Poster Presentation 33

Abstract Title:	**Inducing Elevated Insulin Signaling via a Constitutively Active Human Insulin Receptor Leads to Alterations in Glucose Metabolism in Cultured Hippocampal Neurons**
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Abstract: Recent studies indicate insulin signaling diminishes with aging and Alzheimer's disease (AD) (evidenced by reduced signaling markers, insulin mRNA, and insulin receptor (IR) density), and also highlight insulin's role in normal brain function. Yet, the mechanisms underlying these processes remain unclear. To address this, we conducted experiments exploring the relationship between insulin signaling, glucose metabolism, and GLUT translocation in hippocampal neurons. Mixed, primary hippocampal cultures were infected with plasmids encoding a red fluorescent protein (mCherry), with or without a constitutively active human IR (IRβ), using a lentiviral system. A synapsin promoter was included to limit expression to neurons. Immunocytochemistry against IRβ was used to confirm expression. Western immunoblots were performed to obtain signaling levels. To assess effects of increased IR signaling on glucose metabolism, 2-NBDG imaging was conducted. Glucose uptake was obtained by measuring initial 2-NBDG fluorescence. Fluorescent signal decay over time was recorded as an indirect measure of glucose utilization. To test if changes in glucose were related to GLUT receptor density, GLUT immunocytochemistry and Western immunoblots were performed. Lentiviral infection was successful for all constructs. Immunocytochemistry showed IRβ in 80% of cells. Western blots provided evidence that IRβ expression confers elevated IR signaling. 2-NBDG imaging indicated IRβ was associated with increased glucose uptake and utilization. IRβ expression correlated with changes in GLUT density. This characterization provides insights into potential mechanisms governing insulin's effect on memory and learning, and highlights the validity of exploring molecular approaches to enhance insulin signaling to combat cognitive decline associated with aging and AD.

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Poster Presentation 34

Abstract Title:	**Dual PI3K/Akt Inhibition to Overcome Blood-Brain Barrier P-glycoprotein and Breast Cancer Resistance Protein**
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Abstract: Glioblastoma is one of the deadliest cancers with a median survival of 15-23 months. Even aggressive treatment including tumor resection, radiation and chemotherapy does not substantially prolong patient survival. One reason for therapeutic failure are the efflux transporters P-glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP) at the blood-brain barrier. P-gp/BCRP are working together to limit anticancer drugs from entering the brain and eradicating remnant tumor cells, resulting in glioblastoma recurrence. While transporter inhibition combined with chemotherapy is a successful treatment option in mice, it is not a viable approach in patients due to severe adverse effects. Thus, new strategies are necessary to improve the brain delivery of anticancer drugs. Here, we are testing a novel molecular switch approach: dual inhibition of PI3K/Akt to decrease blood-brain barrier P-gp/BCRP expression and activity levels. We hypothesize that this approach will provide a window-in-time to deliver anticancer drugs into the brain. To test this hypothesis, brain levels of anticancer drugs were determined with in situ brain perfusion. PSC833 and fumitremorgin C were used to inhibit P-gp and BCRP, respectively. PI3K/Akt were inhibited with LY294002/triciribine. Directly inhibiting P-gp/BCRP increased brain levels of anticancer drugs. Inhibiting PI3K/Akt decreased P-gp/BCRP expression and activity levels, which resulted in increased brain levels of anticancer drugs. PI3K/Akt inhibition is a useful approach to temporarily turn off P-gp/BCRP to improve drug brain uptake. We will test this strategy in mouse glioblastoma models with the goal of reducing tumor size and prolonging survival.

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Poster Presentation 35

Abstract Title:	**Metabolic Reprogramming and Alzheimer's Disease Risk: The Role of Apolipoprotein E4**
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Abstract: Background: Apolipoprotein E (APOE) gene encodes for three different isoforms present in humans (E2, E3, and E4). Homozygous E4 individuals face up to a 15-fold increase risk for developing late-onset Alzheimer's Disease (AD). A hallmark of AD is a regional reduction in cerebral glucose metabolism, alluding to a metabolic component in disease onset and progression. Interestingly, E4 individuals display cerebral glucose hypometabolism decades prior to disease onset, but the underlying biological mechanism remains unknown. Efficient metabolic coupling of neurons and glia is necessary for proper neuronal function, and a disruption in glial energy metabolism has been proposed to contribute to AD pathology and neuronal cell death. One important function of astrocytes to provide energy substrates (mainly lactate) to neurons. Our preliminary results show a reduction in E4 astrocytic glucose uptake, glycolytic activity, and subsequent lactate secretion. Methods: Stable isotope-resolved metabolomics (SIRM) using a 13C-glucose tracer was administered to human APOE mice in vivo using a novel liquid diet delivery protocol. In vitro metabolism of immortalized astrocytes expressing human APOE was measured using SIRM tracer metabolomics with a 13C-glucose supplemented growth media. Results: In vivo metabolic tracing shows evidence for decreased incorporation of glucose to lactate and also a reduction in glycolytic flux, specifically in the TCA cycle, in E4 compared to E2 and E3 brains. In vitro tracer metabolomics in immortalized astrocytes revealed increased glucose-6-phosphate yet decreased pyruvate, suggesting reduced glycolytic flux in E4 compared to E2 and E3. Conclusion: These findings suggest a reduction in glycolytic flux of E4 relative to E2 and E3, potentially altering mitochondrial energetics thus contributing to disease phenotype. Ongoing studies aim to expand our SIRM approach to examine substrate uptake, utilization, and metabolic flux in neuron-astrocyte co-cultures, as well as in purified neuronal and astrocytic mitochondria, and ex vivo brain slices. We hope our studies will illuminate metabolic bottlenecks (i.e. specific enzymes) responsible for APOE associated changes in cerebral metabolism which can be exploited as new potential therapeutic targets.

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Poster Presentation 36

Abstract Title:	**Changes in dorsal hippocampal calcium levels and behavior before, during, and after AD pathology in the 5XFAD and HNE mouse models**
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Abstract: As the projected rise of individuals affected by Alzheimer's disease (AD) is expected to triple by 2050, the need for characterization of associated molecular mechanisms and the development of novel therapeutic treatments remains indispensable. A potential mechanism, highlighted in the calcium hypothesis of brain aging and dementia, describes a state of altered calcium handling in neurons that has an impact on several physiological parameters, including the Ca+2-dependent potassium potential, the afterhyperpolarization (AHP). One hallmark of field CA1 neuronal aging in the hippocampus is an increased AHP, accompanied with elevated levels of intracellular calcium. Though a robust association between calcium and the AHP has been illustrated in normal aging, how the two phenomena contribute to disease-state aging remains largely unknown. Recent work has reported reduced levels of L-type voltage sensitive calcium channels (L-VSCCs) in older APP and PS-1 transgenic mice, suggesting calcium dysregulation in AD mouse models may vary from that seen in aging. In this study, we are identifying the effects of aging on the calcium-dependent AHP and intracellular calcium levels in the 5xFAD and HNE models. Using sharp electrode electrophysiology and calcium imaging (OGB-1), we are beginning to observe an attenuated AHP in the 4 month 5xFAD animals compared to 1.5 months. Analyses of behavior data (MWM) does not show deficit until later time points (6-7 months). These data support the notion that reduced neuronal calcium signaling could be a precipitating factor in the manifestation of behavioral deficits, rather than an increase in neuronal calcium seen in normal aging.

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Poster Presentation 37

Abstract Title:	**Deciphering the Complex Relationships of Periocular Mesenchyme Subpopulations within the Developing Zebrafish Ocular Anterior Segment**
Author(s):	K.L. Van Der Meulen, Department of Biology, University of Kentucky J.K. Famulski, Department of Biology, University of Kentucky

Abstract: The anterior segment (AS) is a complex collection of structures used to project light onto the retina and maintain inner eye homeostasis. Anterior Segment Dysgenesis (ASD) is a spectrum of developmental disorders effecting these structures and resulting in visual impairment. The neural ectoderm, surface ectoderm, and Periocular Mesenchyme (POM) lineages come together in early development to assemble the AS. We believe that misregulation of the molecular machinery regulating incorporation of POM into AS tissues may predispose individuals to ASD. Using transgenic zebrafish embryos expressing GFP in POM cells we have discovered the POM comprise of several subpopulations denoted by unique AS distributions, population sizes, and migratory dynamics. Static and live time-lapse analysis clearly defined significant differences amongst various POM cell migrating onto the AS. This suggests that AS formation is multifaceted. In order to better understand AS development, we sought to characterize each subpopulation on a molecular level. To isolate molecular signatures of POM subpopulations (representing cells regulated by FoxC1b, FoxD3, Pitx2 or Sox10), we used FACS and RNA sequencing to compile their unique transcriptomes. A 4-way comparison of the data indicated ~20,000 genes were similarly expressed. However, more than 2,000 genes were uniquely expressed in the POM-specific FoxC1b population, ~4,000 in the neural crest-specific Sox10 population ~5,000 in the Pitx2 subpopulation and ~370 in the FoxD3 population. Detailed analysis of this data is expected to uncover novel marker gene expression patterns for each subpopulation in addition to common regulators of the POM cell lineage.

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Poster Presentation 38

Abstract Title:	**Escalation and Reinstatement of Fentanyl Self-Administration in Rats**
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Abstract: Purpose: Opioid abuse disorder is characterized by increased intake over time and high likelihood of relapse. This study determined if escalation of fentanyl self-administration over extended (6-hr) sessions enhanced craving as measured by fentanyl- and yohimbine-induced drug seeking following a period of extinction. Methods: Adult male and female Sprague-Dawley rats were trained to self-administer i.v. fentanyl (2.5 ug/kg/infusion) across seven 1-hr sessions, followed by 21 additional sessions of either 1- or 6-hr duration. Both groups then underwent 14 1-hr sessions of extinction. Reinstatement was assessed within-subjects following pretreatment with either fentanyl (0, 10 or 30 ug/kg, s.c.) or the sympathomimetic yohimbine (0, 1 or 2 mg/kg, i.p.). Results: Responding on the active lever increased during acquisition. Across the 21-day period following acquisition, the 6-hr group escalated fentanyl intake; in contrast, there was no change in the 1-hr group. There was no effect of group on extinction. Similarly, there was no effect of group on drug-induced reinstatement. However, the 6-hr group displayed greater stress-induced reinstatement following 2 mg/kg yohimbine. Conclusion: These results demonstrate that fentanyl self-administration escalates with extended access. More importantly, extended access potentiated yohimbine-induced drug-seeking, but not fentanyl-induced drug seeking, indicating that escalation of opioid intake increases vulnerability to stress-induced relapse.

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Poster Presentation 39

Abstract Title:	**Progesterone Pretreatment Decreased Acute Stress Effect on Cognition and Sgk1 expression in Sprague-Dawley Rats**
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Abstract: Stress is highly prevalent, has negative health consequences, and causes deficits in cognitive function. The impact on cognition is thought to be exerted, at least in part, through stress-induced glucocorticoid action in the brain. Serum and glucocorticoid kinase 1 (Sgk1) is a key downstream effector of glucocorticoid's actions. Progesterone antagonizes glucocorticoid signaling at transcriptional and allosteric modulating levels. Here, we investigated if progesterone pretreatment alleviates the cognitive deficits and elevated Sgk1 associated with acute restraint. Thirty-one adult Sprague-Dawley rats (21 males/ 10 females) were trained in the Morris Water Maze. The rats were placed into one of four groups: 1) stressed and progesterone (n=8), 2) unstressed and progesterone (n=7), 3) stressed and vehicle (n=7), and 4) unstressed and vehicle (n=9). After each of the three training days, progesterone-treated groups were dosed with progesterone (10 mg/ kg). On day 4, a 3 hour restraint was applied immediately prior to the water maze probe trial. Progesterone had no effect on training in any group, and had no effect on probe trial performance in unrestrained animals. In restrained animals, stress caused a significant decrease in vehicles that was reversed by progesterone. In a subset of animals (n=20; stressed and progesterone (n=6); unstressed and progesterone (n=4); stressed and vehicle (n=4); and unstressed and vehicle (n=6)), Western blot analysis of hippocampal tissue revealed a non-significant trend towards upregulation of SGK1 with stress, and suppression of that Sgk1 response in stressed, progesterone-treated animals. This data suggests progesterone may have some effect on decreasing stress response.

