter specific astrocyte phenotype that is deleterious for axonal health and that the white matter specific astrocyte phenotype and associated neuroinflammation contributes to cognitive impairments and traditional AD pathology. The aim of the study is to determine if the loss of myelin integrity, by the mutation in a PLP will accelerate amyloid beta, phosphorylated MAPT, astrocyte-induced inflammation, and cognitive deficits, in the APP KI mice. Histopathological assessment of astrogliosis was performed using Aperio ScanScope with a 20x objective. 140 brains of APP X PLP 2x KI mice stained for GFAP. We found striking astrogliosis in the APP X PLP 2x KI mice compared to the APP KI or PLP KI mutation alone. The results suggest that loss of myelin integrity can drive astrocyte dysfunction, in the context of AD-related mutations in the APP gene. Ongoing work will determine if cognitive deficits and accelerate amyloid beta, phosphorylated MAPT are seen in the APP X PLP 2x KI mice

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13th Annual CCTS Spring Conference
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Poster Presentation #170

Abstract Title: **The effect of dehydration on mild traumatic brain injury in mice**

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Abstract: Dehydration is known to have the ability to change the volume of many brain structures, as well as, produce confounding symptoms that resemble concussive injuries. In the present study, we examined the effect of dehydration on brain injuries in C57Bl/6J mice. We hypothesize that dehydration at the time of a brain injury significantly worsens axonal injury and related clinical sequelae. For this study, we conducted the radial arm water maze (RAWM) and passive avoidance post-injury to quantify the effects of the injury on memory and learning. However, neither behavioral assay produced significant differences between the sham and dehydrated mice. These results suggest that dehydration does not increase the damage produced by a concussive brain injury.

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13th Annual CCTS Spring Conference
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Poster Presentation #171

Abstract Title: **Differential Reinforcing Effect of Methamphetamine Isomers**

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Abstract: Methamphetamine is a Schedule II stimulant with a high potential for abuse. Currently there are no pharmaceutical therapies specific to methamphetamine addiction. Although the d-isomer is known to be the active form of methamphetamine (METH), preclinical work in monkeys has shown that the l-isomer of methamphetamine (PAL-1311) has a weak reinforcing effect, and thus may serve as a substitute for stimulants such as METH or cocaine; however, monkeys used in that study were not drug naïve, but instead had a history of cocaine exposure. The current experiment sought to compare the reinforcing effect of METH and PAL-1311 in drug naïve rats. Rats were trained to acquire IV self-administration of either METH or PAL-1311 using a standard 2-lever procedure, with dosing varied between test days to derive a dose-response curve for each isomer. The results showed that while both METH and PAL-1311 engendered reliable self-administration, the dose of PAL-1311 required to elicit maximal responding was approximately 10 times higher for PAL-1311 than for METH. Moreover, although both drugs yielded an inverted U-shaped dose-effect curve, the curve for PAL-1311 was flattened and shifted to the right compared to METH. These results suggest that PAL-1311 has a weak reinforcing effect compared to METH, as demonstrated in previous preclinical work in monkeys. From a clinical perspective, this suggests the possibility that when administered at high enough doses, PAL-1311 may serve as a substitute for METH in those suffering from addiction.

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Poster Presentation #172

Abstract Title: **Cortical correlates of memory accuracy and reaction times in healthy older adults**

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Abstract: Patients with Alzheimer's disease have diminished memory performance, i.e. less accurate, more false alarm, and increased reaction times during a memory task. For many healthy older adults, whether poor performance is part of normal aging or a risk of mild cognitive impairment is not clear. Here we test the hypothesis that functional brain responses in selective regions are correlated with either memory accuracy or response times. 44 older adults (25 females; aged 65-93), from University of Kentucky Alzheimer's Disease Center cohort, participated in the magnetic resonance imaging (MRI) were put through a series of behavioral tasks using the Bluegrass during a short-term memory task. Liner regression analyses were performed on event-related functional MRI and individual performance results. We found that bilateral insula, the right frontal eye field, and bilateral inferior parietal lobe (IPL)showed a significant negative correlation to accuracy, and positive correlation to the number of false alarms (e.g. the right IPL and accuracy $R2=0.2747$; $p < 0.001$). On the other hand, the activity in bilateral hippocampi and the left amygdala significantly correlated to reaction times. These negative correlations indicate that increased activity in a brain is associated with impaired accuracy of the short-term memory. The present results will allow us to test the next step hypothesis whether the performance and brain activity measures are associated with cerebrospinal fluid (CSF) AD biomarkers ß-amyloid (Aß42) and tau-related neurodegeneration (p-Tau181), hallmark for AD pathology.

