BGSFN Spring Neuroscience Research Day

Poster Presentation Abstracts

7th Annual CCTS Spring Conference

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1 **Abstract Title:** **How do Polymorphisms in CD2AP and CD33 Alter Gene Expression to Modulate Alzheimers Disease?**

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Abstract:
Recent genome-wide association studies have identified the SNPs rs9381564 and rs3865444 in CD2AP and CD33, respectively, as modulating Alzheimer's disease (AD) susceptibility. Here, we seek to identify CD2AP and CD33 isoforms expressed in the human brain, quantify expression of these isoforms in brain, and evaluate the correlation between these isoforms and the SNPs that modulate AD risk. Our study's long-term goal is to translate these insights into novel therapeutic agents that replicate the protective allele actions. Preliminary immunocytochemistry analyses suggested that CD2AP is commonly expressed in neurons and microvessels. No variably spliced CD2AP exons were identified. CD2AP expression was quantified in cDNA prepared from 30 AD and 30 control brains as a function of rs9381564 genotype and AD status. Rs9381564 was predicted to be a functional SNP because (i) rs9381564 is in strong linkage disequilibrium with rs9349407, which is strongly associated with AD and (ii) rs9381564 alters a predicted FOXL1 binding site in the CD2AP promoter. However, we did not detect an association between CD2AP quantity and rs9318564 or AD status. CD33 typically contains seven exons. Characterization of CD33 splicing in the brain identified multiple variably spliced exons and introns, including exon 2 deletion, intron 1 and 4 retention, and two alternate final exons. Several of these variations are predicted to drastically affect protein sequence and functionality, particularly due to codon frameshifts. Our next steps will be to analyze total CD33 expression and expression of each splicing motif as a function of AD-associated SNPs. Our overall results will be presented.

Supported by: NIH Award P01AG030128
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2 **Abstract Title:** **Identifying Different Argonaut Complexes in Alzheimer's and Normal Brain**

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Abstract:
Alzheimer's disease (AD) is the most prevalent form of dementia, currently affecting 5.4 million Americans. Unfortunately, there is still no effective AD treatment or prevention. MicroRNAs (miRNAs) are endogenous small, non-coding RNAs (21-25 nucleotides in length) that play critical roles in many biological processes such as development, differentiation, and many human diseases. Our previous work demonstrated that the expression of a particular miRNA (microRNA-107, or has-miR-107) decreases in AD brains, and may accelerate the disease progression through regulation of beta-site amyloid precursor protein-cleaving enzyme 1 (BACE1). MiRNAs negatively regulate gene expression by partial sequence complementary to the target mRNAs resulting in mRNA translational inhibition. The execution of miRNA function relies on effector- Argonaute (AGO) protein complex, termed microribonucleoprotein, or miRNP. MiRNAs bind to AGO and other proteins and guide the miRNP to specific targeted mRNAs. Besides the essential AGO proteins, MiRNPs contain several other major proteins, for example, TRPB and GW182. Investigating the role of miRNA and the regulation of miRNA in AD pathology could hold a key in further understanding the regulatory pathways of AD. The goal of this study is to analyze the components of the miRNP in normal and AD brains by purifying different miRNP complexes using size exclusion chromatography. We aim to isolate the possible components involved in modifying brain miRNA expression and function in AD brains.

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3	**Abstract Title:**	**The Long-term Effects of Antipsychotic Drug Treatment in Rats after Treatment Discontinuation: Back to Normal or a New Normal?**

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Abstract:
Antipsychotic drugs (APDs) are commonly prescribed for the treatment of manychronic psychiatric disorders, but little research has ascertained whether there are lasting effects of treatment after discontinuation.This research determined the persistence of behavioral changes observed after prolonged antipsychotic drug (APD) treatment in rats. Specifically, locomotor activity was measuredin female and male adult rats on a weekly basis for several weeks after the cessation of a five-week regimen of daily risperidone (1.0 or 3.0 mg/kg) administration. Activity was also monitored once a week over the course of risperidone treatment in order to track the emergence of possible drug-induced changes in locomotor activity levels. Immediately after administration each week, locomotor activity was dramatically suppressed in rats treated with either risperidone dose. At 23 hours post-treatment, locomotor activity was increased in treated animals across all five weeks of treatment. At seven and 14 days after risperidone discontinuation, treated animals were hyperactive. By week five after discontinuation, there was no significant difference between treatment groups. None of the testing revealed gender differences in treatment-induced activity. These data demonstrate that behavior changes in animals after the cessation of chronic APD treatment, but only for a period equal to the length of treatment.

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	Abstract Title:	**Transection of a motor nerve results in a rapid synaptic depression**

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Abstract:
For a number of years it has been suggested that motor nerve terminals in vertebrates might release ACh spontaneously in a non-quantal and non-evoked manner (Katz and Miledi, 1981). It has also been reported that the non-quantal release of ACh decreases after the motor nerve is severed while evoked and quantal release can still occur. In vertebrate preparations examined, shortly before a severed nerve terminal fails to be evoked altogether, there is an increase in the spontaneous quantal release. Recently, it was shown in Drosophila larva that evoked synaptic efficacy decreases over 50% in severed motor neurons within 2 hours. Such reports indicate that severed motor neurons show altered behavior in a short period of time. Despite these reported phenomena, information is scant on the underlying mechanism to account for the alterations. To address if there is a joint pre- and post- synaptic contribution to the decrease is synaptic efficacy as well as the time domain in the acute run down in evoked release, we used intra- and inter-animal comparisons while monitoring evoked synaptic function and spontaneous quantal responses. We used the genetic attractable model Drosophila melanogaster for this study since it is being used more often today to address disease states and pathologies common to man. Preliminary studies demonstrate the release is depressed in a time dependent manner to exposure of the severed axon to the bathing saline, where as intact axons do not depress. We postulate that the axoplasm may be compromised by the diffusion of ions in and out of the terminal though the severed axon to account for the acute changes.

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5	**Abstract Title:**	**Neural modulation of the crayfish hindgut: A model system for examining central and peripheral control mechanisms**

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Abstract:
Although, the crayfish hindgut has been a research model for over a century, it is still an excellent model for investigating the generation and regulation of peristaltic rhythms and for describing the mechanisms underlying their modulation, both at the level of neural circuitry and at the level of ion channels within the neurons and muscles. The crayfish hindgut is unique when compared to the smooth muscle in the GI tract of vertebrates, as this invertebrate system not only contains striated muscle with gap junctions but also has the ability to generate intrinsic pacemaker activity. We first investigated the influence of the ventral nerve cord (VNC) and, in particular, the sixth abdominal ganglion on the activity of the hindgut of Procambarus clarkia by measuring the force and frequency of GI contractions. Then we examined the influence of neuromodulators selectively on the drive to the hindgut from sixth abdominal ganglion as well as the whole chain of abdominal ganglia (A1-A6). In addition, we assessed the effects of applying neuromodulators directly to the brain (cerebral ganglia) on descending drive of the hindgut and effects of direct application to the hindgut. Serotonin, octopamine and dopamine (1 uM) all enhance the rate of contractions when the VNC or the GI is directly exposed. Direct application of neuromodulators to the GI produced more forceful contractions and a faster rate than exposure only to the VNC. Dose-response curves of the various modulators are being examined.

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6	**Abstract Title:**	**Pathophysiological conditions with hypercalcemia: Neuron, CNS, intestine, and behavior.**

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Abstract:
It is a well-known phenomenon that hypercalcemia results in a loss of deep-tendon reflexes in humans. However, there is no readily substantiated mechanistic explanation for this occurrence. If left untreated, hypercalcemia can progress to a loss of consciousness and to coma. Therefore, understanding how to detect hypercalcemia via physical assessment and laboratory evaluation is essential. Acute treatment for urgent conditions presently entails a standard IV infusion. Yet, the phenomenon of a loss of neuronal induced reflexes with high calcium is paradoxical in the sense that ionized calcium is known to enhance synaptic efficacy at neuromuscular junctions and at synapses connecting sensory nerves to interneurons and interneurons to motor neurons. Thus, the question remains: Why in the intact CNS is there a reduction in sensory-evoked motor responses? Determining the mechanism(s) for the loss of deep tendon reflexes associated with hypercalcemia in humans is being addressed through literature searches and discussions with neurologists and other health care providers. The compilation of information will be presented. The crayfish nervous system is feasible to use to study sensory-CNS-motor nerve-muscle circuits. Therein provided are well-defined musculatures which are readily exposed in relatively intact preparations, as well as in the whole animal. The crayfish circuits used in our studies were a superficial flexor muscle circuit comprised of a 'sensory nerve root – ganglia – motor nerve root' and the well-established Telson induced tail flip response. We examined whole animal responses as well as in situ preparations with exposure to various ionized calcium concentrations. The experiments are still in progress at this time, but results will be reported at the meeting.

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7 **Abstract Title:** **Effects of Early-life Risperidone on Differential Reinforcement of Low Rates of Responding in Rats**

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Abstract:
The use of antipsychotic drugs (APDs) in pediatric populations has doubled over the past 15 years despite the relative absence of basic research documenting the long-term effects of such drug exposure. Since these drugs target the prefrontal cortex, it could be expected that prefrontal functions, such as cognitive control, may be altered in adults treated with APDs early in life. The purpose of this study was to determine if one measure of inhibitory control, differential reinforcement of low (DRL) rates of responding, was altered in adult rats administered the APD, risperidone, early in life. Rats received injections of vehicle or 1.0 or 3.0 mg/kg of risperidone from postnatal day 14 – 42. Testing in the DRL task began around 100 days of age. In the DRL task, rats received a food reward for operant responding, but only if responses were limited to once every five seconds or more. This criterion was decreased to once every 15 seconds two weeks later, and decreased to once every 30 seconds after two additional weeks. Females were more efficient (i.e., made fewer bar presses for each pellet received) at each delay. While not statistically significant, males treated with the 3.0 mg/kg of risperidone early in life were the least efficient group at each delay. These mean differences seen between the treatment groups warrant further investigation of cognitive control in adult rats exposed to early-life APD administration.

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8 **Abstract Title:** **The effects of early-life risperidone administration on forebrain neurotrophin expression during adulthood.**

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Abstract:
The antipsychotic drug risperidone has become increasingly popular as a treatment for children with various behavioral disorders, including autism. However, little is known about the long-term effects of early-life risperidone treatment and the permanent effects it may have on a developing brain. The frontal cortex is one of the primary targets of risperidone action in the brain, so the purpose of this study was to determine if early-life risperidone treatment alters neurotrophin levels in the prefrontal cortex during adulthood. Twenty-four rats (13 Female, 11 Male) received daily injections from postnatal days 14-42. Rats were divided into two treatment groups (1.0 and 3.0 mg/kg risperidone) and a vehicle control. Rats were sacrificed on PND 62 and their brains extracted for analysis. Prefrontal cortical tissue from the left hemisphere was examined for neurotrophin expression through the use of a rat neurotrophin RT-PCR array (SABiosciences, Inc.). Sections of prefrontal cortex were also used for immunohistochemistry. PCR results indicated an up-regulation of mRNA levels of the cytokine, Leukemia Inhibitory Factor (LIF), in the low and high dose treatment groups. Immunohistochemistry is currently being carried out using LIF antibody to verify protein level differences between groups. This study indicates that early-life risperidone treatment has the potential to alter the expression of proteins involved in cell growth and differentiation with brain regions critical for cognitive control. The behavioral consequences of these cellular changes is an area that further research should address.

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9	**Abstract Title:**	**Changes in Microglial Morphology within the Primary Somatosensory Barrel Fields of the Cortex Following Experimental Traumatic Brain Injury in Adult Male Rats**

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Abstract:
Upon impact to the central nervous system, resident microglia become activated, the cell bodies enlarge and the processes thickening. A less document morphological response is the elongation of the microglia cell body to form a rod-shaped cell, first described by Franz Nissl in the late 1800s. Similar findings have been observed in our laboratory following an experimental model of diffused brain injury. The observation is predominantly within the primary somatosensory barrel field (S1BF) of the cortex, with rod cells aligning perpendicular to the dural surface as early as 1 day post-injury. Adult male rats were subjected to a single moderate severity midline fluid percussion injury (1.9atm; 6-10 minutes righting reflex time), and perfused at 1, 2, 7 and 28 days post-injury. Neuroscience Associates Inc. were contracted for tissue sectioning and staining for the Iba-1 microglia marker. The unique alignment of microglia afforded a unique microscopic analysis by Fast Fourier Transformation (FFT). FFT represents the pixel intensity of the original photomicrograph as a frequency domain. When adjacent pixels of the original photomicrograph describe a straight line, the FFT plots a straight line though the origin along the orthogonal angle. The summation of pixel intensities in radial coordinates around the origin is used to generate a value of alignment. Alignment was greater at 1, 2, 7, and 28 days post-FPI compared to the sham, 7 days post-FPI also showed a greater alignment compared to 1 day post-FPI ($p<0.05$, One-way ANOVA). We aim to determine the causation of this sub-acute morphological change in microglia and their role within the cortex.

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10	**Abstract Title:**	**Risperidone Treatment Alters Locomotor Activity in Developing Rats**

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Abstract:
Risperidone is an antipsychotic drug that has been approved for use in children with psychiatric disorders. However, little is known about the immediate and delayed effects of risperidone in pediatric populations. Our lab has found that rats treated with risperidone early in life demonstrate hyperactivity as adults. The purpose of this study was to assess the immediate effects of risperidone on activity in younger rats, and to determine when the hyperactivity associated with prolonged developmental treatment emerges. Female and male Long-Evans rats received daily subcutaneous injections of vehicle or two doses of risperidone (1.0 and 3.0 mg/kg) from postnatal days (PND) 14 – 42. Once a week beginning on PND 14, pups were tested for locomotor activity immediately and 23 hours after drug treatment. Rats were also tested one week after treatment for two consecutive days. Throughout treatment, male and female rats were profoundly sedated immediately after risperidone administration. By PND 35 and 42, risperidone-treated rats were significantly more active than controls at 23 hours post-administration. One week after the cessation of risperidone administration, the risperidone-treated rats remained significantly more active than the vehicle-treated rats. Our study indicates that risperidone, as it does in adult rats, powerfully suppresses activity in young rats. After three weeks of risperidone administration, hyperactivity can be seen at 23 hours post-administration. Whether this is a consequence of prolonged risperidone administration, a critical period in brain development at PND 35, or some interaction of these factors remains to be determined.