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Poster Presentation 40

Abstract Title:	**A Systematic Review of Closed Head Injury Models of Mild Traumatic Brain Injury in Rodents**
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Abstract: Mild TBI (mTBI) is a significant health concern. Animal models of mTBI are essential for understanding mechanisms, and pathological outcomes, as well as to test therapeutic interventions. A variety of closed head models of mTBI that incorporate different aspects (i.e., biomechanics) of the mTBI have been reported. The purpose of the current review was to compile a comprehensive list of the closed head mTBI rodent models, along with the common data elements, and outcomes, with the goal to summarize the current state of the field. Publications were identified from a search of PubMed and Web of Science and screened for eligibility following PRISMA guidelines. Papers were included that were closed head injuries in which the author's classified the injury as 'mild' in rats or mice. Injury model and animal-specific common data elements, as well as behavioral and histological outcomes, were collected and compiled from a total of 402 articles. Our results outline the wide variety of methods used to model mTBI. We also discovered that female rodents and both young and aged animals are underrepresented in experimental mTBI studies. Our findings will aid in providing context comparing the injury models and provide a starting point for the selection of the most appropriate model of mTBI to address a specific hypothesis. We believe this review will be a useful starting place for determining what has been done and what knowledge is missing in the field to reduce the burden of mTBI.

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Poster Presentation 41

Abstract Title:	**Siah E3 ubiquitin ligase regulates photoreceptor cell development by targeting CDHR1a for proteasomal degradation during zebrafish eye development.**
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Abstract: E3 ubiquitin ligases mediate orderly and precise targeting of protein degradation to maintain biological homeostasis and coordinate proper development. We recently discovered that Siah family of E3 ligases plays a role in early ocular morphogenesis, in particular fusion of the optic fissure. Interestingly, Siah ligases are also expressed during photoreceptor development and are predicted to target CDHR1a, a cadherin superfamily of calcium-dependent cell adhesion molecules and photoreceptor-specific cadherin. Mutations in this cadherin are associated with inherited retinal dystrophies, such as cone-rod dystrophy. Using whole mount in situ hybridization we detected Siah gene expression in the outer nuclear layer and in the retinal ganglion cell layer. CDHR1a expression overlaps with both Siah genes in the outer segment of the retina at 72hpf. We therefore hypothesized that Siah regulates CDHR1a during photoreceptor development. To test our hypothesis, I first confirmed siah-mediated targeting of CDHR1a for degradation using a mammalian cell culture and western blotting analysis. Second, I created two transgenic zebrafish lines that express Siah1 or a dominant negative (Siah□RING) under the control of the heat shock promoter. Using the heat shock line to overexpress Siah1 at 48hpf and 60hpf, we observed a decrease in the number of developing rods in the dorsal part of the retina at 72hpf. In addition, there was a significant reduction of proliferating (BrdU positive) cells in the outer nuclear layer. Taken together, our results suggest that Siah ubiquitin ligases may control CDHR1a stability and therefore regulate the photoreceptor cell proliferation and differentiation.

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Poster Presentation 42

Abstract Title:	**Neurocognition in post-bilateral globus pallidus interna deep brain stimulation with adjunctive substantia nigra sural nerve graft in Parkinson's disease**
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Abstract: Objective: Globus pallidus interna (GPi) deep brain stimulation (DBS) shows benefit in improving movement and quality of life in Parkinson's disease (PD) and is thought to relatively preserve cognition in domains such as verbal fluency, attention, and executive functioning. A recent pilot study examined whether dopaminergic neurons affected by PD can be regenerated via peripheral nerve graft implantation during GPi DBS. Current results assess the safety of this novel procedure and examine postoperative cognitive decline. Participants and Methods: Data were compared from baseline and 2-year postoperative neurocognitive evaluations for 14 patients with PD undergoing bilateral GPi DBS with unilateral sural nerve graft to the substantia nigra (SN). Paired sample t-tests were conducted and Cohen's d effect sizes calculated to evaluate pre- and post-surgical differences. Results: Performances in phonemic fluency ($t(13) = 2.95$, $p < .05$; $d = .50$, medium effect), working memory (Digit Span, $t(13) = 3.46$, $p < .01$; $d = .76$, medium effect), and processing speed (Stroop Color, $t(13) = 2.24$, $p < .05$; $d = .41$, small effect) significantly declined between evaluations. Conclusions: The magnitude of observed declines was somewhat larger than those for GPi DBS with results suggesting frontostriatal disruption following DBS consistent with the literature. Results also provide initial evidence of SN graft safety. Limitations include small sample size and lack of control for dopaminergic medication dose. Once safety is well-established, future research is needed to further determine cognitive outcomes in GPi DBS with sural nerve graft.

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Poster Presentation 43

Abstract Title:	**Frequency Response of Brain Electrical activity to Cognition and Tempo of Music**
Author(s):	Mohammad Javad Mollakazemi, Department of Biomedical Engineering,U of Kentucky Dibyajyoti Biswal, Department of Biomedical Engineering,U of Kentucky Abhijit Patwardhan, Department of Biomedical Engineering,U of Kentucky

Abstract: It is over 150 years ago that the intimate interaction between the heart and the brain was realized by Claude Bernard, and of all the organs in the human body, the heart is among the ones that have the most extensive neural connection with the brain. In this study, we used cardiacsynchronized EEG segments to investigate the effects of tempo and cognition induced by auditory stimuli. ECG and EEG were recorded from 14 subjects when they were listening to music and during silence (control). The 300-millisecond EEG segments ending at R-peaks of ECG were extracted. The frequency bands of Delta, Theta, Alpha, Beta, Gama and Gamma2 within EEGs were also analyzed to determine which bands were more sensitive to the induced changes. The eigenvalue analysis of covariance matrix of synchronized EEG showed that Alpha band in EEG from parietal zone was the most sensitive band among all other frequency bands to auditory stimuli. The cognition of song had much higher impact on Gamma and Gamma2 bands of EEGs from right hemisphere, which corresponds to music awareness, than tempo of song even though tempo is considered as the most impactful acoustic structural feature of music on physiological variables. The higher impact of local phase randomized version of known song than local phase randomized of unknown song on these bands also verifies the stronger impact of cognition relative to tempo.

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Poster Presentation 44

Abstract Title: **Fibroblast Growth Factor 19 Alters Excitability in the Dorsal Motor Nucleus of the Vagus**

Author(s): Wean, J.B., Smith, B.N.

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 candidate region mediating the observed antidiabetic effects. Here, patch-clamp electrophysiology was used to measure the effects of FGF19 on intrinsic excitability and synaptic currents in vagal motor (i.e., DMV) neurons in brainstem slices. Application of FGF19 (230 pM) either increased (30%), decreased (50%) or caused no change in action potential firing in DMV neurons. Additionally, FGF19 was found to decrease the amplitude of voltage-gated potassium currents in the DMV. The frequency of spontaneous synaptic currents was also altered. 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 45

Abstract Title:	**The Effects of Adolescent Binge Drinking on Astrocyte Maturation and Synaptic Colocalization**
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Abstract: Binge drinking is highly prevalent among today's youth and is associated with increased risk of alcohol dependency later in life. While progress has been made in understanding the consequences of binge drinking on neuronal and subsequent cognitive function, little is known about the role of glial cells, which ensheath synapses and are critical in synapse formation, maturation, and transmission. Here we investigate astrocyte morphology and colocalization with synapses across development and the influence of adolescent binge-ethanol. Male Sprague Dawley rats received intracranial astrocyte-specific adeno-associated virus directly into the medial prefrontal cortex (mPFC) and dorsal hippocampus (dHipp). Brains were collected at PNDs 24, 30, 45-48, and 70 and immunohistochemistry was performed using post-synaptic marker, PSD95. Imaging was performed using confocal microscopy and 3D reconstructions were rendered using IMARIS (bitplane). To investigate the effects of adolescent ethanol exposure, identical procedures were performed on animals that received intermittent ethanol exposure (5g/kg, i.g.) 10 times over 16 days beginning PND 30. Tissue was processed 24 hours after the 10th dose (PND 46) and 26 days later (PND 70). Results show a significant post-adolescent increase in mPFC astrocyte volume and increased colocalization with synapses. Following intermittent ethanol exposure there was a substantial decrease in the colocalization of HIPP astrocytes and synapses following the 10th dose despite no change in astrocyte volume. In adulthood, following ethanol exposure, there was a significant decrease in HIPP astrocyte volume and a decrease in colocalization of astrocytes and synapses when compared to the age-matched controls.

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Poster Presentation 46

Abstract Title:	**Apolipoprotein E Modulates Respiratory Motor Plasticity Following Cervical Spinal Cord Injury**
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Abstract: Each year, 17,700 Americans suffer a spinal cord injury, most of which occur at the cervical level. These injuries can interrupt neural pathways controlling breathing. One approach to promote breathing recovery is by enhancing plasticity in the spinal cord. Activating the latent crossed phrenic pathway can lead to motor plasticity known as long term facilitation (LTF), causing a recovery of breathing motor output. LTF can be induced through intermittent hypoxia (IH) or intermittent serotonin dosing. While a portion of the SCI population responds to IH with an increase in respiratory output, others remain non-responders. This inconsistency suggests that variability in the human population may influence how individuals respond to treatments that aim to enhance plasticity. Therefore, we propose that genetic diversity could be a key factor in determining an individual's propensity for plasticity. Apolipoprotein E (apoE) is a promising candidate gene that could be responsible for this variability. One apoE allele, E4, has previously been shown to reduce synaptic plasticity by decreasing expression of glutamate receptors when compared to the E2 or E3 alleles. This study investigates impacts of human apoE4 on respiratory motor plasticity following SCI. Serotonin dosing induces an increase in diaphragmatic activity in ApoE3-dosed rats, but not in those dosed with E4. Analysis of tissue treated with human apoE protein indicated that apoE also modulates synaptic expression of glutamate receptors, a crucial component of LTF induction. These experiments demonstrate ApoE4's potential to inhibit plasticity following SCI, emphasizing the importance of considering genetic diversity when developing therapeutic strategies.

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Poster Presentation 47

Abstract Title:	**Myelin Modulates Macrophage Inflammatory Responses After Spinal Cord Injury**
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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 persists long after 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 macrophages 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. Next, we hypothesize that myelin-mediated pro-inflammatory potentiation is a result increased activation of the enzyme cytosolic phospholipase A2 (cPLA2) within macrophages. This enzyme can modify lipids present in myelin into pro-inflammatory stimuli. Indeed, inhibition of cPLA2 blocked much of myelin's pathogenic effects. Further, 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 28 days post injury indicating a role for cPLA2 in chronic SCI inflammation. Ongoing studies aim to genetically target macrophage cPLA2 within macrophages after SCI to determine any therapeutic effects, and thereby identify novel therapeutic targets after SCI.

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Poster Presentation 48

Abstract Title:	**Human Apolipoprotein E Isoforms Influence Neurite Outgrowth and Regeneration In Vitro and In Vivo**
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Abstract: Translating spinal cord injury (SCI) therapies from preclinical animal models into the human population is challenging. One potential explanation is that human genetic predispositions may limit the efficacy of treatments which enhance regeneration and sprouting. The ApoE4 (E4) allele, present in about 14% of the human population, corresponds to an increased incidence of Alzheimer's disease. Its role in recovery from SCI is poorly understood despite suggestive data implicating its involvement. Two clinical studies found that SCI individuals with the E4 allele had less motor recovery than individuals without the allele. We hypothesize that ApoE4 can impair sprouting limiting recovery. To test this hypothesis, we investigated the impact of ApoE4 on sprouting and neurite outgrowth. In our experiments, we cultured dorsal root ganglia neurons from mice expressing the human ApoE isoforms—ApoE2 (E2), ApoE3 (E3), or ApoE4—under the control of the mouse ApoE promoter. We analyzed differences in 1) neurite complexity and 2) robustness of outgrowth between genotypes. Our results demonstrate that E3 neurons have more robust outgrowth than E4 neurons, as indicated by a higher total combined neurite length. Analysis of neurite branching indicates that E3 neurons also have higher neurite complexity than neurons expressing ApoE4. Since outgrowth and sprouting partially mediate recovery after CNS injury, impairments in this process can adversely affect recovery. These foundational studies address the possible genetic influence of ApoE4 on recovery from CNS injury, and whether there is a genetic contribution underlying responses to treatment in SCI individuals.