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13th Annual CCTS Spring Conference
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Poster Presentation #173

Abstract Title: **Effects of Solidago Nemoralis on Fetal Alcohol Syndrome: A Water Maze Paradigm**

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Abstract: Alcohol ingestion during pregnancy can be detrimental to developing fetuses and can result in Fetal Alcohol Syndrome Disorder (FASD). FASD presents itself via behavioral, learning and cognition deficits and facial abnormalities. Existing studies suggest that drugs including Solidago nemoralis can reduce the effects of FASD by acting as an agonist on the alpha-7-nicotinic-acetylcholine-receptor. In the present study, ethanol was administered during a period of CNS development that overlaps the third trimester "brain growth spurt" of human pregnancy. ETOH (6g/kg/day) was given to Sprague-Dawley rat offspring on post-natal days (PND) 1-7. On PND 8, offspring were given either Solidago nemoralis or saline injections. To test for spatial learning and memory, a water maze paradigm was used in which the subject had to use external cues and an internal map to find a platform hidden under the water surface conducted on PND 40-45. The group that received ETOH paired with the Solidago nemoralis showed fewer deficits than the group that received only ETOH. The results in this study support the hypothesis that "third trimester" ETOH exposure impairs spatial learning and that a single administration of ETOH can result in deficits. It also showed that deficits associated with fetal alcohol exposure can be treated with Soliadgo nemoralis to help reduce effects of FASD and, in some cases, eliminate effects of FASD. The possible role of the alpha 7 in effects of prenatal ETOH exposure may also suggest that Solidago nemoralis has antioxidant properties. Further research is needed to understand the underlying mechanisms.

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13th Annual CCTS Spring Conference
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Poster Presentation #174

Abstract Title:	**The Effects of Solidago Nemoralis on Balance in Rodents Following 3rd Trimester Ethanol Exposure**
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Abstract: Ethanol (ETOH) exposure during fetal development can have harmful effects on the central nervous system causing a variety of behavioral deficits. Previous studies have shown that following developmental ethanol exposure, drugs acting on the cholinergic system can reduce both behavioral deficits and hippocampal damage. This reduction may be due to the activation of the alpha-7- nicotinic acetylcholine- receptor (a7nAChR). The current study examined the ability of Solidago nemoralis (SN) to reduce behavioral effects. Solidago nemoralis has agonist effects on the a7nAChR receptor, reducing the behavioral effects of ETOH exposure occurring in the "3rd trimester human pregnancy brain growth spurt" in rats. In this study, ETOH (6g/kg/day) was administered to the neonatal rat pups via intragastric intubation on postnatal days (PND) 1-7. Intubated and non-intubated control groups were also included in the process. On PND 8, during ETOH withdrawal, the rat pups received an injection of a flavonoid-enriched extract of SN (50 mg/kg) or saline. Balance was examined in adolescent offspring using a dowel rod. The distance traveled by the rats was measured with numbered increments on the dowel rod. This study indicated that both male and female ETOH exposed offspring showed balance deficits. With the addition of the SN extract, reduction of the deficits was observed relative to ETOH alone. These results support the potential neuroprotective properties of a SN flavonoid injection by decreasing some of the detrimental behavioral effects in this model of "3rd trimester" ETOH exposure.

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13th Annual CCTS Spring Conference
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Poster Presentation #175

Abstract Title: **NPY-Y1 Signaling Pathway Responsible for the Affective Component of Neuropathic Pain**

Author(s): C. R. Wessel, Department of Physiology, U of Kentucky
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Abstract: Background Latent sensitization (LS) is a driving mechanism behind the development of chronic pain after inflammatory or neuropathic injury. LS can remain in remission due to endogenous pain inhibitory controls. A genetic or pharmacological blockade of neuropeptide tyrosine Y1 (NPY-Y1) signaling in the central nervous system produces a reinstatement of hyperalgesic behaviors when administered long after peripheral nerve injury. These hyperalgesic behaviors are models for the stimulus evoked component of pain. Whether LS involves the affective component of pain, which is critical in the human pain experience, remains unclear. Methods In this study we used a spared nerve injury model of neuropathic pain in the mouse in which the common peroneal and the sural branches of the sciatic nerve were ligated and transected (CPxSx model). The CPxSx model produces a period of hyperalgesia that resolves after 4-5 weeks. After 37 days, Y1 receptors were targeted in the spinal cord via a 5ul intrathecal injection of BIBO3304, a high affinity Y1 antagonist. To evaluate the affective component of pain, a conditioned place aversion (CPA) assay was used with a single day of chamber association conditioning. Results: We found that intrathecal BIBO3304 (5ug/5ul) produced CPA in CPxSx mice but not in sham controls. Discussion Although preliminary, our data indicates that LS that is masked by NPY-Y1 signaling in the spinal cord represents not only a long-lasting vulnerability to the sensory/discriminative component of pain, but to the affective component as well. Studies are in progress with additional mice to provide sufficient power to our analyses.