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11 **Abstract Title:** **Alterations of Brain Function during Working Memory in Military Veterans with Mild Brain Injury or Post Traumatic Stress Disorder**

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Abstract:
Differentiating symptoms of post-traumatic stress disorder (PTSD) from sequelae of mild traumatic brain injury (mTBI) is a significant challenge in the clinical setting. Veterans with both disorders may show similar cognitive impairment (e.g. memory loss). We investigated the possible differential brain responses underlying PTSD and mTBI groups. In this pilot study, four clinical groups of military veterans including mTBI (6), PTSD (4), both mTBI and PTSD (6), and neither mTBI nor PTSD (i.e. "combat control") (5) performed a working memory task (delayed match-to-sample) under functional Magnetic Resonance Imaging (fMRI). Event-related fMRI data were collected by using a 3 Tesla Siemens Trio MRI scanner. Multiple regression analyses of fMRI blood-oxygen-level dependent (BOLD) signals were analyzed in several regions of interest critical to cognitive functions. The combat control group significantly differed from patient groups at the anterior cingulate cortex, known for cognitive control (uncorrected $p = 0.003$). The mTBI group shows a significant difference in amygdala activation ($p = .016$), whereas the PTSD group showed no significant difference ($p > 0.2$). Significant repetition effects were found in the frontal BA10 region between the combat control and the PTSD and mTBI groups ($p < 0.01$). In the PTSD group, in frontal BA10 brain activity decreased with repeated repetitions, whereas the other three groups displayed a positive trend. Functional BOLD changes that are associated with repetition priming may clinically differentiate PTSD and mTBI. The pilot results suggest that PTSD and mTBI affected differential as well as overlapping neural mechanisms during a cognitive task.

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12 **Abstract Title:** **Adding Insult to Injury: Hypoxia Following Ethanol Exposure Produces Multiplicative Damage In Vitro**

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Abstract:
Ethanol (ETOH) exposure and oxygen deprivation are serious events and can have detrimental effects for the fetus. The hypothesis that prenatal ETOH exposure and ETOH withdrawal increases the consequences of a hypoxic challenge in the developing brain, due to shared excitotoxic mechanisms has been examined. When the two insults occur together at subthreshold levels, an exacerbation of damage could occur. This hypothesis was examined in two experiments measuring cell damage in an organotypic hippocampal slice culture model. The culture model contains neurons and glial cells and maintains significant elements of the complex circuitry of an intact hippocampus. The first experiment investigated the effects of ethanol or control medium followed by varying levels of hypoxia and the second observed age differences in sensitivity to ETOH and hypoxia. The combination of ETOH and 30 min hypoxia produced significant cell damage in the CA1 and CA3 regions of the hippocampus. Remarkably, there were age differences in sensitivity to ETOH in combination with hypoxia; in younger rats, 30 min hypoxia alone causing cell damage in both the CA1 and CA3 regions relative to controls. The combination of ETOH and hypoxia exaggerated this effect more in the CA3 than the CA1 regions in younger rats. These results show that prior ETOH exposure can leave the CNS vulnerable to subsequent hypoxic challenges and this may help explain the variation sometimes observed in outcome following fetal alcohol exposure or neonatal hypoxia.

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13 **Abstract Title:** **Chronic Preexposure to Cocaine Facilitates Sexual Conditioning and Increases Resistance to Extinction in Male Japanese Quail**

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Abstract:
Previous research suggests that chronic preexposure to cocaine (COC) enhances sexually conditioned approach behavior and facilitates copulatory performance in male Japanese quail. However, the effect of chronic COC preexposure on the extinction of sexually conditioned approach behavior has yet to be examined. In the present experiment, male Japanese quail (N = 32) were administered COC (10 mg/kg ip) once daily for 10 days and locomotor activity was measured. It was hypothesized that chronic preexposure to COC would result in locomotor sensitization. The results indicated that quail that received COC exhibited increased locomotor activity compared to saline controls, $F(1, 30) = 10.21$, $p < 0.05$. After a 10-day withdrawal period, 10 sexual conditioning trials were conducted, one per day, that consisted of presentation of a conditioned stimulus (CS) light followed by sexual reinforcement for paired subjects. Unpaired subjects were treated similarly except they received a copulatory opportunity 3-5 hrs prior to conditioning. Following conditioning trials, all subjects received extinction trials where the CS was presented without a copulatory opportunity. It was hypothesized that a history of COC would facilitate sexually conditioned approach behavior and increase resistance to extinction. The results indicated that previous exposure to COC facilitated conditioned approach behavior to the CS light. Paired subjects that received COC demonstrated greater approach to the CS across conditioning trials compared to paired saline controls, $F(9, 252) = 2.04$, $p < 0.05$. Furthermore, a history of COC increased resistance to extinction. COC paired subjects were slower to extinguish conditioned approach behavior compared to saline paired subjects. These findings may suggest that chronic preexposure to COC enhanced sexual motivation and that enhancement may persist under extinction conditions.

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14 **Abstract Title:** **Lobeline Reduces Fetal Alcohol-Induced Water Maze Memory Deficits in Rats**

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Abstract:
Prenatal alcohol exposure is the leading preventable cause of mental retardation in the United States. Children with Fetal Alcohol Spectrum Disorders typically exhibit hyperactivity as well as various learning and memory deficits similar to those observed with Attention Deficit Hyperactivity Disorder (ADHD). Standard pharmacotherapies for ADHD have shown varying efficacy in those with FASD, suggesting that alternative options are crucial. We have previously shown that lobeline can reduce hyperactivity induced by neonatal alcohol exposure. Here, we evaluated whether lobeline could also reduce learning and memory deficits. Male and female Sprague-Dawley rats were intubated with either ethanol (6g/kg/ day) or milk diet during postnatal days (PND) 1-7 or received no intubation. On PND 40 and 41, animals were injected subcutaneously with lobeline 30 minutes prior to a spatial water maze test. Animals were trained to reach a hidden platform in a maze with multiple choices and then tested again for 24 hr retention. The learning criterion was 2 consecutive errorless trials. Alcohol-exposed offspring showed normal acquisition of the task but were impaired during retention testing. Lobeline either reduced or eliminated these deficits. These findings support further study of lobeline treatment for some symptoms of FASD.

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15 **Abstract Title:** **Novel CNS therapeutic suppresses proinflammatory cytokines in a rodent model of diffuse traumatic brain injury**

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Abstract:
Traumatic brain injury (TBI) is a major public health issue with an unmet need for therapeutic interventions that alter pathology progression and improve longer-term neurologic outcomes. Neurologic damage following TBI is complex and is the result of both the immediate primary impact injury and secondary mechanisms that are more amenable to therapeutic intervention. Post-traumatic glial activation and increased production of proinflammatory cytokines is an early secondary event that contributes to pathology progression in both animal models and human head injury patients. Selective pharmacological attenuation of the acute proinflammatory cytokine surge, therefore, offers the potential for altering pathology progression and later stage neurologic sequelae. 151WH is a novel, CNS-penetrant small molecule drug that selectively restores injury- or disease-induced overproduction of proinflammatory cytokines towards homeostasis, with resultant improvement in neurologic outcomes. We report here that 151WH attenuates the post-injury cytokine surge in a midline fluid percussion model when administered briefly after TBI. Diffuse brain injury was induced by moderate fluid percussion injury in mice. Inflammatory cytokines were found elevated in the cortex within 1 hr post-injury compared to uninjured sham, with significant peak responses occurring within 9 hrs post-injury. By 24-48 hrs post-injury, most of the cytokines were at or below sham levels. Drug administration of 151WH at low doses (1.5 or 5 mg/kg) post-injury led to a significant reduction in the levels of cytokines seen at 6 hrs post-injury. Our results extend the potential clinical utility of 151WH to diffuse brain injury and offers a potential new therapeutic approach to CNS disorders.

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16 **Abstract Title:** **Intranasal Delivery of Long-acting Insulin on Spatial Learning and Memory Processes in the Aged F344 Rat**

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Abstract:
Impaired brain insulin signaling (i.e., type 3 diabetes) may mediate decreased cognitive function with pathological aging. Indeed, intranasal insulin delivery to the brain has yielded promising clinical and pre-clinical results and can alleviate aspects of memory decline in mild cognitive impairment (MCI) and Alzheimer's disease (AD). Similar results are seen in animal models of diabetes or AD. It is unclear, however, how increasing brain insulin levels might alter functional communication and improve learning and memory. We used the F344 rat model of aging to test the hypothesis that long-acting insulin Levimir® could improve cognitive function in aged animals (21 months old). Rats received one of three daily doses of Levimir®, equivalent to those used in clinical trials (0.143, 0.286 or 0.571 IU/ Kg/ day) for 18 days. Ten aged and ten young-adult (3 months old) rats also received saline vehicle. Animals were trained on the Morris water maze spatial task starting on the fifth day. A subset of animals was used to test effects of intranasal insulin on peripheral glucose levels. Similar to clinical trials, animals receiving low dose insulin (10 IU/ day) improved maze performance on the 24 hr recall task. Animals were also tested on 72 hr recall after which brains were collected and biochemical analyses of brain insulin levels, insulin receptor and IRS-1 signaling conducted. Ongoing studies will evaluate electrophysiological components likely to participate in the improved hippocampal learning seen in response to intranasal Levimir®.

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17 **Abstract Title:** **GRM2 C407X rats do not develop mechanical hypersensitivity in an alcohol- and high fat diet-induced model of pancreatitis**

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Abstract:
Alcohol and a diet high in fat are contributing factors to the development of pancreatitis which causes severe abdominal pain in patients. We focused on the role of metabotropic glutamate receptors (mGluR) in this inflammatory process. Typically glutamate is an excitatory neurotransmitter, yet activation of group II mGluRs (mGluR2/GRM2, mGluR3/GRM3) which are coupled to G?i proteins results in activation of inhibitory signaling cascades that are anti-nociceptive in models of somatic pain. Here we investigated the role of mGluR2 in a diet-induced chronic pancreatitis model of visceral pain. GRM2 C407X rats (Transposagen) carrying an N-ethyl-N-nitrosourea-induced point mutation and wildtypes were fed a liquid diet with 6% ethanol and 30% fat. During this time mechanical and heat sensitivity were determined. Primary mechanical sensitivity was characterized by probing the abdomen 10 times each with 4 different von Frey filaments (0.4, 1.2, 5.5, 15 gf) and recording the number of responses. The up-down method was used to determine secondary mechanical sensitivity of the footpads, and latencies in the hotplate (50°C) test for heat sensitivity. Baseline thresholds/latencies were not different. Within 4 weeks wildtypes developed mechanical and heat hypersensitivity. Abdominal stimulation with the 1.2 gf von Frey filament resulted in doubling of responses. Strikingly, GRM2 C407X rats did not develop mechanical hypersensitivity. Both strains developed heat hypersensitivity. These data suggest the GRM2 C407X mutation inhibits the development of inflammation-induced mechanical hypersensitivity in a visceral pain model. We speculate that this mutation results in a soluble truncated form of mGluR2 able to repress glutamate activation. These data suggest that mGluR2 is an interesting target for novel drug therapy development for visceral pain.

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18 **Abstract Title:** **TNFα Receptor R1/R2 Dual Deficient Mice Develop Chronic Arthritis**

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Abstract:
The biological activity of TNFα is mediated by two subtype receptors, TNFR1 and TNFR2, co-expressed on the surface of most cells. Inflammatory stimuli trigger TNFα receptors proteolytic cleavage giving rise to two soluble fragments, p55 sTNFR and p75 sTNFR that inhibit further binding and activity of TNFs. The present study investigated pain related behavioral differences between dually deficient (TNFR1/R2-/-) and matched wild type (WT) mice, in an arthritis model. Monoarthritis was induced by injecting complete Freund's adjuvant into one knee joint cavity. All injected mice exhibited less weight bearing and had swollen joints, shortened thermal paw withdrawal latency (PWL) and lowered mechanical threshold to von Frey fiber stimuli on foot pads. Pain related behaviors in TNFR1/R2-/- mice persisted for 1 - 2 weeks longer than in the WT mice. At week 8, animals were given a second inflammatory insult by colonic mustard oil infusion. All animals again had shortened PWLs and lowered mechanical thresholds on their footpads that persisted for 2 weeks. Whereas WT mice recovered by week 10, TNFR1/R2-/- mice remained hyperalgesic on the foot and re-developed spontaneous pain related posture in the primary CFA injected leg through week 23. The pain related behaviors were effectively attenuated by i.t. P2X7 receptor antagonist, A438079. Protein proteome analysis found a significant increase in serum cytokine/chemokines: TNFα, RANTES, CCL2, CXCL1, CXCL9, and CXCL10 in TNFR1/R2-/- mice with chronic arthritis compared to WT mice. These findings support the concept that TNFR gene alterations play a pivotal role in the persistence of chronic arthritis.

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19 **Abstract Title:** **Acute Pancreatitis, Endothelin and Gene Expression Profiling.**

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Abstract:
Pancreatitis is manifested with abdominal pain. Neural innervation of pancreas is important in the initiation and maintenance of inflammation. Activation of pancreatic sensory neurons causes release of neurotransmitters in the spinal cord and neurogenic activation signals in pancreas producing plasma extravasation. Endothelin-1 (ET-1) a potent vasoconstrictor peptide is increased in inflammation and provokes pain through endothelin-A receptors (ET-A). Hypothesis: Gene expression profile in thoracic spinal cord and dorsal root ganglia (DRG) elucidate translational and posttranslational modifications in neuronal system in pancreatitis model. Rats were i.v. injected with either DBTC or vehicle. Spinal cord and DRG were taken at the peak of inflammation and processed for transcriptional profiling with a microarray biased for rat neuron-specific genes. Animals were treated with ET-A antagonists and ET-B. Spontaneous pain related hypersensitivity were measured. Animals developed pancreatic inflammation and secondary pain-related mechanical and thermal hypersensitivity. Gene expression differentially expressed in nervous tissue of pancreatits animals compared to controls included up-regulated and downregulated unique genes. Treatment with an ET-A antagonist (BQ123) revealed significant protection against inflammatory and pain related behaviors in animals with pancreatitis. Conclusions: Genes identified in the analysis can be selected as future biological markers for the diagnosis of pancreatitis and/or targeted gene therapy. Endothelin-A receptor antagonist (BQ123) protects against inflammatory pain response in this pancreatitis model.