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Poster Presentation 49

Abstract Title:	**Single Cell Analysis of Periocular Mesenchyme During Anterior Segment Development**
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Abstract: Development of the anterior segment (AS) of the eye largely depends on a group of neural crest cells, named periocular mesenchyme (POM). Thus far, only the physiological role of these cells during maldevelopment of AS has been investigated. An understanding about details of their molecular function is largely missing. As such, clinicians lack the opportunity to molecularly screen and treat several diseases associated with the AS, including AS dysgenesis (ASD) and glaucoma. In this study, we used Foxc1b, a known AS determinant, to isolate and characterize POM cell development. A transgenic line of zebrafish, Tg[foxc1b:GFP] was used to isolate AS associated Foxc1b+ cells and subsequently generate single cell transcriptomes using 10X genomics technology. The great advantage of this technology is that reads from individual cells are tagged with a barcode, allowing to analyze gene expression at a cellular level. By comparing data from different developmental time points, we aim to decipher the POM specification program. Our preliminary data show that there are at least four distinct groups of cells marked by Foxc1b at early stages of AS development. This study provides new insights and novel targets for analysis in how and when the AS forms, while providing new candidate genes for analysis of AS development as well as association with various ASD disorders. This new-found knowledge will be crucial for diagnosis and treatment of ocular diseases such as ASD and glaucoma.

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Poster Presentation 50

Abstract Title:	**MANF Protects Purkinje Cells from Alcohol Induced Neurodegeneration**
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Abstract: Purpose: Ethanol exposure can lead to significant neurodegeneration in the developing brain due to elevated endoplasmic reticulum (ER)-stress. Mesencephalic astrocyte-derived neurotrophic factor (MANF) is an ER-stress inducible protein expressed in many cell types including neurons. Evidence has shown that MANF can alleviate ER-stress induced cell damage. We hypothesize that MANF may act to maintain ER homeostasis in response to ethanol exposure and deficiency of MANF renders neurons more susceptible to ethanol-induced neurodegeneration. Methods: To test this hypothesis, Purkinje cell specific MANF knockout mice were generated using Cre/Lox recombination. Mice were intubated (gavage) with 5 g/kg/day ethanol or equal volume of H2O once a day for ten days. Locomotor behaviors including open field, rotarods, and balanced beam were tested. The number and morphology of Purkinje cells were examined. Apoptosis was detected by immunohistochemistry of cleaved-caspase 3 and TUNEL assay. ER-stress markers were detected by immunohistochemistry. Results: MANF deficient mice exhibit deficits in the locomotor behaviors after alcohol exposure. Their Purkinje cells showed reduced numbers, shrank cell body, missing dendrite, and increased apoptosis. ER-stress markers including GRP78, ATF6, p-eIF2α and CHOP were upregulated. Conclusions: MANF deficient Purkinje cells are more susceptible to ethanol-induced neurodegeneration, possibly due to elevated ER-stress, suggesting the neurotrophic role of MANF in protecting neurons from ethanol-induced neurodegeneration is partially through the alleviation of ER-stress.

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Poster Presentation 51

Abstract Title:	**Temporal response of mitochondria enriched microRNAs and inflammatory marker genes following traumatic brain injury**
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Abstract: Traumatic brain injury (TBI) is a major public health burden around the world. Secondary brain injury arises hours to days after the primary insult and leads to further damage due to a cascade of biochemical events, including excitotoxicity, inflammation, oxidative stress, apoptosis, and compromised mitochondria function. These secondary biochemical and pathophysiological events occur at different time points following the initial injury and the outcome could significantly impact the fate of neuronal tissue repair or further deterioration. MicroRNAs (miRNAs) regulate widespread biochemical and molecular events and are associated with secondary brain injury events. We previously reported a dynamic alteration of hippocampal mitochondria-associated, inflammatory related miRNAs following a controlled cortical impact (CCI) injury in rat. Here we report the expression of a subset of inflammatory miRNAs in hippocampal mitochondria and cytosol at 24-, 72-hours and 7 days following CCI, and their impact on the overall expression of pro- and anti-inflammatory macrophage/microglia marker genes. While mitochondria-enriched miR-142-3p, miR-142-5p, and miR-146a display a compartmental shift in abundance from mitochondria to cytosol, the association of other inflammation related miRNA including miR-155 and miR-223 within the mitochondrial fraction was elevated. TaqMan Low Density Array analysis of macrophage/microglia phenotypic gene expression suggested a high activity of these genes, including validated miR-146a targets, up to 24 hours and subsequent reduction after 72 hours post TBI. We further demonstrate a nanoparticle+miRNA delivery strategy that enabled miRNA targeting and pro-inflammatory gene expression in vivo. The manipulation of miRNA may provide a strategy in the intervention of TBI pathogenesis.

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Poster Presentation 52

Abstract Title:	**Hypothalamic oxytocin release induced by DREADDs blocks peer-induced cocaine seeking**
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Abstract: Purpose: Re-association with drug using peers is a common trigger for relapse. Our laboratory has recently developed a rodent model of peer-induced drug seeking. We have shown previously that systemic administration of the neurohormone oxytocin reduces peer-induced cocaine seeking, but the mechanism for this effect is unclear. In the current study we tested the effects of activation of oxytocin neurons in the paraventricular nucleus (PVN) of the hypothalamus on peer-induced cocaine seeking using an oxytocin-promotor targeting DREADD that activates oxytocin neurons when a pretreatment of clozapine is given. Methods: Rats underwent surgery for implantation of a jugular catheter and microinfusion of an adeno-associated virus that targeted the oxytocin promoter and inserted copies of either an excitatory DREADD (hM3Dq-mCherry) or mCherry (a fluorescent protein) alone. Rats then underwent 30 days of twice-daily self-administration training. Each day, one session was with cocaine (1.0 mg/kg/infusion) and a cocaine-associated (S+) peer and the other session was with saline and a saline-associated (S-) peer. After extinction, rats were tested for peer-induced reinstatement (S+, S-, or none) following a pretreatment (1.0 mg/kg clozapine or vehicle). Results: The virus did not affect acquisition or extinction of cocaine self-administration. Rats receiving the activation virus reinstated to the S+ peer following vehicle, but clozapine blocked this effect. In contrast, for rats with the control virus, clozapine had no effect on peer-induced reinstatement. Conclusion: Oxytocin activation within the PVN of the hypothalamus blocks peer-induced reinstatement. Modulating this system may be a valuable target for future drug discovery projects.

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Poster Presentation 53

Abstract Title:	**The Effects of Adolescent Binge-Like Alcohol Exposure on Adult Alcohol and Nicotine Co-Use in Sprague Dawley Rats**
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Abstract: Introduction: Alcohol and tobacco use disorders are highly comorbid, and the earlier one is exposed to either substance, the more likely they are to develop an addictive disorder. To explore the relationship between adolescent exposure to alcohol and poly-substance abuse in adulthood, we utilized a model for ethanol (EtOH) and nicotine co-use in Sprague Dawley rats following adolescent EtOH exposure. We hypothesized that exposure to EtOH during adolescence would lead to higher levels of EtOH and nicotine consumption in adulthood. Methods: Phase 1 Adolescent EtOH Binge: Adolescent male and female Sprague Dawley rats (n=18) were gavaged orally with either an ethanol or control diet, every 8 hours for 48 hours. Phase 2 Young Adulthood EtOH Only: 2-bottle choice between water and an EtOH solution, in hour-long daily sessions occurring in modified operant chambers. Phase 3 Adulthood Nicotine + EtOH: Nicotine self-administration under an increasing fixed-ratio (FR) schedule; nicotine infusions (0.03 mg/kg/inf) and a paired stimulus light maintained responding; 2 bottles containing H2O or 0.2% saccharin/15% EtOH (w/v/v) were available. Results: Contrary to our hypothesis, results from phase 2 suggest male rats exposed to EtOH in adolescence consume less alcohol in adulthood compared to females and controls $F(1,13)=7.172$, $p=0.019$. Preliminary results from phase 3 suggest that male rats who received EtOH during adolescence consume more alcohol and less nicotine as the price (higher FR values) of nicotine increases. Conclusions: Completion of phase 3 and a larger sample size are needed to draw firm conclusions about the outcomes of these studies.

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Poster Presentation 54

Abstract Title:	**Anti-inflammatory treatment restores memory deficits in a mixed dementia model of Alzheimer's disease with comorbid vascular pathology**
Author(s):	D. J. Braun, Sanders-Brown Center on Aging, U of Kentucky D. M. Wilcock, Sanders-Brown Center on Aging, U of Kentucky L. J. Van Eldik, Sanders-Brown Center on Aging, U of Kentucky

Abstract: A concern with amyloid-specific therapies is that they do not address many of the wider pathologies present in patients, thereby limiting their effectiveness in a large proportion of patients. One pathway linking amyloid pathology, vascular damage, and cognitive dysfunction is neuroinflammation. The current project aims to determine whether pharmacological inhibition of pro-inflammatory signaling can rescue pathology in a mouse model of mixed dementia (MD). To generate our MD model, we used a transient dietary hyperhomocysteinemia (HHcy) model to induce vascular dysfunction in the APPswe/PS1dE9 transgenic mouse amyloid overexpression model of Alzheimer's disease (AD). AD mice (and wildtype littermates) were placed on vitamin B-deficient and methionine supplemented HHcy diet for 8 weeks beginning at around 7.5 months of age, after the beginning of plaque deposition. Mice were then recovered on normal chow for 2 weeks, before beginning two weeks of treatment with our novel, brain-penetrant anti-inflammatory MW151 (5 mg/kg, I.P., daily). In the final week of treatment, mice underwent a battery of behavioral testing prior to euthanasia. MW151 successfully reduced several pro-inflammatory mediators, reduced astrogliosis, and ameliorated spatial learning and memory deficits in the radial arm water maze. No effect was seen on short-term hippocampal memory, hyperlocomotion, or intrinsic behaviors. Anti-inflammatory treatments may therefore be useful in patients with comorbid AD and vascular pathologies. This work was supported by postdoctoral fellowships from the Weston Brain Institute and the National Institute on Aging (F32AG058456).

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Poster Presentation 55

Abstract Title:	**Regulation of Mitochondrial Membrane Potential Changes from a Role of Recovery to Pathology with Increased Age at Time of Spinal Cord Injury**
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Abstract: Aging exacerbates spinal cord injury (SCI) through increased reactive oxygen species (ROS) damage, however the contribution of mitochondrial-derived ROS remains unknown. This work investigates if dysregulation of mitochondrial membrane potential (DYm) with age effects ROS production after SCI. Graded doses of 2,4-dinitrophenol (DNP; mitochondrial uncoupler) were delivered for 1-week after SCI in young- (4-month-old; 4-MO) and middle-aged (14-MO) mice. Tissue collected at 7-days post-injury (DPI) suggested that 1-mg/kg/day of DNP exerts therapeutic benefits to 14-MO SCI-mice, but toxic effects to 4-MO SCI-mice. Specifically, 14-MO SCI-mice treated with DNP trended towards improved myelin preservation, reduced inflammation, and lower 3-nitrotyrosine (3-NT; protein nitration product) accumulation. In contrast, 4-MO SCI-mice treated with DNP trended towards worse myelin preservation, increased inflammation, with no effect on 3-NT accumulation. Similarly, treating SCI-mice with DNP improved motor functions of 14-MO mice but worsened motor abilities of 4-MO mice during 28-days of weekly functional monitoring. Next, macrophages were isolated from spinal cords at 6-DPI and analyzed using Seahorse for mitochondrial function. Spared respiratory capacity and maximal respiration were reduced with age, which paralleled effects derived from pro-inflammatory stimulation (LPS and INFg) of macrophages in vitro. Collectively, this data demonstrates that age dysregulates mitochondrial function, which effects SCI injury and repair. Because respiration is collapsed in an age-dependent manner in activated macrophages after SCI, as well as mild-uncoupling exerting opposite effects on SCI pathophysiology with age, we can conclude that increased age changes the regulation of DYm from a role of recovery to a role of pathology after SCI.