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Poster Presentation #176

Abstract Title:	**Potassium Conducting Kv4.2 Expression in the Neuropeptide Y1 Receptor Expressing Spinal Lamina II Neurons Increase After Injury as Revealed by Immunohistochemistry**
Author(s):	S. Schmitt, Department of Physiology, U of Kentucky G. Sinha, Department of Physiology, U of Kentucky L. Zhang, Department of Physiology, U of Kentucky B.Taylor, Department of Physiology, U of Kentucky

Abstract: Neuropeptide Y Y1 receptor expressing neurons are involved in pain processing and pain transmission in the mammals. Combined electrophysiological and anatomical studies from our laboratory have revealed that majority of the neurons in the lamina II region of adult mice are excitatory or glutamate containing interneurons. Patch clamp whole cell recordings in our laboratory revealed that the about 74% of Y1R expressing neurons exhibited Kv4.2 mediated voltage gated potassium currents which result in delayed action potential firing and are also believed to be characteristic of excitatory neurons. Using immunohistochemistry with Kv4.2 primary antibodies, we studied the lamina II region of the Y1-eGFP mouse spinal dorsal horn. Co-localization of about 56% of Y1-eGFP expressing neurons with Kv4.2 antibodies was observed. This provides evidence that majority of Y1 receptor expressing neurons express Kv4.2 channels and putatively are excitatory. In the setting of injury, a pain transmission can be exacerbated by the excitatory neurons. This could happen by phosphorylation and internalization of Kv4.2 channels that would lead to a reduction of the absence or weaker Kv4.2 mediated currents meaning a shorter duration of delay in action potential firing, as verified by electrophysiological recordings. Overall this would lead to an increase in signal transmission. To test this hypothesis, we performed immunohistochemical studies with phosphor-Kv4.2 antibodies. As expected we observed a higher percentage of phosphor-Kv4.2 expression in our injured model compared to sham. In the sham model, about 56% of Y1-eGFP expressing cells also expressed phosphor-Kv4.2, which increased to about 67% in our injury model.

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13th Annual CCTS Spring Conference
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Poster Presentation #177

Abstract Title:	**Reducing Behavioral Deficits and Cellular Damage In Ethanol Exposed Rats Using Flavinoids From the Goldenrod, Solidago Nemoralis.**
Author(s):	M. Knabel, Psychology Department, U of Kentucky L. Pack, Psychology Department, U of Kentucky B. Auvil, Psychology Department, U of Kentucky L. Fields, Psychology Department, U of Kentucky A. Hawkey, Psychology Department, U of Kentucky J.M. Littleton, Psychology Department, U of Kentucky S. Barron, Psychology Department, U of Kentucky

Abstract: Ethanol (ETOH) exposure during development can have a negative impact on the central nervous system causing a variety of behavioral deficits. Previous research shows that drugs that act on the cholinergic system can reduce some of the behavioral deficits following ETOH exposure during development. This protection may be due to activating the alpa-7-nicotinic-acetylcholine-receptor (?7nAChR). This study examined Solidago nemoralis (SN), from goldenrods, and its agonist effects on ?7nAChRs to reduce the behavioral deficits of ETOH exposure during fetal development. Preliminary research from our lab found SN could reduce deficits in some paradigms following developmental ETOH exposure. Previous studies show that SN can reduce neurotoxicity caused by ETOH in cellular models.This study, 6g/kg/day of ETOH was administered to neonatal rats via intragastric intubation on postnatal days (PND) 1-7; a model for exposure during the human third trimester. Intubated/non-intubated control groups were also included. On PND 8, after the last ETOH intubation and during ETOH withdrawal, the pups were injected with a flavonoid-enriched extract of SN (50mg/kg) or saline. Attentional Set Shifting (ASST) was used to assess executive function, which can be affected by prenatal ETOH exposure. Offspring were tested on PND 55-60. ETOH exposed rats displayed deficits during the first phase of the complex discrimination task and ETOH exposed females displayed deficits in reversal learning. The ETOH exposed offspring that also received SN showed reduced deficits compared with ETOH alone. These results provide support for the role of ?7nAChRs as a possible mechanism as well as impairments of prenatal ETOH exposure.