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20 **Abstract Title:** **Calpastatin overexpression protects against traumatic SCI in mice**

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Abstract:
Small molecule inhibitors of calcium-dependent proteases, μ- and m-calpains, protect against neurodegeneration induced by a variety of insults including spinal cord injury (SCI). However, these compounds also inhibit other proteases, which has made it difficult to evaluate the contribution of calpains to neurodegeneration. Calpastatin is a highly specific endogenous inhibitor of μ- and m-calpains, thus eliminating the specificity and efficacy problems associated with small molecule inhibitors. In the present study, we utilized transgenic mice that overexpress human calpastatin under the prion promoter (PrP-hCAST) to evaluate the hypothesis that calpastatin overexpression will reduce calpain-mediated proteolysis, attenuate lesion volume, and improve locomotor function following contusive SCI. Contusion SCI was produced following a T10 laminectomy at 70 kdyn force setting using an Infinite Horizons (IH) SCI device. Western blot analysis demonstrated that calpastatin overexpression reduced α-spectrin breakdown (145 KDa) by 51% at 24 hours post-injury, as compared to wild-type controls ($p<0.05$, n=3/group). PrP-hCAST (n=13) mice displayed a significant improvement in locomotor function at 1 and 3 weeks after contusive SCI compared with the wild-type controls (n=9, $p<0.05$), but were similar at four weeks postinjury (repeated measures ANOVA and Bonferroni post-hoc test). Histological assessment of lesion volume and tissue sparing, performed on same animals used for behavioral analysis, revealed that calpastatin overexpression resulted in a 30% decrease in lesion volume ($p<0.05$) and significant increases in total tissue sparing, white matter sparing, and gray matter sparing at 4 weeks postinjury compared with wild-type animals. The finding that calpastatin overexpression significantly reduces calpain-mediated proteolysis and lesion volume following SCI provides support for the hypothesis that sustained calpain-dependent proteolysis contributes to pathological deficits after traumatic SCI.

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21	**Abstract Title:**	**Tracking the delivery of GDNF to the putamen of Rhesus Monkeys using Tracers of Different Molecular Weights**

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Abstract:
Convection enhanced delivery (CED) has been used in clinical trials to deliver therapeutics for the treatment of neurodegenerative diseases. However, the distribution of the agents is frequently unknown at the time of delivery. Tracers visible on MRI and co-infused with a therapeutic are a possible solution. Our objective was to test the hypothesis that tracers of similar molecular weight to the therapeutic would distribute similarly. Our aim was to test which of two tracers would better represent the distribution of the trophic factor GDNF. To test this hypothesis we developed a chronic, implanted CED system in rhesus monkeys. A multiport catheter was implanted in the putamen using image-guided stereotactic techniques. The infusion system consisted of an implanted pump which maintained the catheter's patency, an in-line "Y" connector which we repeatedly accessed through small skin flap incisions and connected to an external syringe pump to deliver small volumes (~100 µL) of tracer. At the final of several infusions we co-infused a tracer with GDNF (MW = 66-90 kDa). All infusions occured while the animal was imaged using MRI to monitor the development of the infusion distribution. The system was implanted in ten female rhesus monkeys and maintained for several months allowing multiple infusions through the same catheter. Animals were infused either with the low molecular weight tracer, Gd-DTPA (Magnevist) (MW=938 Da) or the high molecular weight tracer Gd-DTPA-albumin (Galbumin) (MW~70 kDa). Comparing the histological and the MR imaging measurements of tracer and GDNF distribution showed that the larger MW tracer better matched the distribution of the GDNF. There was also evidence that the co-infusion of albumin with GDNF facilitated a wider distribution.

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22	**Abstract Title:**	**Pursuing Treatments for Disorders of Consciousness: Theories of Common Neural Mechanisms Underlying Consciousness and Locomotor Activity**

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Abstract:
Following a severe traumatic brain injury, the majority of individuals emerge from coma, but many are slow or fail to regain consciousness. These individuals are diagnosed with a disorder of consciousness, such as vegetative state or minimally conscious state. Currently, we have limited evidence for effective treatments to stimulate arousal and awareness, both components of consciousness. However, an abundance of evidence from basic science and human clinical literature demonstrates that experience-dependent neural plasticity can occur in the injured brain through intensive physical interventions, such as locomotor training with bodyweight support and treadmill. Additionally, a synthesis of this literature with other scientific work, including sleep and respiratory research, reveals a potential overlap in neural substrates and feedback mechanisms underlying consciousness and physical activity. Based on this synthesis, two newly generated "bottom-up" mechanistic theories will be presented: the posture and locomotion theory and the aerobic drive theory. These theories will discuss, at minimum, 1) the role of the locus coeruleus in both sleep-wake cycle regulation as well as static and dynamic postural control, 2) the mesencephalic locomotor region and the reticular activating system as common components of the pedunculopontine tegmental nucleus, and 3) the host of evidence describing enhanced cognition post-aerobic exercise in aging adults and persons with neurological disorders. We hypothesize that physical exercise, in particular locomotor-like activity, is capable of priming the reticular activating system for arousal, thus preparing the cortex to receive, process, and respond to afferent information via thalamocortical pathways.

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23 **Abstract Title:** **The mitochondrial protective effects of an anti-parkinsonian synthetic peptide derived from proGDNF**

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Abstract:
Current treatments of Parkinson's disease (PD) target its chronic disabling multi-symptoms by regulating levels and/or signaling actions of the neurotransmitter dopamine. However, as the disease progresses, dopamine-producing neurons in the substantia nigra pars compacta are continually lost to a point where these existing treatments become ineffective. While the specific cause of PD is unknown, the development of parkinsonian symptoms has been observed in patients following prolonged exposure to environmental toxins and/or genetic alterations that target the mitochondria, thereby triggering a cascade of events leading to defects in cellular bioenergetics and ultimately neuronal death. Thus as a strategy for the longterm treatment of PD, therapeutics are needed that not only restore dopaminergic function, but also provide neuroprotection via the mitochondria. Recently, we demonstrated that an amidated, eleven amino acid propeptide from the glial cell line-derived neurotrophic factor (GDNF), named dopamine neuron stimulating peptide-11 (DNSP-11), produced anti-parkinsonian effects by restoring dopaminergic activity in animal models - likely involving the mitochondria. To test our hypothesis, we measured the protective effects of DNSP-11 in dopaminergic neuronal cell lines (MN9D, B65) against mitochondrial-specific toxins associated with parkinsonism symptoms in patients and animal models, by measuring mitochondrial potential, caspase-3 activity, and TUNEL staining. Furthermore, we examined the protective effects of DNSP-11 on mitochondrial functioning by measuring the reserve respiratory capacity of MN9D treated and non-treated cells exposed to toxins. Collectively, our data support a mitochondrial neuroprotective hypothesis and suggest further evaluation of DNSP-11 as a downstream therapeutic for age-related neurogenerative diseases, like Parkinson's disease.

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24 **Abstract Title:** **Phenelzine Protects 4-Hydroxy-2-nonenal-Mediated Respiratory Dysfunction in Isolated Mouse Brain Mitochondria: An In Vitro Study**

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Abstract:
The potential protective effects of phenelzine, an older MAO inhibitory antidepressant drug, against 4-HNE-induced mitochondrial respiratory dysfunction was investigated. Phenelzine has been shown to be a chemical scavenger of LP-derived reactive aldehydes by virtue of its hydrazine functional group which can covalently react with 4-HNE. Bioenergetics in healthy, Ficoll gradient-isolated mouse total cortical mitochondria were measured using the Seahorse Bioscience XF24 Extracellular Flux Analyzer (Seahorse Bioscience, North Billerica, MA, USA). Complex I (pyruvate+malate substrate) and complex II (succinate substrate)-driven oxygen consumption rates (OCR, pmoles O2/min) were assessed after exposure to 4-HNE which inhibited both in a dose-dependent manner. On the other hand, pretreatment of mitochondria with equimolar concentrations of phenelzine significantly antagonized the respiratory depressant effects of 4-HNE. For instance, 30 μM 4-HNE attenuated complex I function by 37% whereas phenelzine pretreatment reduced this to 17% ($p<0.05$ vs. 4-HNE alone). Complex II was inhibited by 24% by 4-HNE which was completely prevented by phenelzine. Consistent with phenelzine being a scavenger of 4-HNE, western blot analysis demonstrated that the compound decreased 4-HNE binding to mitochondrial proteins in a dose-dependent manner. An investigation is ongoing to evaluate the mitochondrial protective effects of phenelzine in vivo after experimental traumatic brain injury (TBI).

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25 **Abstract Title:** **Analysis of Lipid Peroxidation Biomarkers in Adults with Traumatic Brain Injury**

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Abstract:
Traumatic brain injury (TBI) is a heterogeneous mixture of pathologies which often end with permanent neurologic sequelae. Clinical presentations include subarachnoid hemorrhage, diffuse axonal injury and subdural hematoma, among others. Free radical-induced lipid peroxidation has been demonstrated to be a major secondary injury mechanism in TBI pathophysiology. Recently, we have been exploring the use of the free-radical mediated formation of isoprostanes from arachidonic acid and neuroprostanes from docosahexaenoic acid in cerebrospinal fluid (CSF), blood serum and plasma as potential TBI biomarkers. These markers are elevated in CSF following aneurysmal subarachnoid hemorrhage (SAH) but studies in acute traumatic brain injury patients have shown mixed results. The purpose of this investigation was to determine the profile of lipid peroxidation markers in different matrices following TBI. Samples of serum, urine and CSF were collected from TBI patients serially from hospital admission up to 14 days, when available. Gas chromatography/mass spectrometry was used for 5 & 15 F2t-isoprostanes, F2-isofurans, F4-neuroprostanes analysis and Western Blot was used for analysis of spectrin degradation products. In this preliminary report, all patients [n=3 moderate TBI (2 males) GCS 9-12, 10 severe TBI (7 males) GCS ≤ 8] are included. Five of the TBI patients had associated SAH. Concentrations of each biomarker were determined in each matrix and compared to published values from TBI, SAH and control populations. Preliminary data analysis of this pilot investigation suggests that serum isoprostane and isofuran concentrations are elevated in TBI compared to normal values. In addition, CSF neuroprostane values in TBI are elevated when compared to normal CSF controls and are comparable to values previously seen in patients with aneurysmal SAH or TBI.

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26 **Abstract Title:** **PINK1-deficient Mice as a Potential Model to Study Common Mechanisms of Diabetes and Neuronal Dysfunction**

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Abstract:
Mutations in the gene coding for PTEN-induced kinase 1 (PINK1) cause recessive early-onset Parkinsonism. Here we show that PINK1 ablation in mice results in a combination of neurological, cell signaling and metabolic defects. Mice lacking PINK1 develop mitochondrial dysfunction and age-dependent dopamine loss associated with increased dopamine turnover in the striatum. Embryonic fibroblasts and primary neurons from PINK1-deficient mice display reduced Akt phosphorylation in response to insulin-like growth factor 1 (IGF-1) and insulin. As a consequence, several Akt and/or mammalian target of rapamycin substrates are abnormally regulated in IGF-1-treated PINK1-deficient cells, including glycogen synthase kinase-3β and the transcriptional factor FoxO1. Moreover, IGF-1-mediated protection against apoptosis is abrogated in PINK1-deficient cells, suggesting that impaired cell survival signaling may contribute to recessive Parkinsonism. These results are relevant because Akt phosphorylation is decreased in the substantia nigra of PD patients and the IGF-1/Akt signal transduction pathway protects dopamine neurons in several animal models of PD. Recent studies have suggested that diabetes is associated with a higher risk of future PD, especially in younger patients. Given the importance of Akt signaling in metabolic regulation, we studied the effects of PINK1 deficiency on peripheral glucose metabolism and found that PINK1-deficient mice have a diabetes-like phenotype. In summary, our results show that PINK1 ablation results in dopaminergic, cell signaling and metabolic defects and suggest that the association of diabetes with early-onset PD may be explained by shared pathogenic mechanisms of the two disorders. Accordingly, PINK1-deficient mice may be an attractive model to study common causes and/or mechanistic links between diabetes and neurodegeneration.

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27 **Abstract Title:** **Diffuse Brain Injury Results in Altered Regulation of Thrombospondins and Plasticity Genes in Rats; Implications for Post-traumatic Circuit Reorganization?**

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Abstract:
Despite preventative efforts (e.g., helmets and seatbelts), diffuse traumatic brain injuries (TBI) occur at a staggering rate and frequently result in late-onset post-traumatic neurological impairment, including sensory sensitivity. In rodents, diffuse TBI leads to a late-onset, gain-of-function sensory-sensitivity to whisker stimulation hypothesized to result from maladaptive circuit reorganization in the thalamocortical circuit. For circuit reorganization to manifest in neurological impairment, synaptogenesis is necessary. Here, we hypothesize that midline fluid percussion injury induces a distinct temporal profile of thrombospondin-mediated synaptogenesis which may be a pivotal point to solidify late-onset neurological impairment. Adult male, Sprague-Dawley rats were subjected to a single moderate severity (1.9 atm; 6-10 min righting reflex time) midline fluid percussion injury. Using real-time PCR and western blots, we quantified synaptogenic and thrombospondin-related molecular changes in the somatosensory thalamocortical circuit at multiple time points after mFPI (out to 28 days). Initial experiments support our hypothesis with a significant injury-induced reduction in the gene expression of synaptophysin at 7 days post-injury, which recovered by 28 days compared to sham in the thalamus. Synaptophysin protein expression followed a similar dynamic, indicating a loss of synaptic content immediately after injury followed by synaptic rebuilding. Thrombospondin gene expression was more volatile, demonstrating increases as great as 30 times sham levels between days 1 and 7 post-injury. This is the first indication that thrombospondins may be playing a role in circuit reorganization after experimental diffuse brain injury, as has been reported after experimental stroke. These data will serve to identify a rehabilitative and therapeutic window for diffuse brain injury treatment by focusing on the role of synaptogenesis in circuit reorganization.