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Poster Presentation 56

Abstract Title:	**Ceruloplasmin in aging and Alzheimer's disease neurovascular units**
Author(s):	K. Hargis-Staggs* E. S. Johnson* E. M. Blalock

Abstract: Aging and being female are key unmodifiable risk factors for sporadic Alzheimer's disease (sAD), yet the mechanisms through which either risk factor communicates its increased risk are not clear. Searching across sAD brain transcriptional profiles, we identified a set of robust transcriptional changes that were not only consistent across different labs and measurement platforms, but also showed exacerbated signal in female vs male sAD patients. Ceruloplasmin was found to be among the strongest of these gene expression changes. CP has two splice variants, secretory (sCP) and membrane-bound (mCP). Although sCP is well-characterized, mCP is not, although it is strongly expressed in neurovascular units (NVUs). Prior data suggested that mCP may be more strongly expressed in white matter than gray matter. In preliminary data, we used laser capture microdissection to show that sCP is indeed robustly enriched in white matter. Presently, we are assessing RNA quality in post mortem brain specimens to identify the strongest candidates for laser capture microdissection and analysis of CP expression in gray and white matter NVUs.

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Poster Presentation 57

Abstract Title:	**Novel NFAT inhibitor Q134R protects synaptic deficits in a mouse model of Alzheimer's disease.**
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Abstract: Calcineurin (CN) and its substrate, Nuclear Factor of Activated T cells (NFAT), are active at early stage of Alzheimer's disease (AD). We previously reported that CN and NFAT4 are activated in reactive astrocytes from AD mouse model. Blocking of CN/NFAT activation by genetic or pharmacologic approaches exhibit anti-inflammatory, anti-amyloid, neuroprotection and/or nootropic properties in AD models. Here, we characterize the effects of Q134R, a novel small chemical compound which had been developed and tested for human use by Avidin Biotechnology, on NFAT signaling pathway. Similar to CN inhibitor-cyclosporine, Q134R suppressed IL-1β- or oligomycin-induced NFAT activation in primary astrocyte and neuron cultures. To test the effect as acute treatment in intact animal, 15 months old APP/PS1 mice were orally administered with the compound for two weeks (4mg/kg and 12mg/kg, twice daily). Nuclear levels of NFAT4 were reduced in the astrocytes from Q134R treated animals. To investigate the beneficial effects of the compound in long-term treatment, WT and APP/PS1 mice were on three months administration program (4mg/kg, twice daily) started from 6 months of age. Compared to vehicle control, Q134R strikingly increased CA3-CA1 synaptic strength and long-term potentiation (LTP) in acute brain slices from APP/PS1 mice. In fact, synaptic indices in WT and Q134R-treated APP/PS1 mice were qualitatively and quantitatively similar. The results demonstrate that Q134R inhibits hyperactive NFAT signaling en route to protecting synaptic function during the progression of AD-like pathology. The findings offer important proof-of-concept support for the use of small chemical NFAT inhibitors, like Q134R, in the treatment of AD and related neurodegenerative disorders.

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Poster Presentation 58

Abstract Title:	**Calpain-5 Membrane Association Is Mediated by the C2 Domain and Cysteine Palmitoylation**
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Abstract: Calpains are calcium-activated thiol proteases. Abnormally high levels of calcium were reported in pathological conditions including traumatic brain injury and neurodegenerative diseases. The pathologically high calcium level hyperactivates Calpains, resulting in the misregulation of downstream processes. Calpain-5 is one of the most highly expressed Calpains in CNS, yet very little is known about its function and regulation. Several classical Calpain substrates are membrane-associated proteins. Calpain-5 associates with the plasma membrane, but the mechanism of this association has not been deciphered. Calpain-5 has a predicted C2 domain, a lipid interaction module. We found that the C2 domain of Calpain-5 was indeed functional and mediated Calpain-5 membrane association. Employing a novel mass tagging approach, we also demonstrated that Calpain-5 was palmitoylated at multiple cysteine residues. Palmitoyl groups are highly hydrophobic, mediating membrane association of proteins. Whereas an intact C2 domain was essential for the palmitoylation of Calpain-5, the catalytic activity of the enzyme was not. Our results suggest that the initial association of Calpain-5 with the membrane is mediated by the C2 domain, followed by multiple palmitoylation events, conferring firm membrane association.

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Poster Presentation 59

Abstract Title:	**Multiphoton imaging reveals that a microglial response to a microinjury is elevated in hippocampal white matter and inhibited by glucocorticoid**
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Abstract: Quantifying whether microglial show an 'aggressive', a 'normal', or a 'quiescent' response to brain damage is an area of some debate because microglial responses are highly dependent on their environment. Dynamic responses can be measured in vitro, but the in vitro preparation itself alters microglial behavior. Post-mortem measures capture the native environment, but lose dynamic responses. Finally, damage magnitude matters. Many experimental models involve large injuries, while many conditions (e.g., microbleeds) induce microscopic changes. Here, we addressed some of these issues by developing a 'microglial response to microinjury' (MRMI) assay. This method images living microglia in an ex vivo environment that closely matches microglial native environment, induces a microscopic injury, and quantifies response. CX3cr1-GFP mouse (produce green fluorescent protein in microglia/ macrophages) hippocampal slices are imaged and a small 30x20 um cylindrical avulsion (microinjury) is created. The resulting MRMI is rapid (5-20 min), elevated in white matter, and inhibited by glucocorticoid, consistent with prior reports. Thus, this method appears appropriate for not only assessing the microglial response to microinjury in the context of various disease states, but also in the context of dissecting out the pharmacology of its facilitation or inhibition by different agents.

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Poster Presentation 60

Abstract Title:	**Effect of Intranasal Insulin on Somatosensory Cerebral Blood Flow in Young and Aged F344 Rats**
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Abstract: Multiphoton calcium imaging techniques are quickly becoming favored approaches for investigations into cellular mechanisms of neurodegeneration as seen in aging and Alzheimer's disease. It is also well documented that intranasal insulin improves age-related cognitive deficits and can reverse calcium electrophysiological correlates of brain aging. Previous work from our lab has also shown that intranasal insulin can increase CBF in the aged animals, providing a novel mechanism for the improved memory function. Whether alteration in cerebral blood flow and calcium activity in neuron are interdependent processes that coexist in the brain in response to intranasal insulin in vivo still remains uncertain. We delivered GCaMP6 AAV to the somatosensory cortex of young aged rat and characterized cerebral blood flow using acute cranial windows and retroorbital delivery of rhodamine dextran. Two-photon microscopy was used to perform line scans and frame scans of the regions of interest across different vascular beds and neuronal layers (depth). Somatosensory cortex activation caused significant changes in vessel diameter that was also sensitive to intranasal insulin more in the younger compared to the older animals. Two types of line-scan images were captured: 1) along the blood flow and 2) cross the vessel. Radon transform routines on segmented images were used to derive measures of velocity and blood flow. As previously reported, in medium-sized vessels (~40 um), an age-dependent reduction in blood flow was noted at baseline. Here, we also describe methods used to extract and interpreted multiphoton imaging data based on calcium and blood flow measures using MATLAB.

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Poster Presentation 61

Abstract Title:	**Theophylline Improves Survival Following Cervical Contusion Injury in Rats**
Author(s):	C.M.Calulot, Spinal Cord and Brain Injury Research Center, Department of Neuroscience, U of Kentucky W.J.Alilain, Spinal Cord and Brain Injury Research Center, Department of Neuroscience, U of Kentucky

Abstract: Cervical spinal cord contusion injuries are the most common form of spinal cord injury (>50%) observed in humans. These injuries can result in the impaired ability to breathe. Previous research in this lab has demonstrated that administration of a 15mg/kg dose of theophylline, an approved for human use respiratory stimulant, 3 weeks after 150 kD C3/4 unilateral contusion can increase utilization of injured respiratory pathways. In the immediate stages after injury, these animals typically require exogenous ventilation to survive. In this study, we examine the effectiveness of theophylline in increasing survivability following that initial contusion injury without ventilation assistance. Subjects received a 15 mg/kg dose of theophylline 30 minutes prior to a 150 kD contusion injury or at the time of injury. Control treated animals received saline vehicle. Our preliminary results indicate that subjects that received theophylline, either prior to or at injury, were able to resume a normal respiratory rate after 10 minutes of recovery. In comparison, saline treated subjects struggled to self-ventilate and expired. Through these experiments, we demonstrate that administration of theophylline is effective in increasing survivability in the immediate and critical stages following cervical spinal cord injury.

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Poster Presentation 62

Abstract Title:	**The Effects of a Bacterial Endotoxin (LPS) on Behavior and Sensory-CNS-Motor Circuits**
Author(s):	O. Istas, Department of Biology, U of Kentucky A. Greenhalgh, Department of Biology, U of Kentucky R. L. Cooper, Department of Biology, U of Kentucky

Abstract: The effect of bacterial sepsis on animal behavior and physiology of the nervous system is complex due to direct and indirect actions of the infectious agents. The most common form of bacterial sepsis in humans is due to gram-negative bacterial strains. The endotoxin (lipopolysaccharide, LPS) secreted from the bacteria is the key agent to induce an immune response, which then produces a cascade of immunological consequences. However, there are direct actions of LPS on cells which are commonly overlooked. This study showed behavioral and neural changes in larval Drosophila fed LPS for 48 hours from Serratia marcescens. Locomotor behavior was not altered, but feeding behavior increased and responses to sensory tactical stimuli were decreased. In driving a sensory-CNS-motor neural circuit in in-situ preparations, direct application of LPS initially increased evoked activity and then decreased and even stopped evoked responses in a dose-dependent manner. With acute LPS exposure (10 minutes) the depressed neural responses recovered within a few minutes after removal of LPS. LPS induces a transitory hyperpolarization of the body wall muscles within seconds of exposure and alters activity within the CNS circuit. Thus, LPS itself has direct effects on tissues without a secondary immune response.

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Poster Presentation 63

Abstract Title:	**Effects of TEA and 4-AP on firing frequency of proprioceptive neurons in crustaceans**
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Abstract: Ion channel pathologies can lead to severe neurological problems because these channels are required for normal electrical function and conduction in neurons. Repolarization of the membrane to resting state in neuronal conduction is typically dependent on potassium channels. These channels can be blocked by drugs such as 4-AP and TEA, though different K+ channels may show differing sensitivities to these drugs. Examining proprioceptive neurons in model animals, while blocking K+ channels which are 4-AP and/or TEA sensitive, can help reveal the contribution of these channel types in the physiological functions related to proprioception. The actions of varying concentrations of 4-AP and TEA independently, as well as combined, were explored in the blue crab propodite-dactylopodite (PD) chordotonal organ. The PD organ monitors joint position in relation to rate of movement and static position. Extracellular recordings were collected and analyzed for changes in firing pattern observed with the application of these drugs. Based on previous research, an alteration of activity in an intact sensory unit was expected to occur with the application of these drugs; the extent to which this occurs was investigated. If sensory neurons are not able to repolarize rapidly, it is expected that the rate of firing will decrease. Blockage of K+ channels may lead to cells remaining depolarized, potentially leading to voltage gated Na+ channels staying inactivated, completely silencing electrical activity in the affected neurons.

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Poster Presentation 64

Abstract Title:	**The Effects of Bacterial Endotoxin LPS on Synaptic Transmission at the Neuromuscular Junction of Larval Drosophila**
Author(s):	M. C. McNabb, Department of Biology, U of Kentucky R. L. Cooper, Department of Biology, U of Kentucky

Abstract: Gram-negative bacterial septicemia is a common cause of death in many animals, including humans. Serratia marcescens and Pseudomonas aeruginosa are two gram-negative bacterial strains commonly found in human cases of septicemia. Gram-negative bacteria contain high levels of lipopolysaccharide (LPS) endotoxin in their outer membrane, which induces an immune response in animals. The direct effects induced by LPS, independent of an immune response, have often been overlooked in endotoxin studies. The mechanisms in glutamatergic synapses at Drosophila neuromuscular junctions (NMJs) have been well-documented and serve as an effective model for the direct action of LPS on presynaptic motor neurons. Studies with LPS exposure was shown to enhance synaptic transmission and hyperpolarize the membrane potential at low doses but block glutamatergic receptors and decrease observable spontaneous events at a high dosage. The dosage effects are LPS type specific. The hyperpolarization is not due to voltage-gated potassium channels or to activation of nitric oxide synthase (NOS).