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Poster Presentation #178

Abstract Title: **The Effects of a Bacterial Endotoxin on Sensory Perception in Larvae**

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Abstract: Humans experience lethargy during bacterial sepsis and even after a sepsis has been treated. The prolonged effects of sepsis make it difficult to distinguish the cause as many factors are involved including immunological actions, treatment protocols, and even potentially direct actions of the bacteria endotoxin on cells. Insects can detect some forms of LPS and avoid eating foods tainted with LPS and even avoid laying eggs in contaminated environments. This suggests a direct action of being able to sense the bacteria. It is known that LPS can have direct actions on sensory and motor neurons in mammals. Larvae of blow flies, which are used as therapy for debriding dead tissue in wound care, are exposed to bacterial endotoxins and few studies have investigated the actions of forms of LPS endotoxins on therapeutic blowflies to assess survival and physiological function. In this study, we examined the effect of touch on the behavioral responses in larvae of Drosophila melanogaster and larvae of blowfly with and without exposure to LPS in their diet over various time periods (24 to 48 hrs). We developed behavioral HAT assays for larvae to assess their reaction to tactile stimuli. Food tainted with 100 µg/ml of LPS from two common strains (Pseudomonas aeruginosa and Serratia marcescens) were used. These are the specific bacterial strains which afflict humans and other mammals who have septicemia or potentially may be receiving maggot therapy for a wound care. Our studies are still ongoing. This presentation will include the results of these studies.

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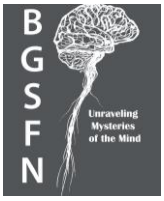

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Poster Presentation #179

Abstract Title:	**Effects of Smoke Exposure on the Expression of Aggression-Related Genes in Honeybee Brains**
Author(s):	J. Harrison, Neuroscience, U of Kentucky C. Rittschof, Department of Entomology, U of Kentucky J. Palmer, Department of Entomology, U of Kentucky

Abstract: The use of smoke to pacify honeybee colonies has been employed by beekeepers for millennia, but the reasons for the effectiveness of this technique are still not well-understood. This ongoing study will be the first to investigate the phenomenon through the lens of gene expression. Past research has identified several environmentally-regulated genes which correlate to levels of behavioral aggression. These genes are up-regulated when a bee detects the odor of alarm pheromone, and a leading hypothesis for the effects of smoke suggests that smoke exposure impairs a bee's ability to sense alarm pheromone. We offer a slight modification to this hypothesis, and postulate that smoke inhalation actively down-regulates aggression-related genes, as opposed to simply masking the perception of aggression-inducing stimuli. By analyzing messenger RNA from the brains of honeybees shortly after exposure to smoke, we look to find significantly less transcription of aggression-related genes when compared to non-exposed bees, thus establishing a genomic basis for the aggression-reducing effects of smoke.

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13th Annual CCTS Spring Conference
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Poster Presentation #180

Abstract Title: **Oxytocin Release and Reward Pathway Activation Following Social Play**

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Abstract: The adolescent stage of development is marked by a heightened emphasis on social interaction. This highly conserved feature of development can be modeled in rodents. Rats engage in social play more during their adolescent stage than at any other point in their lives, and social play is especially rewarding in adolescent rats. Social interaction and bonding cause the hormone oxytocin to be released from the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus. It is possible that the rewarding effects of social play are mediated by oxytocin release to regions of the reward pathway, such as the prefrontal cortex (PFC) and nucleus accumbens (NAc). This experiment sought to determine whether social play activates oxytocinergic neurons in the PVN and how this release affects activity in the reward pathway. Male adolescent Sprague-Dawley rats (n=12) received one 15-minute session to explore a microdialysis chamber for three consecutive days. On the fourth day, half the rats were given another 15-minute session by themselves, while the other half were given a 15-minute play session in the chamber with another rat. Rats that interacted with their peers had a significant increase in the number of oxytocin-releasing neurons that were activated in the PVN. Furthermore, the NAc and the dorsomedial PFC were more active following play. In rats that were isolated, increased PVN activation was associated with decreased NAc activation, but this relationship was not present following play. The results of this experiment provide insight into the connection between oxytocin and the reward pathway.