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28 **Abstract Title:** **Rod microglia differentially express immune receptors and align with neuronal elements**

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Abstract:
Traumatic brain injury (TBI) initiates multiple molecular and cellular cascades, including inflammation. The primary somatosensory barrel field (S1BF) cortex is particularly susceptible to neuropathology after experimental moderate diffuse TBI with consistent axonal injury, neuronal atrophy and consequent neuroplasticity. Microglial cells are known to play a pivotal role in the inflammatory events post-TBI, with documented neurotoxic and neurotrophic roles. At the turn of the last century, Nissl described a specific rod-like morphology of microglia in conditions of infection and toxic exposure. Little research has been conducted on rod-cells and their role within the brain is yet to be elucidated. Here, we test the hypothesis that rod microglia differentially express inflammatory markers following diffuse brain injury. Adult male, Sprague-Dawley rats were subjected to a single moderate severity (1.9 atm; 6-10 min righting reflex time) midline fluid percussion injury (FPI). Iba-1 (ionized calcium binding adapter protein) immunohistochemistry identified all microglia, with their phenotype compared between FPI brain-injured and uninjured animals. Following FPI, a vast proportion of microglia in S1BF of brain-injured animals show elongation and alignment, with a rod-like morphology similar to that described by Nissl. Rod microglia couple together to form trains that span multiple cortical layers. At day 7 post-FPI, some cells in these trains co-localize with other known markers for active microglia, including CD68 and ED-1; however these cells are not CD11b or CD11c positive. In addition, rod microglia align parallel to both neurofilament (NF-M) in axons and microtubule associated protein (MAP-2) in dendrites, but not with astrocytes. Results thus far indicate that diffuse brain injury induces a sub-acute morphological change in microglial alignment with neuronal processes in the S1BF. We speculate that these microglia 'rod-cells' are involved in synaptic stripping associated with late onset behavioral sensory sensitivity.

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29	**Abstract Title:**	**Binge Alcohol Intoxication Results in Aberrant Hippocampal Microglia Morphology and Reduced Microglia Number**

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Abstract:
Earlier studies demonstrated that hippocampal microglia activation occurs 2 days after binge alcohol exposure; however, it is not known if activation begins during or after intoxication. Therefore, we examined the effects of alcohol on hippocampal microglia morphology and number during binge intoxication. Adolescent (n=16) and adult male (n=15) Sprague-Dawley rats were administered 25% (w/v) alcohol or control diet every 8 hours for 2 or 4 days according to the Majchrowicz model. Brains were harvested 2 hours following the last dose and processed for Iba-1 immunohistochemistry to stain all microglia. No time or age-dependent changes in activation were found based on morphological criteria, but binge alcohol intoxication did alter microglia morphology. In both age groups, microglial processes displayed a distinct beaded appearance, which is not typical of activation. Image analysis revealed a significant decrease in microglia number after 4-day binge alcohol exposure. In the dentate gyrus, microglia number decreased by 19% (p=0.04) and 26% (p=0.01) in alcohol-treated adolescent and adult rats, respectively. Similarly in the CA fields, microglia number decreased by 19% (p=0.01) and 27% (p=0.01) in alcohol-treated adolescent and adult rats, respectively. Morphology and cell count data indicate that binge alcohol intoxication alters microglia similarly in adolescent and adult rats. Although our past reports show upregulation of other indices of microglia activation such as CR3 and the 18kDa translocator protein during alcohol intoxication, microglia did not exhibit activated morphology based on Iba-1 staining. Additionally, alcohol reduced microglia number, suggesting that alcohol may have detrimental effects on microglia function during intoxication.

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30	**Abstract Title:**	**Potency Of Neu2000 On Various Reactive Oxygen/Nitrogen Species Using In Vitro Oxidative Model Systems**

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Abstract:
Numerous studies have documented that reactive oxygen/nitrogen species (ROS/RNS) formation and mitochondrial dysfunction are major factors of secondary injury following spinal cord injury (SCI). In this context, compounds that counteract these factors may be effective as a therapeutic treatment for SCI. Recently, Neu2000 [2-hydroxy-5-(2,3,5,6-tetrafluoro-4 trifluoromethylbenzylamino) benzoic acid] was found to be a dual-acting neuroprotective agent functioning as an inhibitor of N-methyl-D-aspartate (NMDA) receptor–mediated excitotoxicity as well as a free radical scavenger. The present study was undertaken to elucidate the pharmacological action of Neu2000 using biochemical/biological in vitro oxidation stress assays. In the biochemical assays, Neu2000 effectively scavenged superoxide, nitric oxide and hydroxyl radicals with an IC50 of 80.22, 130.54, 75.95 µM, respectively. These results also confirmed that Neu2000 scavenges authentic peroxynitrite as well as peroxynitrite derived from SIN-1 in the presence (IC50= 5.57 and 1.15 µM, respectively) or absence (IC50= 1.82 and 0.30 µM, respectively) of physiological concentrations of bicarbonate anion. For the in vitro biological assays, mitochondria from healthy rat spinal cord were chosen as a model to study the actions of Neu2000 on free radical-mediated mitochondrial function and oxidative stress. In these studies, it was demonstrated that Neu2000 strongly counteracted the respiratory chain complex-III inhibitory action of antimycin A that substantially increased mitochondrial total ROS/RNS and hydrogen peroxide (H2O2) generation. Further, lipid radicals derived from Fe(III)/ascorbate-catalyzed mitochondrial lipid peroxidation were also quenched by Neu2000. The calculated IC50 values of Neu2000 were 2.3, 7.3 and 3 µM for total ROS/RNS, H2O2 and lipid peroxidation models, respectively. Finally, Neu2000 reduced hydroxyl and peroxynitrite mediated protein carbonyl formation in mitochondria using the anti-DNP immunoblot assay. Overall, Neu2000 may provide a new class of pharmacological agent by targeting oxidative damage and mitochondria function, and has potential as a novel pharmacological approach for reducing certain secondary injury events after SCI.

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31	**Abstract Title:**	**1,4-Diphenethyl-based Lobelane Analogs: Potent and Competitive Inhibition of the Vesicular Monoamine Transporter-2**

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Abstract:
ADHD is characterized by medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC) and striatal dysfunction. Methylphenidate (MPH), a DAT and NET inhibitor, and atomoxetine (ATO), a selective NET inhibitor, are prescribed for ADHD. Spontaneously Hypertensive Rats (SHR), an ADHD model, given MPH during adolescence, enhanced cocaine self-administration in adulthood. We hypothesize that MPH during adolescence will produce a lasting increase in DAT function and cell-surface expression in mPFC and OFC of SHR, while ATO will not. SHR, Wistar-Kyoto inbred (WKY) and Wistar outbred (WIS) received MPH (1.5 mg/kg, po), ATO (0.3 mg/kg, ip) or vehicle on P28-55. DAT function and cellular expression were assessed during P77-85. Saturation analysis of [3H]dopamine uptake and biotinylation assays were performed using mPFC, OFC and striatal synaptosomes. For each brain region, Vmax and Km values for control rats from each strain did not differ. MPH increased Vmax in SHR mPFC, decreased Vmax in WKY OFC and decreased Km in WIS OFC, and had no effect in striatum. MPH did not alter cellular distribution in any brain region. These results indicate trafficking-independent DAT functional alterations in response to MPH. ATO decreased Vmax and DAT surface expression in SHR OFC, suggesting that NET inhibition decreases DAT function and cell surface expression. ATO decreased DAT Vmax, but not expression in WIS OFC, suggesting trafficking-independent regulation. ATO decreased Vmax in SHR striatum, which was accompanied surprisingly, by increased DAT total expression. Despite sparse NET expression in striatum, ATO altered DAT function and expression in this dopamine rich brain region. In conclusion, adolescent treatment with MPH and ATO produce different brain region specific alterations in DAT function and cellular expression, and thus, may differently influence cocaine vulnerability in adults with ADHD.

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32	**Abstract Title:**	**Insulin Inhibition of Gastric-Related Neurons of the Dorsal Motor Nucleus of the Vagus via KATP-Channels is PI3K-Dependent**

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Abstract:
The dorsal motor nucleus of the vagus (DMV) in the caudal brainstem is comprised mainly of preganglionic parasympathetic neurons that control the subdiaphragmatic viscera and thus participates in energy homeostasis regulation. Insulin, which is critical for glucose metabolism in the body, crosses the blood brain barrier and is transported into the brainstem over twice as rapidly as into whole brain and may affect glucose balance via central mechanisms. Insulin receptors (IRs) are expressed in the DMV, and are located in proximity to gastric-projecting DMV cells. Certain pathologies, including diabetes, can disrupt vagal circuitry and lead to gastric dysfunction. Despite growing evidence that insulin action in the brain is critical for energy homeostasis, little is known about insulin's action in the DMV. We used whole-cell patch-clamp recordings in brainstem slices to identify effects of insulin on synaptic input to gastric-related DMV neurons, identified subsequent to injection of a retrograde label into the gastric wall. Insulin application (1 □M) significantly reduced the frequency of action potential firing (42% decrease; $p<0.05$) and spontaneous excitatory postsynaptic currents (sEPSCs; 59% decrease; $p<0.05$), with no change in amplitude. Insulin effects on sEPSC frequency were eliminated in the presence of a KATP channel antagonist, tolbutamide (200 μM), or the PI3K inhibitor, wortmannin (100 nM), suggesting that insulin inhibition of excitatory input to gastric-related DMV neurons was mediated by KATP channels and depended on PI3K activity. Analogous experiments are also being conducted on DMV neurons of a mouse model of Type-1 diabetes. Insulin regulation of synaptic input in the DMV may influence autonomic visceral regulation and thus systemic glucose metabolism.

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33 **Abstract Title:** **p38α MAPK knockout in microglia reduces TNFα overproduction and rescues cortical neurons against LPS insult in neuron-microglia coculture**

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Abstract:
Aberrant microglia activation has been implicated as playing an important role in the process of neurodegeneration in the CNS. Several lines of evidence suggest that overproduction of the proinflammatory cytokine TNFα from activated microglia induces neuronal death, and an elevated level of TNFα has been consistently observed in postmortem brain tissue from Alzheimer's disease (AD) patients. Our previous study showed that inhibition of p38α MAPK with a selective small molecule p38α MAPK inhibitor suppressed brain proinflammatory cytokine production, and attenuated synaptic protein loss in an AD-relevant mouse model. In the present study, we used primary microglia from either wildtype mice or microglia p38α MAPK conditional knockout mice in co-culture with cortical neurons from wild-type mice. In wild-type microglia-neuron co-cultures, LPS treatment for 72 hr led to a significant increase in TNFα production, accompanied by synaptic protein loss and neuron death. In contrast, p38α MAPK deficiency in microglia protected neurons from LPS-induced death and loss of synaptic proteins, with a reduced TNFα production. The results suggest that stressor-induced activation of p38α MAPK signaling in microglia is a key event promoting cytokine production and neuronal dysfunction in the CNS. The data also suggest that selective targeting of the p38α MAPK signaling pathway should be explored as a potential therapeutic strategy for the treatment of neurodegenerative disorders.

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34 **Abstract Title:** **Aβ- and NMDA-induced neuronal damage: potential involvement of p38α MAPK**

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Abstract:
Alzheimer's disease, a chronic neurodegenerative disease, is the most common cause of dementia among the elderly. Progressive neuronal degeneration including synaptic loss, dendritic damage and neuronal death is observed in AD brain. There is increasing evidence that aberrant regulation of beta-amyloid (Aβ) and neuronal calcium homeostasis can contribute to neuronal degeneration in AD. The important signaling kinase, p38α MAPK, is activated early in the progression of the disease and has also been implicated in neuronal dysfunction. Therefore, we explored a potential involvement of p38α MAPK in regulation of neuronal degeneration, as well as in the regulation of voltage-gated Ca2+ channels (VGCCs) linked to impaired neuronal function and viability. We induced neuronal damage by treatment of mouse primary cortical neuron cultures with oligomeric Aβ1-42 or with NMDA, an agonist of glutamate-gated cation channels that are highly permeable to calcium. Excessive activation of NMDA receptors can lead to excitotoxicity and neuronal loss in neurodegenerative diseases. We assessed neuronal damage by measuring the reduction of dendritic branches and neuronal survival after Aβ or NMDA exposure. Interestingly, selective p38α MAPK inhibitors blocked VGCC currents by up to 35% in cultured cortical neurons, supporting a potential involvement of p38α MAPK in aberrant neuronal responses in AD.

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35 **Abstract Title:** **Calpain Levels in Mild Cognitive Impairment and Alzheimer's Disease**

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Abstract:
Increases in intracellular calcium are thought to contribute to the two hallmark pathologic features of AD; 1) presence of neurofibrillary tangles (NFTs) composed of tau, a microtubule associated protein, and 2) senile plaques composed primarily of β-amyloid (Aβ) protein. These pathologies are predominately examined in the hippocampus, but can occur in other vulnerable brain regions such as the pre-frontal cortex. Calpains are calcium-activated proteases thought to contribute to NFT and plaque formation making them a target for AD prevention and treatment. Previous studies in a mouse AD model revealed increased expression of calpains 2 and 10 in the hippocampus. However, the relationship between calpain expression and AD pathology in the human brain is unknown. I hypothesize calpain expression increases in brains from MCI and AD patients as compared to age-matched controls. mRNA and protein was isolated from the cerebellum, motor cortex, pre-frontal cortex and posterior cingulate from age-matched controls (n=6), MCI (n=6) and AD (n=6) post-mortem brain samples. Calpains 1, 2, 5, 7 and 10 mRNA and protein expression was evaluated by qPCR and western blot, respectively. In AD, but not MCI, calpain 2 and 10 mRNA levels increased only in the posterior cingulate. Calpains 1, 3, 5 and 7 mRNA levels and calpain 10 proteins levels were unaffected. Calpain 1 autolysis occurred in AD, but not in MCI, suggesting its activation in AD. Elevations in calpain 2 expression and activation of calpain 1 are correlated with AD, but not MCI, suggesting that the conversion of MCI to AD may involve calpain activation.