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Poster Presentation 65

Abstract Title: **Is avian incubation reinforced by opioid activation?**

Author(s): Melissa Burns-Cusato, Arpit Rana, Will Hawkins, and Josh Rieskamp

Abstract: Contact comfort is a type of reward that stems from physical contact with another organism (Machin & Dunbar, 2011). In mammals and birds, contact comfort results in release of endogenous opioids and an associated feeling of euphoria and contentment (Nelson & Panksepp, 1998). In mammals, contact comfort can also arise from stimuli associated with nests (Machin & Dunbar, 2011). However, it is unknown if aspects of avian nests (e.g. warm eggs) can also elicit opioid-mediated contact comfort. If so, the pleasurable effects of contact comfort may explain why birds return to the nest for their daily bout of nest sitting. We injected incubating male ring neck doves (Streptopelia risoria) with the opioid receptor antagonist (naloxone) or saline. Time spent off the nest following each injection was measured. Doves injected with naloxone spent significantly more time off the nest than doves injected with saline. In the second experiment, we used a condition-placed preference paradigm to determine whether naloxone creates an aversive internal state. Naloxone was paired with one visually distinct context and saline with another on alternating days. On test days, the door between training contexts was removed so subjects could move freely throughout apparatus. Subjects did not show a conditioned aversion to the context that had been paired with naloxone. These results suggest that the disruption in incubation seen in the first experiment was not due to naloxone creating a physical discomfort. Taken together, the results from both experiments provide support for the opioid hypothesis for avian incubation.

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Poster Presentation 66

Abstract Title:	**Pharmacological Identification of Cholinergic Receptor Subtypes: Modulation of Locomotive and Feeding Behavior and Neural Circuit Excitability in Drosophila Larvae**
Author(s):	Eashwar Somasundaram, Department of Biology, University of Kentucky Cole Malloy, Molecular Neurophysiology and Biophysics, NIH, Bethesda, MD Aya Omar, Veterinary Medicine, North Carolina State University, Raleigh, NC Umair Bhutto, Department of Biology, University of Kentucky Robin Cooper, Department of Biology, University of Kentucky

Abstract: Acetylcholine (ACh) is a neurotransmitter and neuromodulator in many species. In Drosophila melanogaster ACh is the neurotransmitter used in peripheral sensory neurons and is a primary excitatory neurotransmitter and neuromodulator within the central nervous system (CNS). Cholinergic receptors can be further subdivided into the nicotinic acetylcholine receptors (nAChRs) and the muscarinic acetylcholine receptors (mAChRs). This receptor classification is shared in mammals and insects; however, the pharmacological and functional characterization of these receptors for Drosophila has lagged behind its mammalian model counterparts. We used a behavioral and electrophysiological approach to assess cholinergic modulation of locomotion, feeding, and sensory-CNS-motor (sensorimotor) circuit excitability to identify the impact of ACh receptor subtypes in regulating the performance of select neural circuits within the larval CNS. We exposed intact and semi-intact 3rd instar larvae to ACh receptor agonists and antagonists to observe their roles in behavior and regulation of neural circuit excitability and to investigate AChR pharmacological properties in vivo. We combined this with AChR RNAi-mediated knockdown to identify specific receptor subtypes facilitating ACh modulation of circuit function. We reveal that chronic ACh exposure enhances locomotion but reduces mouth hook extensions and acute application excites the sensorimotor circuit. Nicotine reduces the efficacy of each behavior upon acute and long-term exposure and inhibits sensorimotor circuit activity at high doses. Moreover, chronic muscarine exposure reduces locomotion and feeding, but acute exposure enhances sensorimotor circuit excitability. These results suggest a role for both nAChRs and mAChRs in modulating these circuits and illuminates important pharmacological properties of cholinergic receptor subtypes in vivo.

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Poster Presentation 67

Abstract Title:	**Pharmacological and Genetic Identification of Cholinergic Receptor Subtypes in Modulation of Neural Circuits in Drosophila Melanogaster**
Author(s):	U.F. Bhutto, Department of Biology, University of Kentucky E.V. Somasundaram, Depart of Biology, University of Kentucky C.A. Malloy, Molecular Neurophysiology and biophysics, NIH, Bethesda, MD A. Omar, Veterinary Medicine, North Carolina State University, Raleigh, NC R.L. Cooper, Department of Biology, University of Kentucky

Abstract: Acetylcholine (ACh) is an abundant neurotransmitter and neuromodulator in many species. In Drosophila melanogaster ACh is the neurotransmitter used in peripheral sensory neurons and is a primary excitatory neurotransmitter and neuromodulator within the central nervous system (CNS). The receptors that facilitate cholinergic transmission are divided into two broad subtypes: the ionotropic nicotinic acetylcholine receptors (nAChRs) and the metabotropic muscarinic acetylcholine receptors (mAChRs). This receptor classification is shared in both mammals and insects; however, both the pharmacological and functional characterization of these receptors within the Drosophila nervous system has lagged behind its mammalian model counterparts. In order to identify the impact of ACh receptor subtypes in regulating the performance of neural circuits within the larval CNS, we used a behavioral and electrophysiological approach to assess cholinergic modulation of locomotion sensory-CNS-motor circuit excitability. We exposed intact and semi-intact 3rd instar larvae to ACh receptor agonists and antagonists to observe their roles in behavior and regulation of neural circuit excitability and to investigate AChR pharmacological properties in vivo. We combined this with targeted AChR RNAi-mediated knockdown to identify specific receptor subtypes facilitating ACh modulation of circuit efficacy. We identify a contribution by both mAChRs and nAChRs in regulation of locomotive speed and reveal that they play a role in modulation of the excitability of a sensory-CNS-motor circuit. We further reveal a conspicuous role for mAChR-A and mAChR-C in motor neurons, directly, in modulation of their input-output efficacy in response to evoked sensory-CNS input, which is also manifested in alterations in locomotive speed.

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Poster Presentation 68

Abstract Title:	**Can Dietary Supplements (βeta-hydroxybutyrate) Improve Cognitive Performance?**
Author(s):	J. Kizhakkadathu, Department of Kinesiology and Health Promotion, University of Kentucky D. Y. Han, PsyD, Departments of Neurology, Neurosurgery, Physical Medicine & Rehabilitation, and Spinal Cord and Brain Injury Research Center, University of Kentucky A. C. Glueck, PhD, Department of Neurology, University of Kentucky

Abstract: βeta-hydroxybutyrate (BHB) is a ketone body produced by the liver in a process known as ketogensis. During periods of fasting or carbohydrate caloric restriction, ketones are used as an alternative fuel throughout the body, including in the brain. Although glucose is the brain's principal energy source, when limited, ketones derived from fats become the major energy source. Exogenous BHB is safe to administer orally, and enhances energy and physical performance. While growing evidence from basic science indicates significant cognitive improvement in animal models following ketone elevation, and in clinical human samples such as in Alzheimer's disease and severe traumatic brain injury, there is limited literature demonstrating beneficial neurocognitive effects of exogenous administration of ketones in non-clinical and mild traumatic brain injury (mTBI) samples. As a proof of concept pilot, we present twelve non-clinical participants and a clinical mTBI patient who underwent a single exogenous administration of 11.7g of BHB. After ingestion, non-clinical participants performed significantly better in attentional accuracy compared to pre-intervention scores ($p < 0.05$; $d = 0.65$). The clinical subject demonstrated improvements in visuo-motor reaction time, learning acquisition, attention, spatial memory, and a reduction in headache severity. The results of this pilot study suggest exogenous administration of BHB may have positive effects on attention in nonclinical participants, and poses the possibly of global neurocognitive improvement post-mTBI. More research is needed to further explore the potential benefits of exogenous BHB administration in nonclinical participants and clinical mTBI patients. Accordingly, continued subject recruitment is underway.

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Poster Presentation 69

Abstract Title:	**The role of peptidoglycans receptors in the response to bacterial endotoxin LPS on body wall muscle and synaptic transmission in larval Drosophila**
Author(s):	Carly Ballinger-Boone, Doug Harrison, Robin L. Cooper Department of Biology, University of Kentucky

Abstract: Gram-negative bacteria produce and release endotoxins in the form of lipopolysaccharides (LPS). The different forms of LPS produce varying secondary immune responses. The direct effect of LPS itself, which occurs in seconds, has not been well studied; however, the receptors which bind LPS were first identified in Drosophila which lead to their discovery in mammals. We continue to use Drosophila as a model in these studies. Exposing the heart of larval Drosophila to LPS (500 µg/ml) from Serratia marcescens causes the heart rate to initially increase and then slow down. Whereas exposing the body wall muscle, while stimulating the motor nerve, results in hyperpolarization. Evoked as well as spontaneous excitatory junction potentials become depressed with the presences of LPS. The decrease in synaptic transmission is likely due to the postsynaptic glutamate receptors being blocked by LPS. However, the mechanism to explain the hyperpolarization of the body wall muscle and alterations in heart rate has yet to be determined. We set out to determine if there was an alteration in the rapid effects upon exposure to LPS in RNAi expressing lines for the peptidoglycan recognition proteins (PGRPs) PGRP-LC and PGRP-LE in body wall muscle and cardiac muscle. These receptors are known to bind LPS in Drosophila. Knocking down the receptor expression for PGRP-LC and PGRP-LE did not alter the acute effects of LPS exposure to the body wall muscle and effects on synaptic transmission or heart rate in larval Drosophila. Thus, it has yet to be determined the mechanism by which LPS is causing these rapid cellular changes. This is significant to address potential effects in human and other animals exposed to gram negative bacterial infections.

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Poster Presentation 70

Abstract Title:	**The Influence of Endotoxic LPS on Primary Sensory Neurons in Crustaceans**
Author(s):	C. Stanley, Department of Biology, U of Kentucky S. Akhtar, Department of Biology, U of Kentucky M. Stanback, Department of Biology, U of Kentucky A. Stanback, Department of Biology, U of Kentucky R. Basham, Department of Biology, U of Kentucky B. Chithrala, Department of Biology, U of Kentucky B. Collis, Department of Biology, U of Kentucky B. A. Heberle, Department of Biology, U of Kentucky E. Higgins, Department of Biology, U of Kentucky A. Lane, Department of Biology, U of Kentucky S. Marella, Department of Biology, U of Kentucky M. Ponder, Department of Biology, U of Kentucky P. Raichur, Department of Biology, U of Kentucky A. Silverstein, Department of Biology, U of Kentucky K. Vela, Department of Biology, U of Kentucky R. L. Cooper, Department of Biology, U of Kentucky

Abstract: Many types of gram-negative bacteria are responsible for serious infections, such as septicemia. Lipopolysaccharides (LPS), the endotoxins released from these bacteria, are responsible for inducing the immune response of organisms. Much is known about the crustacean immune system, such as the conservation of Toll-like receptors (TLRs), but little is known about the direct impact LPS has on primary sensory neurons apart from this immune reaction. Previous studies have supported the idea that motor neurons increase both spontaneous and evoked firing frequencies with LPS, but differences have been observed across species. This project investigated how LPS from two strains of gram-negative bacteria (Serratia marcescens and Pseudomonas aeruginosa) affects the firing frequency of primary sensory proprioceptors in the crab propodite-dactylopodite (PD) organ and crayfish muscle receptor organ (MRO). These sensory organs correlate to mammalian proprioception, as the MRO is analogous to the mammalian muscle spindle, and the PD organ allows for the separation of motor nerve function from sensory neuronal transduction. Results indicated that there is no strong statistically significant impact on sensory transduction through the application of LPS; however, in the crab PD organ, the application of LPS from both strains typically decreased nerve activity except when LPS from both bacteria was applied together. It was also noted that, in the crayfish MRO, there usually was an increase in nerve activity. Interestingly, the MRO muscle fibers often contracted upon addition of LPS, perhaps indicating that the known impact of LPS on motor nerve function is partially responsible for the results obtained.