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13th Annual CCTS Spring Conference
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Poster Presentation #181

Abstract Title: **Oxytocin as Preclinical Treatment for Socially-Induced Reinstatement**

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Abstract: In patients recovering from drug addiction, social cues are a common cause of relapse. Preclinically, relapse is modelled using the self-administration and reinstatement paradigm. For example, in cue-induced reinstatement, a drug-associated light can trigger cocaine seeking. However, few preclinical reinstatement studies have focused on social influences, and thus it is not known if cue-induced reinstatement generalizes to socially induced relapse. Oxytocin may play an important role in social-induced relapse because this neuropeptide increases social recognition and decreases cue-induced reinstatement. The purpose of this experiment was to find if oxytocin would also decrease reinstatement triggered by encountering a cocaine-associated peer. Over the course of 28 days, male Sprague-Dawley rats self-administered cocaine (0.1 mg/kg/infusion) in the presence of one partner (S+ peer) and saline in the presence of a different partner (S- peer) during randomly presented twice-daily sessions. Each infusion was paired with a 20-s timeout period, signaled by the illumination of a light (CS). Next, the rats underwent extinction and cue-induced reinstatement tests with a pretreatment of either saline or 0.3 mg/kg oxytocin. Each rat received four reinstatement tests (no cue or peer, CS present, S+ present, and CS/S+ present). The rats that received oxytocin had significantly reduced reinstatement compared to the group given saline for all tests. The effects of oxytocin did not differ between cue and social-induced reinstatement. These results show that oxytocin has clinical potential to be used for preventing relapse triggered by exposure to drug-associated peers

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Poster Presentation #182

Abstract Title:	**Can Solidago nemoralis Reduce Spatial Learning Deficits following '3rd' Trimester Ethanol Exposure in a Rodent Model?**
Author(s):	B.M. Auvil, Psychology Department, U of Kentucky L. Pack, Psychology Department, U of Kentucky M. Knabel, Psychology Department, U of Kentucky L. Fields, Psychology Department, U of Kentucky J.M. Littleton, Psychology Department, U of Kentucky S. Barron, Psychology Department, U of Kentucky

Abstract: Alcohol consumption during pregnancy can harm the developing offspring and can result in Fetal Alcohol Spectrum Disorders (FASD). Offspring with an FASD can display behavioral, learning and cognitive deficits. Recent preliminary data from our laboratory suggests that flavonoids including Solidago nemoralis can reduce some of the effects of fetal alcohol exposure by acting as an agonist on the alpha-7-nicotinic-acetylcholine-receptor or by its anti-inflammatory actions in in vivo and in vitro rodent models. In the present study, ethanol was administered during a period of CNS development that overlaps the third trimester "brain growth spurt" of human pregnancy. ETOH (6g/kg/day) was given to Sprague-Dawley rat offspring on postnatal days (PND) 1-7. On PND 8, offspring were given either Solidago nemoralis or saline injections. To test for spatial learning and memory, a water maze paradigm was used in which the subject had to use external cues and an internal map to find a platform hidden under the water surface. Subjects were tested on PND 40-45. The group that received ETOH paired with the Solidago nemoralis learned the spatial task more quickly than the group that received only ETOH. These results support the hypothesis that "third trimester" ETOH exposure impairs spatial learning and that a single administration of Soliadgo nemoralis can help improve performance. Further research is needed to understand the underlying mechanisms and whether this generalizes to other behaviors.

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BGSFN Unraveling Mysteries of the Mind

13th Annual CCTS Spring Conference
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Poster Presentation #183

Abstract Title: **Effect of Long-term Dietary Vitamin D3 Supplementation on Cognition in Aging Male and Female F344 Rats**

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Abstract: Previously, we reported mid-aged F344 male rats on an enhanced, long-term vitamin D supplemental diet (cholecalciferol, VitD3: 10,000 IU/kg chow) showed improved cognition and elevated hippocampal gene expression compared to rats on standard and low VitD3 diets (Latimer et al. 2014). Here, we compared the long-term effects (6 months) of the enhanced VitD3 diet to the standard AIN-93 diet (1,000 IU VitD3/Kg) on cognition in mid-aged female and male F344 rats. Cognition was determined using the Morris water maze. Animals were trained for 3 days to find a submerged platform followed by a probe trial. Then, animals were trained for 1 day to find a new platform location (spatial reversal) followed by a reversal probe. There was no difference in pathlength and latency to the platform according to sex or treatment (2-way ANOVA) on training days 1 and 2. On training day 3 pathlength was significantly less in females (18%) and latency increased in VitD3 treated animals (19%). The probe test showed that enhanced VitD3 treatment significantly reduced (P = 0.01; ~70%) pathlength and latency to the platform in females but not males. Next, the one day of reversal training indicated no effect of sex or diet. The reversal probe, conducted three days later, indicated that VitD3 treatment significantly reduced pathlength and latency (P<0.05; ~60%) in males but not females. These results strengthen the hypothesis that optimal blood levels of vitamin D are important for healthy brain aging. Furthermore, vitamin D may affect cognitive pathways in a sex specific manner.