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36 **Abstract Title:** **Comparison of Brain Sub-Regional Mitochondrial Homeostasis in Monkey Model of Aging**

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Abstract:
Background: Impairment in mitochondrial bioenergetics together with altered calcium buffering capacity has been hypothesized to underlie cellular senescence and may promote age-related neurodegenerative disorders. In the present study, we assessed brain sub-regional [cortex (CTX), hippocampus (HP), substantia nigra (SN) and putamen (PUT)] mitochondrial ATP synthesis rates in a nonhuman primate (Macaca mulatta) model of human aging. We measured mitochondrial complex I, IV and pyruvate dehydrogenase complex (PDHC) enzyme activity [in CTX, HP and PUT] and in situ mitochondrial calcium uptake capacity [in CTX and PUT] in young (average age 7 years) and aged (average age 23.5 years) female rhesus monkeys (n=6/group). Principle Findings: As compared with young group, aged monkeys had significantly reduced ATP synthesis capacity (-15-40%). In particular, SN (-40%) was the most significantly impaired followed by HP (-32%), PUT (-23%) and CTX (-15%). When measured individual enzyme activities, aging significantly impaired PDHC enzyme activity in CTX (-60%) and PUT (-51%), whereas complex I and IV activities were remain unchanged. Aging significantly decreased mitochondrial calcium uptake capacity in PUT (-27%); whereas CTX (-27%) show non-significant decreased trend in aged monkeys as compared with young group. Conclusions: Impairments in mitochondrial bioenergetics together with altered calcium buffering capacity were demonstrated in aged nonhuman primates may be partially responsible for onset of age-associated neurodegenerative diseases like Alzheimer's Disease (AD) and Parkinson's Disease (PD).

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37 **Abstract Title:** **Environmental Enrichment Failed to Attenuate Methamphetamine Self-Administration**

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Abstract:
While environmental enrichment is known to reduce the sensitivity to the reinforcing properties of various abused drugs, little is know about its effects on methamphetamine (METH) self-administration or reinstatement of METH seeking. The present experiment examined whether environmental enrichment alters METH self-administration, extinction or cue-induced reinstatement. Male Sprague-Dawley rats were reared in either enriched or isolated conditions from postnatal day 21 to 65 and then were implanted with jugular catheters. Following recovery, rats underwent an autoshaping procedure to learn to lever press for methamphetamine (0.1 mg/kg/infusion) for 10 days. All rats were then run in a dose-response experiment with 8 METH doses (0.0, 0.001, 0.01, 0.03, 0.05, 0.1, 0.3 or 0.5 mg/kg/infusion) presented in semi-random order. A 14-day extinction procedure was then conducted and followed immediately by cue-induced instatement. The amount of METH self-administered by enriched rats did not significantly differ from the isolated controls at any of the doses tested. During the extinction procedure, enriched rats exhibited lower levels of drug seeking behavior and showed lower levels of cue-induced reinstatement compared to isolated controls. Thus, while enriched rearing does not attenuate the reinforcing effects of methamphetamine, it reduces methamphetamine seeking after a period of extinction. These preclinical findings may have important implications for reducing relapse among METH abusers

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38 **Abstract Title:** **Conditionally Knocking Out Beta-Catenin in GFAP-Positive Cells Reduces Astrocyte Proliferation and New Neurons After Traumatic Brain Injury**

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Abstract:
β-catenin, known for its role in mediating the canonical Wnt signaling pathway and cell-cell interaction, can influence cell proliferation and differentiation. Astrocyte and neural stem/progenitor cell (NSC) division rely on β-catenin, as does NSC differentiation into their progeny: additional quiescent or amplifying neural progenitor cells (NPCs), new neurons, or new astrocytes. These cell populations are profoundly affected by traumatic brain injury (TBI) using a controlled cortical impact (CCI) model. Specifically, CCI results in a dramatic loss of immature neurons while stimulating proliferation of quiescent NPCs and astrocytes, both of which stain positively for glial fibrillary acidic protein (GFAP). We hypothesize that β-catenin signaling is critical for both astrocyte and NPC proliferation following CCI injury. The present experiments utilize a novel transgenic mouse in which β-catenin signaling is conditionally knocked out in GFAP expressing cells using the Cre-lox system, thereby affecting NPCs, their progeny, and astrocytes. Adult mice were injected with 5-bromo-2-deoxyuridine (BrdU) 4h prior to euthanasia at 72h post-injury, to label dividing cells at a time point of peak hippocampal proliferation. Dual immunofluorescent labeling for BrdU and cell-specific markers was used to identify cell phenotype. Astrocyte proliferation was significantly decreased in β-catenin knockout (n=6) versus wild-type mice (n=5) following CCI. Numbers of newborn neurons were also diminished in β-catenin knockout mice following CCI, presumably due to a decreased NPC proliferation and differentiation. Future studies will explore downstream effects of β-catenin-dependent neuron and astrocyte proliferation after trauma, including neurodegeneration, synaptic/cognitive alterations, and blood-brain barrier integrity. Increasing the understanding of this basic proliferation pathway will contribute to the development of novel therapeutics for TBI.

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39 **Abstract Title:** **Intervention in Aging-Dependent Calcium Dyshomeostasis Using Hippocampal Micro-Injections of Viral Vectors Targeting FK506 Binding Protein 1b (Fkbp1b)**

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Abstract:
We have previously reported (Gant et al., 2011) that small interfering RNA against the gene encoding FK506 binding protein (FKBP) 12.6/1b, or the immunosuppressant drug rapamycin (which displaces FKBP1b from ryanodine receptor 2 [RyR2] and disinhibits intracellular Ca2+ release), can recapitulate in young rats many of the Ca2+-related electrophysiological changes that are seen in hippocampal pyramidal neurons with aging. These aging changes include increases in the slow AHP, Ca2+ transients, Ca2+-induced Ca2+ release and L-type Ca2+ channel activity. Here, we tested whether AAV-mediated expression of the Fkbp1b open reading frame (ORF) can counteract Ca2+ dyshomeostasis and altered gene expression in the hippocampus of aging rats; leading to improved memory function. Aged (22 mo) F344 rats received unilateral or bilateral injections of 2µl of AAV bearing the Fkbp1b construct or Empty vector containing GFP at a rate of 0.2µl/min. Young (3 mo) rats were either injected with control virus or underwent sham surgery. Following 2-3 weeks of recovery the rats were behaviorally tested and/ or hippocampal tissue was utilized for qPCR, immunohistochemistry, slice electrophysiology or microarray analysis. Measures included performance in the Morris Water Maze, levels of RNA associated with Ca2+ dyshomeostasis, quantification of Fkbp1b, calcium channel and RyR2 protein levels and electrphysiological measures of the post-burst AHP. Results show that the AAV microinjections impact several biomarkers and improve memory in aged rats, suggesting that Fkbps may be novel therapeutic targets for interventions aimed at protection against unhealthy brain aging or Alzheimer's disease.

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40 **Abstract Title:** **Effect of N-acetylcysteine amide (NACA) on mitochondrial bioenergetics and hindlimb functional recovery following severe contusion spinal cord injury**

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Abstract:
The present study evaluated the neuroprotective efficacy of a glutathione precursor, N-acetylcysteine amide (NACA), following contusion spinal cord injury (SCI). Adult female SD rat spinal cords were contused (250 kdyn @ L1/L2) using the IH impactor. Vehicle (saline) or one of 4 NACA dosages (75, 150, 300 and 600 mg/kg) were administered (i.p.) 15 min post-injury, followed by booster of this antioxidant after 6 hrs. At 24 hr post-injury, synaptic, non-synaptic and total mitochondrial populations from naïve and injured spinal cords were isolated and assessed for mitochondrial respiration capacities and activities of NADH dehydrogenase, cytochrome oxidase and pyruvate dehydrogenase. Compared to the naïve group, SCI resulted in significantly compromised mitochondrial bioenergetics. Conversely, NACA treatments improved mitochondrial integrity, with maximum restoration of respiration and enzymatic activities ($p<0.05$) at 300 mg/kg dosage. Subsequent long-term hindlimb functional recovery and tissue sparing was studied using this dosage, administered 15 min post-injury along with osmotic pumps (s.c.) inserted for 7 days to deliver 300 mg/day NACA. Beginning 7 days-post SCI, the NACA-treated rats showed improved hindlimb movements compared to saline. Critically, after 7 weeks they were consistently plantar stepping with hindlimb weight support compared to vehicle-treated rats that demonstrated frequent dorsal and only occasional plantar stepping. Collectively, our results show that NACA treatment after SCI significantly maintains mitochondrial bioenergetics, in all three mitochondrial populations examined, and that prolonged continuous treatment with NACA significantly improves the recovery of hindlimb locomotor function. Ongoing experiments include histological assessments of spinal tissues from behavioral studies, as well as quantitative analysis of glutathione and oxidative markers from mitochondria isolated from the acute experimental groups.

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41	**Abstract Title:**	**Microglial p38α MAPK is a key regulator of proinflammatory cytokine up-regulation induced by toll-like receptor (TLR) ligands**

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Abstract:
Overproduction of proinflammatory cytokines has been implicated as an important contributor to pathophysiology progression in both acute and chronic neurodegenerative diseases. Therefore, it is critical to elucidate intracellular signaling pathways that are significant contributors to cytokine overproduction, especially pathways amenable to drug interventions. The serine/threonine protein kinase p38αMAPK is a key enzyme in the parallel and convergent intracellular signaling pathways involved in stressor-induced production of IL-1β and TNFα in non-CNS tissue inflammation, and is a drug development target for peripheral tissue diseases. However, much less is known about the quantitative importance of microglial p38αMAPK in stressor-induced cytokine overproduction and the potential of microglial p38αMAPK to be a druggable target for CNS disorders. Therefore, we examined the contribution of microglial p38αMAPK to TLR ligand-induced cytokine production. Microglial cytokine response to TLR ligands 2/3/4/7/8/9 was attenuated by treatment with the CNS-penetrant p38αMAPK inhibitor, MW01-2-069A-SRM. Specifically, increased IL-1β and TNFα production by the BV-2 microglial cell line was inhibited in a concentration dependent manner by the p38αMAPK-targeted inhibitor. These data demonstrate that p38αMAPK is an important contributor to the increased microglial production of proinflammatory cytokines induced by diverse disease-relevant stressors.

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42	**Abstract Title:**	**Individual differences in impulsive action and impulsive choice associated with dopamine and serotonin transporter function in rat medial prefrontal and orbitofrontal cortex**

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Abstract:
Recent studies suggest a prominent role for impulsivity as a factor in drug abuse vulnerability. Impulsivity is a multifaceted construct, broadly divided into impulsive action and impulsive choice. Dysregulation of dopamine (DA) and 5-hydroxytryptamine (5-HT) systems in the prefrontal cortex have been implicated in impulsivity. The frontal cortex is subdivided functionally into the medial prefrontal cortex (mPFC), which has been implicated in both reward seeking and impulsivity, and the orbitofrontal cortex (OFC), which has been implicated primarily in impulsivity. Extracellular DA and 5-HT concentrations are dependent upon both presynaptic release and uptake processes. The DA transporter (DAT) and 5-HT transporter (SERT) may be important molecular targets underlying individual differences in impulsivity and drug abuse vulnerability. The current study examined the potential role of DAT and SERT in explaining the association of individual differences in impulsive action and impulsive choice. Across 21 days, rats (n=36) were tested in a counter balanced order for impulsive action using the cued go/no-go task in which responding during a go cue was reinforced, whereas responding during a no-go cue was not reinforced. Rats were also tested for impulsive choice using a delay discounting task in which a choice was made between a small, immediate reward and a larger, delayed reward. Following behavioral evaluation, Km and Vmax were obtained from kinetic analysis of [3H]DA uptake (DAT function) and [3H]5-HT uptake (SERT function) assays using synaptosomes prepared from mPFC and OFC obtained from each individual rat. Results showed that Vmax at DAT in OFC was positively correlated (Pearson correlation $r = 0.562$, $p<0.05$) with variable interval responses/extinction responses in the cued go/no-go task. No significant correlations were observed between DAT function in mPFC or OFC and mean adjusted delay determined in the delay discounting task. Vmax for SERT in OFC, but not in mPFC, negatively correlated (Pearson correlation $r = - 0.581$, $p<0.05$) with mean adjusted delay determined in the delay discounting task. Thus, increases in impulsive action are associated with decreases in DAT function in OFC, and increases in impulsive choice are associated with increases in SERT function in OFC. Taken together, the results suggest that both DA and 5-HT systems in OFC play a role in mediating individual differences in impulsivity.

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43 **Abstract Title:** **Microelectrode Arrays: Advancing Toward Clinical Recordings**

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Abstract:
Monitoring electrophysiological and neurochemical signals in the CNS with precise spatial targeting and rapid temporal resolution is needed to improve patient outcomes while minimizing the invasiveness of procedures. Presently, electrophysiological recordings are limited in the number of channels and the spatial area covered. Meanwhile, direct neurochemical monitoring with microdialysis is fraught with problems of limited spatial and temporal resolution. Our two pronged approach in developing both a ceramic-based tapered site microelectrode array (MEA) and modifying an existing clinically approved depth electrode, with up to 65 micro-contacts, will serve to advance the state of the art for intraoperative electrophysiological and neurochemical recordings. The MEA is designed for use in aiding in a more precise placement of Deep Brain Stimulation (DBS) electrodes with fewer invasive insertions than the single wire electrodes routinely used. Current DBS surgeries may require 2-3 invasive procedures which could affect patient health. The tapered conformal MEA can provide targeting information simultaneously in 2 axes for more efficient DBS probe placement. Meanwhile, the micro-contact probe can be custom fabricated creating recording sites around the surface of the cylindrical probe, thus, simultaneously yielding a 360° recording field at various depths. Preliminary recordings using the micro-contact probe in rats showed dynamic responses to in vivo glutamate challenges and resting glutamate levels of 1.8 µM under isoflurane anesthesia that decreased by 78% to 0.4 µM with urethane anesthesia. These two electrode designs provide minimally invasive techniques for potentially measuring electrophysiological and neurochemical signals in the human CNS.