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Poster Presentation 71

Abstract Title:	**Sonic Hedgehog signaling promotes Siah E3 ubiquitin ligase expression to promote choroid fissure closure.**
Author(s):	S. E. Veith, Department of Biology, U of Kentucky W. P. Piedade, Department of Biology, U of Kentucky J. K. Famulski, Department of Biology, U of Kentucky

Abstract: Vertebrate eye formation involves an intricate interplay of neural tissue, which ultimately forms the semi-spherical eye. A critical aspect of this process is fusion of the choroid fissure located in the ventral area of the developing eye. Failure of choroid fissure closure is known to result in congenital blindness. Recent work in our lab identified Siah1 and Siah2 E3 ubiquitin ligases as regulators of Nlz2 protein stability which in turn regulates gene expression of a critical factor in choroid fissure closure, Pax2a. In order to expand on these findings, I sought to explore the upstream regulators of Siah expression. Sonic hedgehog (Shh) signaling is involved in fissure closure and was the focus of our study. We hypothesized that Shh positively regulates the expression of Siah genes during eye formation. To test our hypothesis, I performed whole mount in situ hybridization on Danio rerio embryos treated from 5.5 – 24 hours post-fertilization (hpf) with either Purmoraphamine (to increase Shh signaling) or Cyclopamine (to decrease Shh signaling), to assay for expression of Siah1, Siah2, and Pax2a. Expressions of these genes was increased in purmorphamine-treated embryos while decreased in cyclopamine-treated embryos. To confirm my results I also analyzed expression in Smo -/- mutant embryos which lack Shh signaling. The observed changes in gene expression were quantified by qPCR. Taken together, my results support the hypothesis that Shh regulates Siah E3 ubiquitin ligase expression. Future work will concentrate on the molecular mechanism of regulation and relation to eye development.

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35th Annual BGSFN Spring Neuroscience Day

Poster Presentation 72

Abstract Title:	**Delayed Pioglitazone administration provides neuroprotective effects following severe TBI**
Author(s):	K. Spear, Department of Neuroscience, U of Kentucky; Spinal Cord and Brain Injury Research Center M. Spry, Spinal Cord and Brain Injury Research Center J. Gooch, Spinal Cord and Brain Injury Research Center W. B. Hubbard, Department of Neuroscience, U of Kentucky; Spinal Cord and Brain Injury Research Center P. G. Sullivan, Department of Neuroscience, U of Kentucky; Spinal Cord and Brain Injury Research Center

Abstract: Traumatic brain injury (TBI) affects millions of people each year in the US. It is characterized by neuronal disruption primarily due to impact. This primary injury is further exacerbated by secondary pathways which includes mitochondrial dysfunction that leads to elevated reactive oxygen species and can cause cell death. In this study, Pioglitazone, an FDA approved anti-diabetic which targets the mitochondrial protein mitoNEET, is evaluated to examine its efficacy as delayed treatment for TBI. To test this, C57B/6 mice received either a sham or severe (1.0mm) controlled cortical impact (CCI) followed by the initiation of Pioglitazone administration at either 3h or 18h post injury, which included a bolus injection of Pioglitazone or Vehicle. Osmotic mini pumps (20mg/kg/day) of either Pioglitazone or Vehicle were inserted, and booster injections were given every 24h. At 7d post injury, animals were euthanized and brains sections (35 mm) were mounted and stained with Cresyl Violet. Tissue sparing analysis was used to calculate cortical tissue loss following injury and treatment. Stereology was then done to measure the number of neurons present in the dentate gyrus and the CA3 regions of the hippocampus. Pioglitazone treatment at 18h after TBI resulted in a significant increase ($p<0.05$) in tissue sparing compared to Vehicle treatment. This was highlighted by, on average, a 12% cortical loss in vehicle treated animals in comparison to a 6% loss in Pioglitazone treated animals. No significant difference was found after 3h initiated treatment. Pioglitazone is a possible translational treatment for TBI due to its therapeutic window.

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Poster Presentation 73

Abstract Title:	**Effects of Repeated Concussive Brain Injury on Progression of Tau Hyper-Phosphorylation**
Author(s):	N. Saleem, Spinal Cord, and Brain Injury Research Center, Univ. of Kentucky and Department of Neuroscience, Univ. of Kentucky W. Brad Hubbard, Spinal Cord, and Brain Injury Research Center, Univ. of Kentucky, Department of Neuroscience, Univ. of Kentucky, and Department of Physiology, Univ. of Kentucky A. DeSana, Spinal Cord and Brain Injury Research Center, Univ. of Kentucky and Department of Physiology, Univ. of Kentucky S. E. Meier, Department of Physiology, Univ. of Kentucky and Sanders-Brown Center on Aging, Univ. of Kentucky K. E. Saatman, Spinal Cord and Brain Injury Research Center, Univ. of Kentucky and Department of Physiology, Univ. of Kentucky

Abstract: Tau is a protein that stabilizes microtubules in neurons, astrocytes, and oligodendrocytes. In some neurological diseases, tau undergoes a greater degree of phosphorylation, known as hyper-phosphorylation, which promotes aggregation of insoluble tau into tangles resulting in the degeneration of neurons. Chronic traumatic encephalopathy (CTE), one of these diseases, is linked to repeated head injury. To better understand the effects of repeated mild traumatic brain injury (TBI) on the progression of tau pathology, a mouse model of closed head injury (CHI) was used to produce a concussive impact, similar to concussion observed in humans. Transgenic mice overexpressing human tau (rTg4510) and wild-type mice were given two CHIs at a 24h interval before euthanasia at 3d, 2wk, or 2mo. Using immunohistochemistry (IHC), we first examined a human tau specific antibody (HT-7) to confirm genotype of each animal. The pathology of phosphorylated tau was examined using the antibody AT-8, which detects hyper-phosphorylation at Ser396/404. In rTg4510 mice with sham injuries or 2 CHIs, we observe progression phosphorylated tau deposition in an age-dependent manner. In both cortex and sub-regions of the hippocampus, qualitative observations suggest increased numbers of AT-8 positive cells after CHI compared to sham at 3d and 2wk post-injury. Therefore, our model is a possible platform to examine pathology related to CTE and therapeutic targeting tau pathology for the goal of mitigating cognitive decline.

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Poster Presentation 74

Abstract Title:	**Examining the effects on neural developmental and behavior with altered gravitational sense in developing larval Drosophila.**
Author(s):	N. deCastro, Lafayette Senior High School, Lexington, KY. R.L. Cooper, Dept of Biology, Univ. of KY, Lexington, KY.

Abstract: Neuronal plasticity has been a topic of intense study for decades. Postsynaptic and presynaptic alterations in response to changes in activity which signals cellular machinery is well-documented. Chronic alterations during develop examines neural plasticity in a form which lends itself to compensatory mechanisms which can lead to hard wired changes. Neural ablation studies are permeant and do not allow reversal of the original conditions. However, even acute studies of sensory deprivation can have long lasting behavioral changes. It may be feasible to tackle complex neural circuits by examining some of physiological and anatomical changes in the less complex neural systems in which even basic behaviors such as reflexive behaviors can be quantified. Drosophila have a relatively simple sensory system in the larval central nervous system (CNS). A means to reduce or increase neuronal function with altering gravitational sense is possible with magnetic control on developing larval Drosophila. Preliminary runs are just now being performed by feeding Fe3O4 and exposing larvae to magnetic fields at different times and intensity during larval development. Testing the effect of feeding Fe3O4 without magnetic fields is also being examined. We are working out various conditions of altering sensory activity in establishing the long- and short-term consequences on neural development when the sensory system for gravitational touch sensation is decreased or over excited.

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Poster Presentation 75

Abstract Title:	**The Effects of Moderate Prenatal Ethanol Exposure on Anxiety in a Rodent Model**
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Abstract: Fetal Alcohol Spectrum Disorders encompass a range of behavioral and structural consequences following exposure to ethanol in utero. The goal of this study was to develop a model of voluntary ethanol consumption by rats prior to and throughout pregnancy to assess the consequences of a low dose exposure. Rats were exposed to a voluntary regime of 5% ethanol in a sweetened saccharin solution for 4 hours daily, during the dark cycle. Exposure began prior to breeding and was continued until 48 hours prior to parturition. Pair-fed and non-treated control groups were also included. A marble burying test was used to assess whether prenatal ethanol exposure altered the levels of anxiety in rats. Offspring were tested between postnatal days (PND) 42-46. Cages were filled with 5 inches of bedding with 4 rows of 5 marbles. Rats were given 5 min to habituate to the test room and then placed in the test cage for 20 min Increased marble burying is significantly correlated with increased anxiety. Females exposed to ethanol prenatally buried more marbles than controls. However, this ethanol-related effect was not apparent in males. Our data show that marble burying is differentially affected across sex following prenatal exposure to a low dose of ethanol. This may indicate females may be more anxious than males in a novel environment following exposure to a low dose of ethanol. Very little data is currently available on the consequences of low dose ethanol and so further work is clearly needed.

Supported by:

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Poster Presentation 76

Abstract Title:	**The role of Sox10 expressing neural crest cells in the organization of POM Subpopulations**
Author(s):	P. Poffenberger, Undergraduate department of Biology, U of Kentucky K. Van Der Meulen, Department of Biology, U of Kentucky J. Famulski, Department of Biology, U of Kentucky

Abstract: The Anterior Segment (AS) of the eye maintains homeostasis and is vital for vision. When development of the AS does not occur properly, anterior segment dysgenesis (ASD) can occur. This disorder can increase the risk of congenital glaucoma, corneal opacity, Axenfeld-Rieger Syndrome, and more. AS development is thought to depend on migration of neural crest (NC) cells, which differentiate into periocular mesenchyme (POM) cells. POM cells populate the AS, but the regulation of their differentiation and migration are unknown. Sox10, a key regulator of NC fate, may play a critical role in POM differentiation. Our hypothesis is that in the absence of Sox10 function, NC cells will fail to migrate and specify into the POM, ultimately producing an ASD-like phenotype. To test our hypothesis, we utilized a Sox10 mutant line of zebrafish, colorless (cls), to analyze the consequences of Sox10 loss of function on POM cell specification. Embryos from CLS heterozygote in-crosses were analyzed for POM expression using whole mount in situ hybridization (WISH). A Sox10 probe along with POM markers FoxC1a, FoxC1b, and FoxD3, were examined. At 24/48hpf we did not observe any changes in expression for POM markers, but did observe a reduction of Sox10 mRNA. NC markers Twist1a, Twist1b, and Twist2 were also analyzed with WISH, and showed a lower expression in mutant embryos at 24/48hpf. This suggests that we are in fact affecting NC cell migration with a Sox10 knockout, but POM cells may not arise specifically from a Sox10 dependent NC progenitor.

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Poster Presentation 77

Abstract Title:	**DBS Plus for Parkinson's disease: 3D subcortical brain mapping of nerve grafts and trajectories in correlation with the functional outcome**
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Abstract: Parkinson's disease (PD) is characterized by the loss of dopaminergic neurons in the midbrain, which causes worsening rigidity, tremors, and bradykinesia. In the United States, the total direct and indirect cost of PD treatment is $25 billion per year. Currently, there is no cure available to stop or slow the progression of this disease. In our two recent clinical trials, NCT01833364 and NCT02369003, autologous peripheral nerve grafts containing "repair" Schwann cells were implanted into the midbrain during Deep Brain Stimulation (DBS) surgery. The hypothesis is that grafts act to supply the damaged cells with a neurotrophic environment, thus promoting their survival and regeneration. The aim of the following research is to reconstruct the grafts' trajectories, map their final targets, and correlate the functional anatomy with the motor outcomes. Using the Lead-DBS software, the pre-operative and post-operative MRI and CT scans of 64 DBS Plus patients will be co-registered and normalized to a common space. After correcting for brainshift bias, the trajectories will then be manually reconstructed to determine their effect on the subcortical connectomes using an ATAG atlas. The placement of the grafts will also be analyzed in correlation to the clinical outcome, based on Unified Parkinson Disease Rating Scale III motor scoring. Mapping the graft locations will help optimizing the grafting technique and potentially identifying new functional targets to improve the motor outcome of patients with PD.