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13th Annual CCTS Spring Conference
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Poster Presentation #184

Abstract Title:	**Exploring Approaches to Promote Respiratory Motor Plasticity Through Varied and Fixed Intermittent Hypoxia**
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Abstract: Fixed interval intermittent hypoxia treatment (FIH) consists of the repeated, alternating exposure of a subject to hypoxic and normoxic conditions which persist for a consistent and equal duration of time. In animals, this treatment is often utilized to induce a prolonged increase in phrenic motor output, a type of respiratory motor plasticity known as Long Term Facilitation (LTF). This treatment exhibits similarity to the psychological construct of operant conditioning and as such, each interval of hypoxia can be construed as the period during which the subject responds with heightened respiratory drive and is subsequently reinforced by an interval of normoxia. We therefore hypothesize that classical intermittent hypoxia procedure is a form of operant conditioning which can be optimized. Specifically, varying the duration of hypoxia and therefore the schedule of reinforcement is predicted to produce a more extinction-resistant behavior, in this case promoting a more long-lasting increase of phrenic motor output. Here we utilized the widely accepted technique of diaphragm electromyographic recording to assess breathing motor output. Preliminary data suggests that exposure of C2 hemisected rats to VIH results in functionally insufficient plasticity when compared to maximal diaphragm output induced by nasal occlusion. In naïve rats treated by VIH, spinal cord application of serotonin depressed breathing motor output, an effect opposite from that observed after FIH. These data inspire further analysis of our construed operant procedure, as they suggest that FIH may actually promote a higher level of respiratory motor plasticity than VIH.

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Poster Presentation #185

Abstract Title: **The Contribution of AMPA Receptor Subunits to Chronic Pain States**

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Abstract: Central sensitization (CS) of neurons in the dorsal horn of the spinal cord (DHSC) contributes to hyperalgesia and chronic pain. CS is mediated at least in part through increased neuronal calcium permeability. An undiscovered area of this mechanism is the vulnerability to hyperalgesia that remains after CS, which is known as latent sensitization. Alpha-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) mediate fast excitatory transmission and play a critical role in synaptic plasticity of spinal cord neurons. AMPAR channels are tetramers that consist of a combination of four subunits (GluA1-GluA4). AMPARs are present in nearly all excitatory synapses in the DHSC. Channels that contain a GluA2 subunit are Ca2+-impermeable (CI), while GluA2-lacking AMPARs are Ca2+-permeable (CP). Under normal conditions, AMPARs in the dorsal horn are mostly Ca2+ impermeable, but perturbations can alter subunit composition and thereby increase AMPAR Ca2+ permeability. We hypothesized that an inflammatory insult involving the intraplantar injection of complete Freund's adjuvant (CFA), would increase either GluA1 or GluA4 expression at the post-synaptic density (PSD), followed by a subsequent increase in GluA2-lacking CP-AMPARs, ultimately leading to an increase in spinal pain transmission. To test this idea, mechanical threshold was tested on C57BL/6 mice using von Frey filaments on days 0, 2, 14, and 21 after CFA (5 µl). Hyperalgesia was observed at day 2 and resolved by days 14-21. PSD was isolated from the total homogenate and confirmed using anti-PSD95 and anti-synaptophysin antibodies. We used western blotting techniques to identify GluA1, GluA2, and GluA4 within the total homogenate and PSD. Our results indicate that CFA increases GluA1 expression at day 2 (100 ± 4.32% for naïve vs. 174.1 ± 23.18% for d2; $p = 0.01$, $n = 7$). We also observed a significant increase in GluA4 expression at 21d post injury (100 ± 5.52% for naïve vs. 143.2 ± 12.39% for d21; $p = 0.03$, $n = 6$-7). Although correlative in nature and thus preliminary, these data suggest that the expression of GluA1 and GluA4 in dorsal horn synapses potentially contribute to the development and/or maintenance of neuropathic pain.

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