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44 **Abstract Title:** **Dysfunctional Glutamate Regulation in the Spontaneously Hypertensive Rat Model of ADHD**

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Abstract:
Attention-deficit/hyperactivity disorder (ADHD) is theorized to be a disorder of catecholamine dysfunction; however, clinical studies show increased levels of glutamate in the striatum and prefrontal cortex of individuals with ADHD. Previously, our group has shown that the spontaneously hypertensive rat (SHR) model of ADHD combined type (ADHD-C) has decreased dopamine release in the striatum versus a model of ADHD inattentive type (ADHD-PI, WKY/NCrl) and the SHR has faster dopamine uptake in the striatum and nucleus accumbens core versus control (WKY/NHsd). Studies of signaling interactions between the dopamine and glutamate systems demonstrate that dopamine D2Rs are involved with inhibition of the NMDAR. We hypothesized that altered dopamine dynamics in the striatum are leading to a more active NMDAR resulting in increased glutamate in the striatum, nucleus accumbens, and prefrontal cortex in the SHR. Here, tonic and evoked release of glutamate were investigated in the striatum, nucleus accumbens, and prefrontal cortex of 8 week old WKY/NHsd, WKY/NCrl, and SHR/NCrl (n=8/strain). Using glutamate sensitive microelectrode arrays, we determined that the SHR exhibited increased tonic and potassium-evoked release of glutamate in discrete sub-regions of the striatum, the nucleus accumbens core, and the prefrontal cortex – all areas implicated in ADHD (two-way repeated measures ANOVA, $p<0.05$). The SHR displayed increased levels of glutamate in all regions compared to the control; however, there were no significant differences between the models of ADHD in the infralimbic cortex, suggesting a role for this region in the ADHD behavioral phenotypes. These findings demonstrate the need for further exploration of glutamate regulation in ADHD as well as pursuing it as a possible target for novel therapies.

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45 **Abstract Title:** **Methylphenidate vs. atomoxetine during adolescence on dopamine transporter function and cellular expression during adulthood in an ADHD model**

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Abstract:
ADHD is characterized by medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC) and striatal dysfunction. Methylphenidate (MPH), a DAT and NET inhibitor, and atomoxetine (ATO), a selective NET inhibitor, are prescribed for ADHD. Spontaneously Hypertensive Rats (SHR), an ADHD model, given MPH during adolescence, enhanced cocaine self-administration in adulthood. We hypothesize that MPH during adolescence will produce a lasting increase in DAT function and cell-surface expression in mPFC and OFC of SHR, while ATO will not. SHR, Wistar-Kyoto inbred (WKY) and Wistar outbred (WIS) received MPH (1.5 mg/kg, po), ATO (0.3 mg/kg, ip) or vehicle on P28-55. DAT function and cellular expression were assessed during P77-85. Saturation analysis of [3H]dopamine uptake and biotinylation assays were performed using mPFC, OFC and striatal synaptosomes. For each brain region, Vmax and Km values for control rats from each strain did not differ. MPH increased Vmax in SHR mPFC, decreased Vmax in WKY OFC and decreased Km in WIS OFC, and had no effect in striatum. MPH did not alter cellular distribution in any brain region. These results indicate trafficking-independent DAT functional alterations in response to MPH. ATO decreased Vmax and DAT surface expression in SHR OFC, suggesting that NET inhibition decreases DAT function and cell surface expression. ATO decreased DAT Vmax, but not expression in WIS OFC, suggesting trafficking-independent regulation. ATO decreased Vmax in SHR striatum, which was accompanied surprisingly, by increased DAT total expression. Despite sparse NET expression in striatum, ATO altered DAT function and expression in this dopamine rich brain region. In conclusion, adolescent treatment with MPH and ATO produce different brain region specific alterations in DAT function and cellular expression, and thus, may differently influence cocaine vulnerability in adults with ADHD.

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46 **Abstract Title:** **Long-Term Water Maze Training Increases Deep Sleep in Aged Rats.**

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Abstract:
Small molecule inhibitors of calcium-dependent proteases, μ- and m-calpains, protect against neurodegeneration induced by a variety of insults including spinal cord injury (SCI). However, these compounds also inhibit other proteases, which has made it difficult to evaluate the contribution of calpains to neurodegeneration. Calpastatin is a highly specific endogenous inhibitor of μ- and m-calpains, thus eliminating the specificity and efficacy problems associated with small molecule inhibitors. In the present study, we utilized transgenic mice that overexpress human calpastatin under the prion promoter (PrP-hCAST) to evaluate the hypothesis that calpastatin overexpression will reduce calpain-mediated proteolysis, attenuate lesion volume, and improve locomotor function following contusive SCI. Contusion SCI was produced following a T10 laminectomy at 70 kdyn force setting using an Infinite Horizons (IH) SCI device. Western blot analysis demonstrated that calpastatin overexpression reduced α-spectrin breakdown (145 KDa) by 51% at 24 hours post-injury, as compared to wild-type controls ($p<0.05$, n=3/group). PrP-hCAST (n=13) mice displayed a significant improvement in locomotor function at 1 and 3 weeks after contusive SCI compared with the wild-type controls (n=9, $p<0.05$), but were similar at four weeks postinjury (repeated measures ANOVA and Bonferroni post-hoc test). Histological assessment of lesion volume and tissue sparing, performed on same animals used for behavioral analysis, revealed that calpastatin overexpression resulted in a 30% decrease in lesion volume ($p<0.05$) and significant increases in total tissue sparing, white matter sparing, and gray matter sparing at 4 weeks postinjury compared with wild-type animals. The finding that calpastatin overexpression significantly reduces calpain-mediated proteolysis and lesion volume following SCI provides support for the hypothesis that sustained calpain-dependent proteolysis contributes to pathological deficits after traumatic SCI.

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47 **Abstract Title:** **Neurocognitive Testing in Concussion Management**

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Abstract:
Public and healthcare provider awareness of the acute effects of mild traumatic brain injury (mTBI) or concussion has increased rapidly in recent years. While imaging and lab work are typically insensitive to concussions, neuropsychological testing has been shown to detect impairment or decreased cognitive functioning, especially in the acute phase (Levin et al., 1987). One area of particular interest is the use of cognitive testing as an indicator of neurologic recovery, which may be best accomplished by a serial assessment approach (Moser et al., 2007). We review the case of a 15-year-old young man with a history of two probable concussions 1.5 months apart. Both were football-related. The patient reported confusion and post-traumatic amnesia for the events but no loss of consciousness. Neuropsychological screening was conducted at 3 weeks (Time 1) and again at 7 weeks post-injury (Time 2). Time 1 test results revealed suspected declines in the domains of reaction time, mental flexibility, visual motor speed, and verbal fluency. Mental flexibility and reaction time were most notably below expectancy. Memory performances were also below the expected range. Time 1 profile was consistent with a Postconcussional Disorder. Results from Time 2 revealed improved mental flexibility and verbal fluency. However, reaction time and visual motor speed remained borderline impaired (see Figure 1). Results at Time 1 and Time 2 were generally consistent with parent report but not the patient report. This case report demonstrates the utility of serial neurocognitive assessments in gauging cognitive recovery in a more objective fashion.

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48 **Abstract Title:** **Calpain5 is Predominantly Expressed in Neuronal Mitochondria and Nuclei in the CNS, and May be Involved in Caspase-Dependent Cell Death Pathway**

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Abstract:
Calpain 5 (Capn5) is an atypical member of the Calpain family. Unlike typical calpains (Capn1 & 2), it has only one subunit, which possesses a conserved non-EF hand Ca2+ binding catalytic domain and a unique domain T. The Capn5 orthologue, TRA-3, is involved in neuronal cell death in C.elegans. Capn5 along with calpains 1, 2, 7, 10 & 13 are ubiquitous and present in the CNS. However, the relative expression of these calpains in the CNS is unknown, as is the CNS localization and developmental regulation of Capn5. We compared relative mRNA and protein levels from pre and postnatal developmental periods in rats, revealing that Capn5 is prominently present in adult brain and spinal cord. In adult CNS, Capn5 was found to be the second most highly expressing calpain, after Calpain 2. In the CNS, Capn5 is predominantly expressed in neurons and is localized to neuronal mitochondria and nuclei. This was evaluated using lacZ staining of Capn5tm1Dgen/Capn5 brain sections, double-label immunohistochemistry, probing Capn5 in fractions obtained through differential centrifugation of rat cortex, and confocal microscopy against mHsp70 and Capn5 in SHSY-5Y-neuroblastoma cells. Proteinase K treatment of mitochondria from rat B35-neuroblastoma cells and rat synaptic mitochondria indicates that Capn5 localization is similar to AIF protein, which is anchored on the inner mitochondrial membrane. Preliminary data reveal a decrease in caspase activation and cell death in Capn5 deficient SHSY-5Y cells following staurosporine treatment, suggesting the possible involvement of Capn5 in caspase-dependent cell death pathways in neurons.

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49 **Abstract Title:** **Renewal of Methamphetamine-Induced Hyperactivity and Dopamine Release in the Nucleus Accumbens Shell**

Author(s): K. M. Alvers, Department of Psychology, U of Kentucky
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Abstract:
Contexts associated with drug use can engender drug craving and, by extension, may also motivate relapse to drug-seeking. One mechanism of relapse is renewal, described as the recovery of an extinguished behavior that occurs when the context is changed after extinction. Renewal has been demonstrated with several drug classes using operant conditioning procedures, and has been shown to be dependent on activation of the dopamine (DA) receptors in the nucleus accumbens shell. The current study examined renewal of conditioned locomotor hyperactivity using Pavolovian conditioning procedures, while measuring extracellular DA release in nucleus accumbens shell using in vivo microdialysis. Rats were given repeated administration of methamphetamine (1 mg/kg, i.p.) in the presence of a white noise conditioned stimulus (CS). Conditioned locomotor activity to the white noise CS was extinguished in the same context as conditioning (SAME group) or a distinctly different context (DIFFERENT group). On the test day, rats were presented with the white noise CS in their original conditioning context, followed by a methamphetamine challenge injection. We failed to observe renewal of conditioned locomotor hyperactivity when rats were returned to a previously drug-paired context. However, return to the drug-paired context and presentation of the CS elicited greater DA release in the DIFFERENT group compared to the SAME group. There were no differences in locomotor activity or DA release between the groups following the methamphetamine challenge injection. The results suggest that renewal of drug-seeking using operant conditioning procedures may not generalize readily to Pavlovian conditioning of drug-induced hyperactivity.

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50 **Abstract Title:** **Brain Stimulation and Robotic Gait Orthosis in Chronic Stroke: a Feasibility Study**

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Abstract:
Hypothesis: Applying transcranial direct current stimulation (tDCS) to the lower extremity motor cortex coupled with a locomotor training approach using a robotic gait orthosis treadmill system will improve locomotion and cortical excitability more than sham tDCS and locomotor training in subjects with chronic stroke. Number of Subjects: 8. Procedures: A double-blind, sham-controlled, randomized study design was used. Subjects were stratified based on lower extremity motor function then randomly allocated to the intervention group (active tDCS and gait training) or control group (sham tDCS and gait training) for 12 sessions over one month. Primary outcomes: 10-meter walk test (10MWT) and cortical excitability measured by transcranial magnetic stimulation (TMS). Secondary outcome measures: Functional Ambulation Category (FAC), Timed Up and Go (TUG), Berg Balance Scale (BBS), and Stroke Impact Scale 16 (SIS). Outcomes measured at baseline, immediately post-intervention and at one-month follow-up. Statistical Analyses: An analysis of variance model fitted to each dependent variable to evaluate group main effects. Significance accepted at $a < 0.05$. Results: Data analysis shows trends towards improvement for both groups, with the active group showing marked improvement over the sham group in all measures except BBS (FAC p=0.028, TUG p=0.066, SIS p=0.062, 10MWT p=0.19, BBS p=0.919). TMS recruitment curves were recorded in one subject and indicate increased neuroplasticity following the intervention and at the one-month follow-up. Important Findings: This study demonstrates the feasibility of using tDCS coupled with locomotor training using a robotic gait orthosis in enhancing gait recovery and cortical excitability in people with chronic stroke.

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51 **Abstract Title:** **Packaging and physiological separation of the RRP and RP of vesicles within various types of presynaptic terminals**

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Abstract:
The reserve pool (RP) and readily releasable pool (RRP) of synaptic vesicles within presynaptic nerve terminals, at crayfish and Drosophila neuromuscular junctions (NMJs), is being investigated to examine if they are physiologically differentiated into distinctly separate functional groups. This was addressed in glutamatergic nerve terminals by inhibiting the proton pump function of Vacuolar-type H+-ATPase (V-ATPase) with Bafilomycin A1 (BA1). In crayfish preparations, 20 Hz and 40 Hz continuous electrical stimulations were compared, as well as 2.5 hours pre-expose and no pre-expose. 8µM, 4µM, 400nM and 4nM of BA1 were examined. 8µM is lethal for all the preparations. Among 4µM treatments, the 20Hz continuous stimulation with 2.5 hours incubation decreased the EPSP amplitude to 50% in about 30Mins while controls can last for 3Hrs. The 20Hz continuous stimulation without 2.5Hrs incubation induces 50% run down in about 1Hr in the presence of BA1 but there was a large variation in this group. Therefore, 20Hz continuous stimulation with 2.5 hours incubation was used as a standard stimulating paradigm in all the crayfish studies. 400nM had a similar effect as 4 µM. The effect of 4nM BA1 (50% depression time is 111Mins) was between the control and 4µM to 400nM treatments. After BA1 induced synaptic depression, the EPSPs were rapidly revitalized by exposure to serotonin (5-HT, 1µM) in the preparations tested ($p<0.05$). At this nerve terminal 5-HT promotes an increase probability of vesicular docking and fusion. Thus, 5-HT was able to recruit unused vesicles from the RP that were not rapidly depleted by acute BA treatment and electrical stimulation. The results support the notion that the RRP is selectively activated during rapid electrical stimulation sparing the RP; however, the RP can be recruited by 5-HT.