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Poster Presentation 78

Abstract Title:	**Combat related alterations in neural processing speed during working memory**
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Abstract: Military deployment is associated with increased risk of mild traumatic brain injury (mTBI) and posttraumatic stress disorder (PTSD). Although the etiology of the neurocognitive compromise in combat veterans are still under debate, combat stress related memory deficits have been well documented. In this study, we investigated neural processing speed and brain responses during a working memory task in 25 veterans (15 mTBI and/or PTSD, and 10 health combat controls). We hypothesize that differential neural processing deficits and poorer cognitive performance will be observable in veterans with mTBI and/or PTSD. We collected 32-electrode scalp-EEG recordings, as well as MRI images of grey and white matter integrity of the brain, during 20 min delayed match-to-sample task. Each subject also completed neuropsychological tests that assessed attention, processing speed, and executive function. Preliminary results reveal reduced bilateral P300 amplitude, indicative of cognitive processing, for individuals with PTSD when compared to healthy combat controls during retrieval of memory targets. This trend was also displayed in retrieval of nonmatch distractors at left and right frontal electrodes in PTSD versus combat controls. Functional MRI results also showed reduced brain responses in the PTSD group. For the mTBI group, left frontal electrodes show reduced mean P300 amplitude during retrieval of memory target when compared to combat controls. Preliminary results showed distinct deficits of white matter integrity in mTBI compared to combat control. Our next step is to examine fractional latency of the late positive component during memory retrieval and measures of white matter in the brain.

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Poster Presentation 79

Abstract Title: **The Effects of Prenatal Ethanol Exposure on Activity and Anxiety**

Author(s): J. Kieckhefer B. Auvil E. Punzal A. Rowell R. Shellhaas S. Barron, Department of Psychology, U of Kentucky

Abstract: Hyperactivity, learning deficits, and altered response to stress have been associated with Fetal Alcohol Spectrum Disorders (FASD). Rodent models have documented many of the same characteristics as that seen in FASD although the majority of these studies have used paradigms built on exposure to high doses of ethanol. This study used a voluntary ethanol consumption model with low ethanol doses consumed prior to and throughout pregnancy. Ethanol was administered in a sweetened saccharin solution daily for 4 hours during the dark cycle. Control groups were also included. Offspring were tested on PND 75-85 in a round open-field chamber. Using ANY-maze motion tracking software, activity was recorded in one 20 minute session per day for two days. Activity was measured as time spent in motion and movement between the center and outer zone of the test chamber. Prenatal ethanol exposure did not alter overall activity; however, an interesting pattern for entries into the center zone of the apparatus emerged. For both control groups, female offspring entered the center zone more frequently than males. For the ethanol exposed group, this pattern either was non-existent (day 1) or reversed (day2). Entries in the center zone is typically used as a measure of anxiety, so these findings suggest that even low dose ethanol consumption during pregnancy may result in an altered response to stress. Further work will examine if these findings generalize to other paradigms and help establish the underlying mechanisms.

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Poster Presentation 80

Abstract Title:	**Myelin modulates macrophages depending on inflammatory activation state**
Author(s):	K. E. Bethel, College of Arts and Sciences B.S. Neuroscience Program, University of Kentucky T. J. Kopper, Department of Physiology, University of Kentucky J. C. Gensel, Department of Physiology, University of Kentucky

Abstract: A notable contribution to spinal cord injury (SCI) pathology is chronic inflammation and macrophage activation. Depending upon environmental stimuli, macrophages can broadly adopt reparative (M2) or pathological (M1) properties in the injured spinal cord. A Detrimental pro-inflammatory M1 macrophage response is sustained post-injury, while the reparative M2 response is diminished after 1 week. It is not clear why the M1 phenotype predominates, but the presence and clearance of myelin debris may be a potentiating factor. There is controversy as to the role of myelin in macrophage activation. One clarifying hypothesis is that macrophages respond differently to myelin depending on their stimuli-induced activation state. Bone marrow-derived macrophages (BMDMs) were induced with M1 or M2 stimulants or left unstimulated (control) and treated with and without myelin in vitro. Differential responses to myelin depending on activation state were observed via RT-PCR; myelin was shown to potentiate M1 pro-inflammatory mRNA targets (IL1b, TNF-a, Marco) while M2 and control BMDMs were largely unresponsive. Supernatant from myelin-treated M1 BMDMs led to decreased dorsal root ganglia neuron differentiation, axon length, and neuron health. Supernatant from myelin-treated M1 BMDMs also led to increased neuron toxicity. There was no significant difference in myelin phagocytosis between groups, suggesting that this is not the mechanism of action. Our results suggest that myelin could be contributing to M1 phenotype predominance. Current studies underway are investigating potential molecular mechanisms of action including in vivo studies using knockout mice. Understanding these mechanisms may lead to decreased secondary injury progression and ultimately neuronal regeneration.

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Poster Presentation 81

Abstract Title:	**Position of Lever, Relative to a Social Peer, Affects Cocaine Self-Administration**
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Abstract: Purpose: Although preclinical drug-seeking models typically focus on non-social factors, relapse in humans is often triggered by re-associating with drug-using peers. The difference between social and non-social cues could be key to understanding discrepancies between clinical and preclinical data. We investigated the role of oxytocin on peer-induced relapse using a dual-compartment apparatus, in which rats self-administered cocaine by pressing on one of two available levers while a peer was in an adjacent chamber, connected by a wire mesh partition. Because one lever was close to the peer and other was far from the peer, this experiment sought to determine if lever position altered the results obtained. Methods: Sprague-Dawley rats underwent jugular catheter implantation and virus microinfusion (oxytocin-activating DREADD or control) into the hypothalamus. Each rat was pseudorandomly assigned to self-administer in one compartment of the apparatus, with either a left or right active lever. Thus, rats were assigned to self-administer far from the mesh partition and peer (e.g., left lever in left compartment) or close. Rats underwent 30 days of twice-daily self-administration training, receiving cocaine (1.0 mg/kg/infusion) and saline each day. Results: During the first 10 sessions, rats consistently took more cocaine when the active lever was close to the mesh partition. Following acquisition, these rats pressed more for both cocaine and saline, relative to rats with a far lever. Discussion: Rats lever press more when the active lever is close to a peer, regardless of infusion type. Care should be taken to design experiments that take this into consideration.

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Poster Presentation 82

Abstract Title:	**Chronic intermittent hypoxia induces robust astrogliosis in an Alzheimer's disease-relevant mouse model**
Author(s):	E. Higgins, Spinal Cord & Brain Injury Research Center, University of Kentucky T. Macheda, Spinal Cord & Brain Injury Research Center, University of Kentucky K. Roberts, Spinal Cord & Brain Injury Research Center, University of Kentucky A.D. Bachstetter, Spinal Cord & Brain Injury Research Center, University of Kentucky

Abstract: Objectives: Sleep disturbances are a common early symptom of neurodegenerative diseases, including Alzheimer's disease (AD), and emerging evidence suggests that poor sleep may be an important contributor to development of amyloid pathology. There is a strong correlative link between sleep apnea and AD; yet, almost no experimental research is available exploring the mechanisms of this link. Previous studies have found that astrogliosis is a contributor to neuropathology in models of chronic intermittent hypoxia (IH) and AD; therefore, we hypothesized that a reactive astrocyte response might be a contributing mechanism in the neuroinflammation associated with sleep apnea. Methods: 10-11-month-old wild type (WT) and APP/PS1 KI mice were exposed to 10 hours of chronic intermittent hypoxia (an experimental model of sleep apnea), daily for four weeks. At the end of four weeks brains were analyzed from amyloid burden and astrogliosis. Results: No effect was found for chronic IH exposure on amyloid load in the APP/PS1 KI mice. A significant increase in GFAP staining and astrocyte activation genes was found in the APP/PS1 KI mice following chronic IH exposure, but not in the WT mice. Conclusions: Our results suggest that the otherwise healthy brain is likely resilient to a short period of IH exposure, but the brain may have less resilience to IH exposure if amyloid pathology is present. In particular, the presence of amyloid pathology can have an additive effect with IH exposure on reactive gliosis.

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Poster Presentation 83

Abstract Title: **Effects of Low-Dose Prenatal Ethanol Exposure on Problem-Solving in Rats**

Author(s): R. Shellhaas, B. Auvil, J. Kieckhefer, E. Punzal, A. Rowell, & S. Barron

Abstract: Prenatal ethanol exposure in high doses can cause severe behavioral and physiological deficits in offspring. Our understanding regarding the effects of lower doses are not well understood although this is likely applicable to a larger human population. This study looked at the effects of low-dose, voluntary ethanol consumption during pregnancy on a problem solving task in rodent offspring. Female rats were given access to a low dose of ethanol (5%) in a sweetened solution for 4 hours daily during their dark cycle prior to conception and throughout pregnancy. Pair-fed and non-treated control groups were also included. Offspring were tested as adults (approximately 90-110 days of age) in a digging maze task. This paradigm required subjects to use a species typical behavior (digging) to solve a novel problem (gaining access to water). While subjects from all three treatment groups were able to solve the problem, more ethanol exposed offspring failed to learn the task relative to controls. Subjects that failed to learn the task on the first trial were given a second trial. All control subjects solved the problem on the second trial. In the ethanol-exposed group, 75% of subjects who failed on trial one also failed on trial two. These results suggest that in utero exposure to a low dose of ethanol can affect performance on a problem-solving task. This task has been shown to be sensitive to cortical and subcortical damage. Further work is needed to better understand how low-dose ethanol exposure in utero affects these CNS regions.

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Poster Presentation 84

Abstract Title:	**The Mechanotransduction-Dependent Stability of the Stereocilia Cytoskeleton in Auditory Hair Cells Does Not Require Myosin XV**
Author(s):	A. I. López-Porras, Department of Physiology, U of Kentucky A. C. Vélez-Ortega, Department of Physiology, U of Kentucky

Abstract: The hair cells of the inner ear detect sound waves through the deflection of their stereocilia – modified microvilli with mechanotransduction (MET) channels at their tips. In mammals, the auditory hair cells do not regenerate or renew thus they need to maintain their stereocilia bundles for up to several decades. We have previously demonstrated that the constant entry of calcium ions through MET channels is fundamental for the maintenance of the stereocilia length and thickness, but the proteins involved in this calcium-dependent process are still unknown. Given that the non-conventional myosin XV is required for the normal growth and maintenance of the stereocilia bundle, we wondered whether this myosin is necessary to deliver the molecular machinery involved in the calcium-dependent stability of the stereocilia actin cytoskeleton. Here we show that, even in the absence of functional myosin XV, the stability of the stereocilia actin cytoskeleton still requires a resting MET current. Shaker-2 mice lack myosin XV in the stereocilia bundles but do exhibit MET currents. We found that the blockage of the MET channels in cochlear explants from these mice leads to the thinning and shrinking of stereocilia in the auditory hair cells. These results indicate that the molecular machinery involved in the calcium-dependent stability of the stereocilia cytoskeleton does not depend on myosin XV.

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Poster Presentation 85

Abstract Title: **The Effects of Moderate Prenatal Alcohol Consumption on a Novel Location Test**

Author(s): Auvil, B., Rowell, A., Kieckhefer, J., Punzal, E., Shellhaas, R., Peng. D. & Barron, S.

Abstract: Ethanol ingestion during pregnancy can be detrimental to the developing fetus and can result in a range of Fetal Alcohol Spectrum Disorders (FASD). These can include behavioral, learning and/or cognitive deficits. Rodent models have given significant insight into the consequences of high levels of ethanol (ETOH) exposure during development, but less is known about moderate exposure. The purpose of this study was to look at the effects of moderate ETOH consumption both prior to conception and throughout pregnancy in Sprague-Dawley rats. ETOH was available for 4 hours daily during their dark cycle. Treatment groups included ETOH treated, pair fed and non-treated control. Offspring (75-85 days of age) were tested in a novel location paradigm in which subjects had to recognize that an object was moved between Day 1 and Day 2 of testing. All treatment groups spent more time in the novel location than the familiar location. Sex differences were observed for the two control groups although the pattern was different. In contrast, no sex differences were observed in the ETOH exposed group. These results provide further support for including both males and females when studying the effects of prenatal ETOH exposure. These results also convey the importance of including both pair fed and non-treated controls. While there may be little ETOH-related effects other than an absence of sex differences in this paradigm, additional studies in our lab suggest that there are effects of moderate prenatal ETOH exposure on levels of anxiety.