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52 **Abstract Title:** **Effect of DREADD receptor activation in Drosophila neurons and muscle on synaptic transmission**

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Abstract:
Second messengers play a key role in synaptic transmission. The role of G-protein coupled receptors (GPCRs) involve in synaptic transmission is not fully understood. In this study, we investigate the role of GPCRs in synaptic transmission by using modified receptors called Designer Receptors Exclusively Activated by a Designer Drug (DREADDs). The DREADD receptors used in this study are modified GPCRs that have lost affinity to their natural ligand, but have high affinity and efficacy for the designer ligand, clozapine-N-oxide (CNO). Moreover, by using this approach we can rule out the possibility of off-target effects of the natural ligand. Five different Drosophila strains are used in this study: UAS-M1D (Gq), UAS-M3DBar (Gs), UAS-M4D (Gi), Gal4-Ok6 and Canton-S. When crossed with GAL4-Ok6 flies, the DREADD receptors are only expressed in motoneurons. The excitatory postsynaptic potential (EPSP) and resting membrane potential (RP) were recorded in muscle 6 of third instar larvae in response to different concentrations of CNO. We predict that after crossing the three parental transgenic strains with GAL4-Ok6 strain that activation of the Gs receptor with CNO will increases EPSP amplitude. The activation of Gs receptors leads to activation of adenylate cyclase which converts ATP to cAMP, which in turn activates protein kinase A. We predict that Gi receptor activation will decrease EPSP amplitude because of decreasing cAMP concentration in the cytosol. Also it is possible that activation of Gi, through G-beta and gamma, leads to opening of potassium channels, and membrane hyperpolarization leading to a reduced synaptic responses On the other hand, we postulate activation of Gq receptor will increase EPSP amplitude because of production of IP3 that binds to IP3 receptor on endoplasmic reticulum and induces the release of Ca+2 into the cytosol to activate more voltage gated calcium channels (VGCC). Alternatively activation of Gq and promoting calcium signaling can leads to closing of membrane potassium channels, and a reduction of membrane depolarization.

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53 **Abstract Title:** **Diet-induced Obesity: Dopaminergic and Behavioral Mechanisms as Predictors and Outcomes**

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Abstract:
Obesity and drug abuse share common neural circuitries, which includes the dopamine (DA) reward system. The current study identified neurobehavioral predictors and outcomes of diet-induced obesity using a rat model. Individual differences in impulsivity and food-motivated behavior were determined using a delay discounting task and progressive ratio (PR) schedule for high-fat reinforcers, respectively. Individual differences in striatal DA transporter (DAT) function were determined using in vivo no-net flux microdialysis, in vitro kinetic analysis of DA uptake and DAT cellular expression. PR breakpoint prior to an 8-wk exposure to a high-fat diet predicted subsequent body weight gain; whereas, delay discounting performance, DA extraction fraction and basal extracellular DA concentration did not predict weight gain. Obesity prone rats, which had greater body weights when fed the high-fat diet for 8 wks, continued to exhibit higher PR breakpoints, and in addition became less impulsive, relative to the obesity resistant rats. Also, obesity prone rats had 42% lower striatal D2 receptor density, 30% lower total DAT immunoreactivity, 40% lower DAT function (Vmax for [3H]DA uptake in vitro and DA extraction fraction in vivo), 45% greater basal extracellular DA concentration and 2-fold greater methamphetamine-evoked [3H]DA overflow from striatal slices relative to obesity resistant rats. Thus, the current results indicate that motivation for high-fat food both predicts diet-induced obesity and persists after the development of obesity, and that diet-induced obesity is associated with decreased striatal DAT expression and function, in addition to decreased D2 receptor density.

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54 **Abstract Title:** **A Cross-Sectional Analysis of Behavioral and Trait Impulsivity Among Young Adult Intermittent and Daily Cigarette Smokers**

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Abstract:
Previous studies have indicated that young adults who are more impulsive tend to initiate tobacco use and escalate to tobacco dependence at higher rates. Impulsivity is a multi-faceted construct that is assessed using both subject-rated trait assessments and performance on tasks of behavioral inhibition. Further research is needed in order to determine how the facets of impulsivity inform vulnerability to tobacco dependence. The aim of this study was to compare young adults who varied in smoking status on different measures of behavioral and trait impulsivity in order to determine which facets of impulsivity were most closely associated with tobacco use and dependence. Young adult first-year college students between the ages of 18-24 (N=512) were assigned to one of three groups based upon smoking status as determined by a life history calendar (non-smokers, intermittent smokers, or daily smokers). Participants completed several measures of impulsivity including self-report trait (e.g. UPPS-P) and behavioral measures, along with assessments of substance use. Group differences were analyzed using ANOVA. Scores on all facets of the UPPS-P (i.e. positive and negative urgency, premeditation, perseverance, sensation seeking) were greater in both smoking groups compared to the non-smoking group, and both negative and positive urgency were greater in daily smokers than intermittent smokers. In addition, inflations per balloon on the BART were greater in intermittent smokers than non-smokers, though performance did not differ between non-smokers and daily smokers. There were no group differences in delay-discounting or cued go/no go task performance. These results are consistent with prior studies indicating that smokers are more impulsive than non-smokers on trait and behavioral measures of impulsivity, and suggest that urgency might be a risk factor for transitioning from intermittent to daily tobacco use patterns among young adults.

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55 **Abstract Title:** **Circadian Rhythms and Clock Gene Expression in an APPxPS1 Knock-in Mouse Model for Alzheimer's Disease**

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Abstract:
In addition to cognitive deficits, Alzheimer's disease (AD) patients suffer circadian rhythm and sleep disruptions. To investigate whether an AD mouse model exhibits similar disruptions, we monitored circadian wheel running rhythms in APPxPS1 knock-in mice developed on a CD-1/129 background. The study included male wild-type (WT) and homozygous APPNLH/NLH/PS-1P264L/264L (APPxPS1) knock-ins of three ages (months): 4, 11, 15 (n=6/age/genotype), as β-amyloid deposits accumulate by 6 months and increase thereafter. After rhythm assessment, mice were euthanized at two times of day and in situ hybridization for the clock gene Per2 was conducted on sections prepared from the brains. APPxPS1 mice ran relatively less during the light phase ($p<0.01$), with a marginal genotype difference in the early light phase ($p=0.056$). Activity patterns among all groups of mice were largely bimodal, with activity extending into the light phase. SCN Per2 expression varied by time of day ($p<0.0001$) and was higher in WT mice ($p=0.022$). A marginal interaction was observed between genotype and time of day ($p=0.074$), and this interaction was age-dependent, the difference being strongest in older mice. Contrary to observations in AD patients, activity rhythms in APPxPS1 mice were not phase delayed and were more consolidated in the dark phase (normal active phase) than activity rhythms WT mice. SCN Per2 expression data in aged APPxPS1 mice suggested changes in either the amplitude or timing of this rhythm. The atypical activity patterns of the background strain (CD-1/129) confound the use of this APPxPS1 model mouse for studying circadian rhythm disturbances in AD pathology.

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56 **Abstract Title:** **Microglial activation precedes an increase in microglia following binge ethanol exposure in the hippocampus**

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Abstract:
Excessive alcohol intake, characteristic of an Alcohol Use Disorder, results in neurodegeneration especially in the hippocampus. One possible mechanism for this neurotoxic event is neuroinflammation. Because microglia activation is characteristic of inflammation, this study examines microglia reactivity using [3H]PK-11195 autoradiography and microglial number using Iba-1 immunohistochemistry. Adult male rats received ethanol or control diet via intragastric gavage every 8 hours for 4 days. Each rat received an initial dose of 5g/kg, but later doses were based on individual intoxication levels. Brains were collected at various time points following the binge: T0, T2 (2 days), T4, and T7. A 3-fold increase in [3H]PK-11195 binding ($p<0.01$) was observed in ethanol animals versus controls at all time points in all subregions analyzed. Analysis of Iba-1 immunoreactivity showed a >35% increase in the number of microglia in the dentate gyrus and the CA3 at T7 only ($p<0.01$). This data suggest that the increase in binding of [3H]PK-11195 is due to an increase in the expression of the 18kDa translocator protein on individual microglia rather than an increase in the number of microglia at early time points. Our previous observation of proliferating microglia at T2 supports the recent finding of microglia number increase at T7. These data support our previously reported data that microglia are only partially activated following binge ethanol exposure in corticolimbic regions. Partially activated microglia have been linked to neuroprotection and may play a role in the alcohol induced neurogenesis seen 7 days after abstinence in this model.

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57 **Abstract Title:** **Sox11 is required for proper optic fissure closure and photoreceptor development**

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Abstract:
PURPOSE: The SRY-Box transcription factor Sox11 is expressed in developing central and peripheral nervous system and regulates the development and differentiation of several organ systems. Sox11-/- mice are micropthalmic and exhibit anterior segment dysgenesis, suggesting that Sox11 is required for proper ocular morphogenesis. Here, we have investigated the role of Sox11 in ocular development in zebrafish, using an in vivo knockdown approach. METHODS: Translation blocking morpholinos were injected into 1-cell stage zebrafish embryos. The embryos were collected at various time-points and processed for whole-mount in situ hybridization (WISH), histological and immunohistochemical (IHC) examination. Cell proliferation and cellular differentiation were analyzed via IHC. Area measurements of the eye were taken by outlining the entire eye and the lens using Nikon Elements software. RESULTS: WISH showed that zebrafish sox11 paralogs, sox11a, and sox11b, have overlapping yet distinct expression patterns in the developing eye. Furthermore, both transcripts are present in mitotic retinal progenitor cells as well as post-mitotic precursor cells. Knockdown of both Sox11a and Sox11b resulted in micropthalmia, delayed lens development, coloboma, and specific reduction of photoreceptor cells. These phenotypes were rescued by co-injection of mRNA specific for zebrafish sox11a, sox11b, and human Sox11. CONCLUSIONS: Our results show that reduction in the levels of Sox11 causes abnormal ocular morphogenesis as well as coloboma. Furthermore, our results suggest that human SOX11 can functionally compensate for the loss of zebrafish Sox11 and is likely to be conserved across vertebrate ocular development. Studies are ongoing to determine the mechanism of optic closure defect in the Sox11 deficient eyes.

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58 **Abstract Title:** **Forward-masked Binaural Intensity Discrimination**

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Abstract:
The ability to discriminate the intensity of a sound is an important function of auditory system to accurately process sound signal information. Previous studies have only reported monaural intensity-discrimination in humans. However, in natural acoustical environments, humans discriminate sound intensity and spatial information binaurally. In the present study, the just noticeable difference (JND) in sound intensity of test tones were measured binaurally with and without a forward masker. The intensity and the spatial azimuth of both forward masker and test tone were manipulated by changing the average binaural level (ABL) and the interaural level difference (ILD) of the sounds. Compared with the JNDs without masker, low-level maskers did not markedly change the JNDs of the test tones. However, moderate- and high-levels (ABL > = 40 dB) maskers significantly increased the JNDs of the test tones. The JNDs were monotonically increased with increasing intensity of the forward masker. When the intensity of the forward masker was constant, the effect of the forward masker on the JNDs of test tones was decreased with increasing intensity of test tones. This effect was not significant when the intensity of test tone was high. The present study found a non-significant effect of ILD difference between the masker and the test tone on the JNDs of test tones.

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59	**Abstract Title:**	**Increased Amygdala Functional Connectivity during Working Memory among Patients with Mild Cognitive Impairment**

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Abstract:
Patients with mild cognitive impairment (MCI) recruit additional neural networks to facilitate cognition. Cortical and affective neural networks interplay in compensation has been proposed. We hypothesize that patients with MCI have altered neuroaffective pathway connectivity during cognitive processing. We examined functional magnetic resonance imaging (fMRI) responses during a non-emotional memory task with patients with MCI and an age- and education-matched control group (NC). 12 NC and 10 participants with MCI performed a delayed-match-to-sample task during event-related fMRI scanning. Full brain high-resolution images of structural (1x1x1 mm) and functional images (4x4x4 mm) were collected, and percent change in blood-oxygen-level dependent (BOLD) signal was analyzed at selected regions implicated in cognition and affect processing. Correlations between the amygdalae and other regions were computed with significance threshold set to $p < 0.001$. The group with MCI was less accurate overall and showed slower RT between working memory conditions (ps < 0.05). Significant group differences in BOLD change were observed at the amygdalae (ps < 0.02). Among the patients with MCI, significant correlations were observed between the amygdalae and ventral temporal cortex, hippocampi, and inferior pre-frontal regions (all ps < 0.001). No significant correlations were found for these regions in NC with the exception of a significant correlation between the left amygdala and left hippocampus ($p < 0.001$). Patients with MCI exhibited increased BOLD amygdala responses and connectivity to visual working memory regions. Our results extend reports that patients with Alzheimer dementia show such changes during cognitive tasks relative to NC.

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60	**Abstract Title:**	**Spinal Neuronal Sensitization in the Zucker Diabetic Fatty Rat Model of Type 2 Diabetes Contributes to Pain-like Behavior**

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Abstract:
A subset of type 2 diabetic patients experience painful diabetic neuropathy, which is traditionally characterized as a peripheral pathology. However, the spinal mechanisms resulting in painful diabetic neuropathy are not well known. Therefore we used an inbred genetic model of type 2 diabetes, the Zucker Diabetic Fatty rat (ZDF/Crl-Leprfa/fa), to test the hypothesis that glial activation and neuronal sensitization in the spinal cord dorsal horn contribute to pain-like behavior. We found that diabetic animals developed hyperglycemia and were hypersensitive to noxious pressure, hot, and cold stimuli compared to their heterozygous littermate controls. Diabetic animals presented with elevated blood glucose at 6 weeks of age and remained hyperglycemic until the end of the study at 18 weeks of age. Hyperalgesia appeared at 14 weeks of age (after establishment of hyperglycemia) and persisted for the remainder of the study. Immunohistochemistry of lumbar spinal cord revealed that noxious hindpaw pressure stimulation resulted in expression of phosphorylated extracellular signal-regulated kinase (pERK). The number of pERK-positive cells in lamina I and II of the dorsal horn was greater in diabetic animals than controls indicating central sensitization. However, neither microglial or astrocytic activation was different between diabetic and control animals as assessed via Iba1 and GFAP expression, respectively. Because 90% of pERK-positive cells colocalized with NeuN, a marker for neurons, this suggests that neuronal sensitization in the superficial dorsal horn may contribute to pain-like behavior in diabetic rats.