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Poster Presentation 86

Abstract Title:	**Improving Breathing Motor Ability After Cervical Spinal Cord Injury by Exploration of a Novel Intermittent Hypoxia Paradigm**
Author(s):	A. L. Silverstein, Department of Neuroscience, U of Kentucky D. R. Stoltz, Department of Neuroscience, U of Kentucky L. E. H. Strattan, Department of Neuroscience, U of Kentucky C. M. Calulot, Department of Neuroscience, U of Kentucky R. S. J. Maggard, Department of Neuroscience, U of Kentucky E. E. Huffman, Department of Neuroscience, U of Kentucky K. J. Ritter, Department of Neuroscience, U of Kentucky W. J. Alilain, Department of Neuroscience, U of Kentucky

Abstract: Spinal cord injury (SCI) most often occurs at the cervical level and frequently causes life-threatening diaphragm paresis and breathing difficulty. Intermittent hypoxia (IH) treatment is often utilized in preclinical models of cervical SCI to induce diaphragmatic recovery following injury through a type of spinal cord plasticity termed long term facilitation (LTF). IH consists of the cyclical exposure of subjects to alternating intervals of hypoxia and normoxia, exhibiting similarity to operant conditioning (OC), described as behavioral training through reinforcement. If IH is construed as OC, our targeted behavior of heightened respiratory drive occurs during hypoxia and is reinforced during normoxia. Because intervals of typical IH are of fixed duration, such procedure can be renamed fixed interval treatment (FIH). Furthermore, FIH can be improved through exchanging fixed for varied intervals of hypoxia between reinforcements, as supported by seminal findings in OC literature. We further hypothesized that varied interval hypoxia (VIH) treatment would induce greater LTF than FIH after injury, utilizing the C2 hemisection model in rats. To test this hypothesis, we treated injured animals with VIH or FIH for 5 days at 1-week and 8-weeks post-injury, conducted diaphragm electromyograph recordings immediately after treatment to assess breathing motor output, and compared respiratory motor activity between treatment groups. Contrary to our hypothesis, results in 1-week post-injury animals suggest that FIH induces greater recovery than VIH—a trend approaching significance in 8-week animals following injury. Future directions include modification of IH paradigms and immunohistochemistry of spinal cord tissue.

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Poster Presentation 87

Abstract Title:	**Clearing Up Phrenic Motor Neuron Survival After Cervical Spinal Cord Injury**
Author(s):	E. E. Huffman, Department of Neuroscience, U of Kentucky A. L. Silverstein, Department of Neuroscience, U of Kentucky D.R. Stoltz, Department of Neuroscience, U of Kentucky W.J. Alilain, Department of Neuroscience, U of Kentucky

Abstract: The diaphragm is the major muscle involved in breathing. Innervated by the phrenic nerve, it is controlled by phrenic motor neurons (PMNs), which receive descending inputs from the medulla. When these bulbospinal-pathways are damaged or severed in spinal cord injury (SCI), the external effects of injury are seen immediately, as the diaphragm becomes paralyzed and the individual loses the ability to breathe. However, the effect of injury on the internal circuitry, specifically PMN survival, is largely unknown. Contradictory evidence has surfaced, suggesting that there is large PMN death after injury, or conversely, that there is an absence of PMN death. The histological techniques utilized in these studies, however, have exposed the data to factors through which certainty cannot be guaranteed. These discrepancies are important to parse out because characterization of PMN survival is integral to studies of plasticity. The present study attempted to bridge this gap in knowledge and used XClarity clearing methods to accurately determine PMN survival after cervical SCI. XClarity transforms the tissue into a transparent medium. This allows for the whole spinal cord to be analyzed without tissue loss, as is common in other histological techniques. Sprague-Dawley rats were hemisected at the second level of the cervical spinal cord (c2Hx) and injected bilaterally with a retrograde tracer that labels PMNs. Cords were processed with XClarity; PMN survival was characterized with Lightsheet microscopy. Analysis of PMNs is ongoing, however, preliminary data suggests that XClarity techniques are the preferable route to characterize PMN survival after injury.

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Poster Presentation 88

Abstract Title:	**The association of dystrophic microglia with aging in humans without neurodegenerative disease**
Author(s):	R. Higdon, College of Arts and Science, University of Kentucky A.D. Bachstetter, Spinal Cord And Brain Injury Research Center, University of Kentucky

Abstract: Dystrophic microglia, a subtype of microglia, has been indicated in Alzheimer's disease pathogenesis. There is limited information on the presence of these microglia in various neurodegenerative diseases. The presence or absence of dystrophic microglia was scored on IBA1 immunohistochemically stained slides for the human hippocampus. Dystrophic microglia were found to account for a substantial proportion of the microglia cells in the hippocampus of both demented and cognitively intact aged individuals (total n=39 cases). We hypothesized that aging could be a defining feature in the occurrence of dystrophic microglia. To test this hypothesis, a series of autopsy cases (total n=168 cases), which covered the adult lifespan from 20 - 100+ years old, were included in the study. The results demonstrated that aging is strongly associated with an increase in microglia density.

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Poster Presentation 89

Abstract Title:	**The effects of social and nonsocial discriminative stimuli in a rat model of cocaine seeking**
Author(s):	Marie J. Clancy Lindsey R. Hammerslag Emily D. Denehy Michael T. Bardo

Abstract: Aim: Re-association with a drug using peer can lead to relapse in humans, but rat models traditionally rely on nonsocial cues. In rats, we have recently found that reinstatement of drug seeking can be initiated by exposure to a cocaine-associated peer. In the current experiment we compared the traditional model of relapse, with nonsocial cues, to our peer-induced reinstatement model. We hypothesize that social stimuli may be more salient than nonsocial stimuli, leading to a greater effect on reinstatement of cocaine seeking. Method: Rats self-administered for 28 days with twice-daily sessions where either saline or cocaine (1 mg/kg/infusion) was self-infused. Rats had two discriminative stimuli (DS), either nonsocial (light/tone) or social (same-sex peer), indicating cocaine (S+) or saline (S-) availability. There was a 20-s timeout following each infusion, signaled by a cue light (CS). After extinction, reinstatement was assessed for a combination of DS (S+, S-, none) and CS (present, absent), with 4 extinction sessions between tests. Results: Lever presses decreased for saline and increased for cocaine in both social and non-social groups during self-administration. Rats with social DS had a shorter latency to 1st injection when the S+ was present, suggesting discrimination between the peers; no difference was seen for nonsocial stimuli. During the reinstatement tests, the social S+ had a significantly greater effect on drug seeking, compared to the S- or nonsocial S+. Conclusion: Social stimuli had a greater effect on reinstatement than nonsocial stimuli, emphasizing the importance of social stimuli in preclinical models of drug relapse.

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Poster Presentation 90

Abstract Title:	**Balancing Neuroprotection with Functional Recovery: The Role of the Perineuronal Net in Preventing Excitotoxicity after Spinal Cord Injury**
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Abstract: In spinal cord injury, initial mechanical trauma causes debilitating primary damage to neural cells and blood vessels. Following this, secondary cascades of downstream events occur, including inflammation, ischemia, and excitotoxicity. Additionally, there is an upregulation of the PNN, a lattice-like structure of the extracellular matrix which normally modulates neural communication and homeostasis. Interestingly, the PNN is partially composed of very negatively charged CSPGs. While CSPGs can stabilize plasticity and neuronal growth during development, these molecules become inhibitory to regeneration, sprouting and plasticity after injury, as well as contribute to the glial scar. However, administration of the bacterial enzyme ChABC can digest the PNN and CSPGs, ultimately promoting functional recovery. What remains unknown are the other impacts of removing the PNN at very acute stages of injury. We hypothesize that the PNN and its negatively charged CSPGs are upregulated after SCI as a neuroprotective response that attenuates excitotoxicity by acting as a buffer against excessive Ca2+. To test our hypothesis we induced excitotoxicity by injecting a threshold dose of the glutamate receptor agonist, NMDA, with or without ChABC, into the cervical spinal cord of rats. Following this administration, we collected tissue and evaluated damage and cell loss. Qualitative preliminary findings suggest that subjects lacking the PNN have exacerbated cell death versus those with an intact PNN. Collectively, these findings indicate that PNN upregulation after injury could be a conserved mechanism to promote cell survival and tissue preservation at the expense of CNS regeneration and plasticity.

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Poster Presentation 91

Abstract Title:	**Differential Responses to Psychostimulants in Rats: Sex differences**
Author(s):	G. C. Clark, Department of Psychology, Morehead State University C Felts, Department of Psychology, Morehead State University I. M. White, Department of Psychology, Morehead State University, Morehead, KY

Abstract: This study compared behavioral changes in male and female rats after repeated administration of psychostimulants, methamphetamine (METH) and cocaine. Following 5 repeated daily administrations of METH, cocaine, or saline, we measured spontaneous activity and simple learning in fixed-ratio 5 (FR5), which required five lever-presses to earn each food pellet. METH increased activity in both males and females, with higher activity in females. For simple learning, METH decreased response latency and run time, reflecting impulsivity. Compared to males, however, female rats showed smaller decreases in response latency and run time, reflecting differential METH effects on motivation. Cocaine produced a higher activity in males than females. In the simple learning task, cocaine increased response latency and runtime similarly in both sexes, with markedly longer runtime in females, reflecting greater effects on motivation. Neither drug affected pellet retrieval or consumption. Our findings suggest that repeated use of psychostimulants may produce different effects in males and females, with a greater abuse potential in males and a greater impact on motivation in females.

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Poster Presentation 92

Abstract Title:	**Sexual Profiling for Extent and Duration of Oxidative Damage Following Experimental Traumatic Brain Injury (TBI) in Young Adult Rats**
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Abstract: Neurological damage following TBI is incurred through various biochemical cascades which occur in response to both primary and secondary injury mechanisms. These mechanisms generate oxidative damage, an imbalance between pro-oxidant and anti-oxidant agents that exacerbate cellular damage. Little has been done to explore TBI in females, as TBI is more common in males than females. But, as females begin to increase their participation in contact sports, as well as a preexisting likelihood of both sexes to experience TBI due to unforeseen incidents, the need to conduct research determining differences in therapeutic window, severity and duration due to the sex of the subject is necessary. Therefore, our goal was to explore sex-based differences in markers relating to the balance between oxidative damage and activation of endogenous anti-oxidant mechanisms following TBI. Specifically, we looked at: 1) reactive aldehydes 4-HNE and acrolein resulting from the peroxidation of lipids by reactive species; 2) downstream markers of Nrf2-mediated activity: NQO1, HO-1 and GPx4; 3) Ca2+-mediated enzymatic degradation of spectrin, a neuronal cytoskeletal protein. As higher levels of estrogen in females have been postulated to be neuroprotective, we hypothesize that there will be significant sex-dependent differences between one-day and seven-days post TBI. We found that the extent and duration to which secondary damage occurs in the brain, was significantly different in the females, compared to males following TBI. These findings help us to understand how sex-related characteristics may affect recovery and assist in the development of sex-specific pharmacological strategies for clinical use in TBI patients.

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Abstract Title:	**Validating DREADDs to Assess the Mechanism of Oxytocin as a Treatment for Stimulant Abuse**
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Abstract: Purpose: Designer Receptors Exclusively Activated by Designers Drugs (DREADDs) are chemogenetically engineered proteins that alter the activity of target neurons only in the presence of “designer” ligands, primarily clozapine-n-oxide (CNO) or clozapine. Since oxytocin treatment has shown promise as a potential pharmacotherapy for stimulant abuse, we sought to determine if a DREADDs approach could be used to activate oxytocin neurons in selective regions of the rat brain. The long-term goal is to validate methods for using an oxytocin-targeting DREADD in order to test the future hypothesis that oxytocin activation will reduce cocaine seeking in rats. Methods: Rats were microinjected into the paraventricular nucleus (PVN) with a control virus or an activation virus that increases the activity of oxytocin-secreting cells when stimulated via the hM3Dq DREADD. Across two repeated tests, rats received an injection (0.1 mg/kg clozapine or vehicle) immediately prior to a locomotor test. Rats were perfused 90 minutes after the second test injection and PVN was examined with immunofluorescence. Results: No combination of DREADD or pretreatment affected locomotor activity. However, clozapine increased the percentage of PVN cells that expressed cFos, an immediate early gene that indicates recent cell activity, in rats that received the activation virus but not the control virus. Conclusion: These results indicate the clozapine serves as an effective and specific ligand for DREADDs. Since oxytocin is being evaluated as a treatment for stimulant abuse, we intend to use this approach to determine if activation of oxytocin neurons will reduce relapse to cocaine seeking.

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