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61 **Abstract Title:** **A modifier of spinal muscular atrophy may be involved in motor behavior and stress response**

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Abstract:
We are characterizing behavioral and physiological defects associated with spinal muscular atrophy (SMA) and an associated gene in Drosophila. In humans SMA is a progressive disorder that causes degeneration of motoneurons leading to muscle dysfunction. This autonomic recessive form of the disease is associated with loss of a copy of the survival of motor neuron gene (SMN). Severity of SMA was found to be worse in patients when an adjacent gene, called Serf, was deleted in addition to SMN. To test the hypothesis that Serf is a modifier of SMA we created null dSerf mutants using transposon-mediated excision. These animals develop normally with life spans similar to controls, but they exhibit deficits in motor behavior and response to heat shock relative to controls. Preliminary data also suggest that high frequency synaptic transmission between the giant fiber pathway and the flight muscles is effected in Serf mutants, with no difference in the amplitude of excitatory junction potentials. The smne33 flies are hypomorphic SMN mutants that exhibit muscular phenotypes similar to patients with SMA, particularly in the flight muscles. By testing for genetic interactions between these alleles in the behavior and stress assays we hope to gain a better understanding of the genetic and physiological mechanisms of SMA.

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62 **Abstract Title:** **Molecular Analysis of Variable Splicing of the Alzheimers-Associated Gene BIN1 in Human Brain.**

Author(s): C. D. Van Sanford, Physiology Department and Sanders-Brown Center on Aging, U of Kentucky
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S. Estus, Physiology Department and Sanders-Brown Center on Aging, U of Kentucky

Abstract:
Recent GWAS have identified a series of single nucleotide polymorphisms (SNP)s that are associated with Alzheimer's disease (AD). Several of these SNPs are near or within the gene Myc box-dependent-interacting protein (BIN1). We hypothesized that two AD-associated BIN1 SNPs, rs1060743 in exon 6, and rs7561528, in the BIN1 promoter region, are associated with BIN1 splicing and/or expression. To evaluate this hypothesis, we performed PCR of 60 brain cDNA samples and visualized the products on acrylamide gels. This analysis suggested a possible association between the rs1060743 SNP and inefficient inclusion of exon 7 (delta-7 BIN1). To investigate this possibility more rigorously, we developed a real-time PCR (RT-PCR) assay and found a positive correlation between the rs1060743 major allele (T) and delta-7- BIN1 expression. The functional significance of these changes is unclear at this time; however, the BIN1 protein encoded by delta-7- BIN1 is missing 31 amino acids from the BIN1-amphiphysin-Rvs167 domain which has been previously identified as critical to BIN1 function. We are currently planning to quantitatively measure the effect of rs1060743 on exon 7 splicing by using Ion Torrent RNA-SEQ . We will also investigate the effects of rs7561528 on total BIN1 expression and splicing in brain. Results of this ongoing work will be presented.

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63 **Abstract Title:** **Calpain Cleaves Methionine Aminopeptidase-2 in a Rat Model of Ischemia/Reperfusion**

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Abstract:
Ischemic stroke results in multiple injurious signals within a cell including disregulation of calcium homeostasis. Consequently, there is an increase in the enzymatic activity of the calpains, calcium dependent cysteine proteases that are thought to contribute to neuronal injury. In addition, cellular stress, due to ischemia/reperfusion triggers a decrease in protein translation through dissociation of methionine aminopeptidase 2 (MetAP2) from eIF2α. We hypothesized that calpain would cleave MetAP2, because levels of phosphorylated eIF2α (an indication of dissociated MetAP2) are increased in ischemic stroke, and also due to the fact that calpain cleaves eIF4γ, another component of the translation initiation complex formed in partnership with MetAP2. To test this hypothesis homogenates of control brain tissue were prepared and digested with either calpain 1 or calpain 2. In addition, rats underwent ischemic stroke for one hour, with harvest of tissue at either one or 24 hours post-surgery. Samples were analyzed for the presence or absence of proteolysis by Western blot. In vitro and in vivo studies both showed the production of a stable 50 kDa calpain-mediated fragment. In a rat MCAO model of ischemia-reperfusion a 57 kDa fragment was produced. These data suggest that calpain activation in stroke may play a role in the MetAP2 mediated decrease in protein translation leading to induction of apoptosis and thus have a larger role in the cellular stress response than previously determined. (p=0.0211 by paired two tailed t test, CI=95%, n=4).

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64 **Abstract Title:** **Calpastatin Overexpression Reduces Posttraumatic Behavioral Deficits Without Sparing Cortical Tissue**

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Abstract:
Calpains are activated early after traumatic brain injury (TBI), with subsequent proteolysis of substrates including cytoskeletal components and membrane receptors. Early cellular damage contributes to overall neuronal death and behavioral dysfunction hours to days following the injury. Prolonged activity of calpains after trauma suggests that endogenous action or levels of its inhibitor, calpastatin, may be insufficient to fully inhibit calpains' proteolytic activity. Therefore, we hypothesize that calpastatin overexpression in transgenic (TG) mice will reduce calpain-mediated substrate proteolysis and behavioral deficits after TBI. The posttraumatic cleavage of the calpain substrate, collapsin-response mediator protein-2 (CRMP-2), was investigated due to the protein's close association with the cytoskeleton and axonal growth-promoting function. Immunoblot analysis of cortical and hippocampal demonstrated the appearance of CRMP-2 fragments following brain injury (n=3-5/genotype/condition). Calpastatin overexpression resulted in a statistically significant decrease of CRMP-2 fragments in the cortex at 6 or 24h post-injury ($p<0.05$ and $p<0.0005$, respectively). Within the hippocampus, CRMP-2 breakdown was reduced at 24h post-injury ($p<0.005$). Considering the structural and functional importance of CRMP-2 and other calpain substrates, preservation of these proteins by augmenting calpastatin levels after injury may contribute to a reduction in behavioral dysfunction. Using a modified neurological severity score assessment, brain-injured TG mice (n=20) showed significantly improved motor function compared to WT mice (n=19) across a 7d post-injury period ($p<0.005$). Furthermore, calpastatin overexpression attenuated the injury-induced impairment in the ability to recognize a novel object at 10d post-injury ($p<0.0005$). Despite this improvement, calpastatin overexpression did not result in cortical neuroprotection as assessed by cortical lesion size in injured WT and TG mice (n=12/genotype). The reduction in calpain-mediated proteolysis and behavioral abnormalities suggests a protective role of calpastatin following CCI injury that does not affect cortical cell sparing. Given its beneficial behavioral outcome, calpastatin may be a potential therapeutic avenue for the treatment of TBI.

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65 **Abstract Title:** **Transcriptional Regulation During Vertebrate Retinal Development and Regeneration**

Author(s): S. G. Wilson, Department of Biology, University of Kentucky
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Abstract:
Coordinated gene expression patterns during developmental and regenerative processes of the retina are mediated by an array of transcription factors, many of which are directly controlled by the Notch-Delta signaling pathway. Notch signaling is a type of cell-cell communication that is dependent upon physical interaction between the signaling and signal-receiving cell, and is highly conserved throughout all metazoans. Some members of the Hairy/Enhancer of Split Related (her) family of basic-helix-loop-helix transcriptional repressors are direct targets of Notch-signaling. To determine whether her4, her9, and her12 are downstream effectors of Notch-signaling, we pharmacologically inhibited the signaling pathway and assayed for changes in expression of the her genes. To investigate whether her genes are expressed during development and of the zebrafish retina, we performed in situ hybridization experiments using labeled anti-sense RNA probes for each of the her genes in a time-course of wild-type embryos. To investigate the role of Her in retinal regeneration, in situ hybridization experiments and immunohistochemistry were performed in a transgenic fish line that undergoes a continual cycle of rod photoreceptor degeneration and regeneration. We found that her4 and her12 are Notch responsive, and that her genes are expressed throughout retinogenesis in overlapping but distinct patterns. Candidate genes of her mediated repression were identified by pharmacological inhibition of Notch signaling. Dual luciferase assays were conducted to show that her directly represses expression of downstream genes. We conclude from these data that Notch signaling plays a role in the development and regeneration of the zebrafish retina by directly influencing the expression of her, which in turn regulates the expression of downstream genes.

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66 **Abstract Title:** **Improving motor function with peripheral nerve stimulation in severe hemiparesis: preliminary results**

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Abstract:
Hypothesis: Upper extremity (UE) motor training paired with active peripheral nerve stimulation (PNS) will lead to more improved motor function in the affected UE than sham PNS paired with UE motor training in subjects with severe post-stroke motor deficit. Number of Subjects: 34. Procedures: Double-blind, sham-controlled, randomized design. We define “severe motor deficit” as virtually no wrist and finger movement. For 10 consecutive weekdays, 18 subjects with severe post-stroke motor deficit received active PNS for 2 hours daily to the ulnar, median, and radial nerves simultaneously, preceding 4 hours of intensive, task-oriented UE motor training. The remaining 16 subjects underwent an identical protocol except that they received sham PNS. Fugl-Meyer Assessment Scale (FMA) was a primary outcome measure in evaluation of UE motor performance at baseline, completion, and 1-month follow-up testing. Statistical Analyses: We compared baseline measures for active versus sham groups. An analysis of variance model with repeated measures was fitted to each variable. Significance level was set at 0.05. Results: Active PNS led to greater change in FMA score from baseline than sham PNS as measured at completion (p=0.07) and 1-month follow-up (p=0.01). Conclusions: Results suggest that UE motor performance in severe post-stroke hemiparesis improves when active PNS is coupled with intensive, task-oriented UE motor training.These results demonstrate that PNS has promising adjuvant effects with motor training to enhance functional recovery in individuals with severe post-stroke motor deficit.

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67 **Abstract Title:** **Molecular characterization of splicing of the Alzheimers-associated gene PICALM in the human brain.**

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Abstract:
Recent GWAS have identified a series of single nucleotide polymorphisms (SNP)s that are associated with Alzheimer's disease. One of the SNPs, rs3851179 (G/A), is near the gene phosphatidylinositol-binding clathrin assembly protein (PICALM). To evaluate whether this SNP is associated with PICALM expression, we quantified PICALM mRNA in 61 brain cDNA samples. When we analyzed PICALM expression relative to rs3851179, or AD status, an association with PICALM was not detected. Immunostaining indicates that the majority of PICALM is expressed in brain microvessels. Consistent with this finding, PICALM expression in the brain samples correlated strongly with the expression of two mRNAs restricted to endothelial cells, vWF and CD31. Linear regression analyses of PICALM that included vWF, CD31, AD and rs3851179 found that only the vWF and CD31 were associated with PICALM expression. To gain clarity into other possible SNP mechanisms, we searched brain cDNA for PICALM splice variants. We identified several PICALM splice variants involving exons 13-18 (Ensembl gene: ENSG00000073921). To gain an estimate of their relative abundance, we PCR-amplified across exons 13-20 in cDNA from six individuals, three rs3851179 GG individuals and three rs3851179 AA individuals. Sequencing the cloned isoforms we found that PICALM lacking exon 13 (delta 13) is the most abundant isoform. Moreover, a portion of delta-13 isoforms also lack exon 14. This may be functionally significant because isoforms lacking exon 13 and 14 do not encode the DPF and FESVF protein motifs, respectively, that have been shown to mediate binding to AP-2 (Meyerholz et al.). Other isoforms detected included deletion of exon 18 and a partial and full deletion of exon 15. The functional significance of these changes is unclear at this time. In current work, we are comparing the pattern of splice variants as a function of AD-associated SNPs. Results of this ongoing work will be presented.

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68 **Abstract Title:** **Facilitation of glutamate release through presynaptic NMDA receptors in the DMV**

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Abstract:
The dorsal vagal complex (DVC) is a critical component of autonomic regulation. Viscerosensory afferent input is integrated in the nucleus tractus solitarius (NTS) and transmitted to the dorsal motor nucleus of the vagus (DMV), from which efferent preganglionic motor axons project to postganglionic neurons controlling abdominal gastrointestinal organs. A variety of metabolic disorders, including complications experienced by diabetic patients, have been linked to synaptic dysregulation within the DVC. Mice with steptozotocin-induced type 1 diabetes express increased miniature excitatory post synaptic current (mEPSC) frequency in DMV neurons recorded in acute brainstem slices. An increase in mEPSC frequency suggests a presynaptic increase in the glutamate release probability. Activation of presynaptic N-methyl-D-aspartic acid (NMDA) receptors can increase glutamate release elsewhere in the brain, suggesting the potential for positive feedback control of glutamate release in the DMV. To test the presence of presynaptic NMDA receptors in the DMV, whole-cell patch-clamp recordings were performed in acute brainstem slices. Application of NMDA resulted in an increase in mEPSC frequency, while the NMDA receptor antagonist AP-5, decreased the mEPSC frequency (n=6). No change in amplitude or decay time was observed. Application of AP-5 increased the paired-pulse ratio under stimulated conditions. Together, these findings suggest that presynaptic NMDA receptors on glutamate terminals function to increase the probability of glutamate release onto DMV neurons. Altered glutamate release may contribute to regulation of autonomic output during behaviorally-relevant changes in autonomic motor function critical for maintaining energy homeostasis under normal or diseased states.

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69 **Abstract Title:** **Transcriptional Regulation by Insulinoma-Associated 1 (Insm1) During Retinal Development**

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Abstract:
Development of the vertebrate eye is the result of a complex series of carefully coordinated gene expression events. Proper timing of signaling and expression permit a single layer of neural ectoderm to proliferate and differentiate into a highly conserved and organized tissue. Coordination of this signaling is tightly controlled by multiple cascades of transcription factors; each regulating and being regulated by a host of other transcription factors. Insulinoma-associated 1 (Insm1) is an evolutionarily conserved zinc-finger transcription factor. Insm1 expression is restricted to the developing nervous and pancreatic systems, and is implicated in cell fate specification, axon guidance and cell migration. Additionally, Insm1 is upregulated in response to chronic rod photoreceptor degeneration and in cancers. While Insm1 has known functions in pancreatic beta cell development, its role in ocular development and photoreceptor regeneration have not been determined. Similarly, few targets of Insm1 regulation have been identified, and little is known about the mechanism of regulation. By combining misexpression studies in the zebrafish model system with an in vitro cell culture assay, we have begun to determine the role Insm1 plays during retinal development and to identify direct targets of Insm1 regulation. Knockdown of insm1 caused a significant decrease in differentiated rod and cone photoreceptor cells (at 3 days post fertilization), with no reduction in other retinal cell types. This suggests that Insm1 is directly required for the development of photoreceptor cells. Using an in vitro luciferase assay, we have determined that Insm1 is (in part) directly regulated by genes of the Notch-Delta pathway and we have identified potential Insm1 targets.

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70 **Abstract Title:** **A 'NEET' mitochondrial target: Pioglitazone and mitoNEET in Brain Injury**

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Abstract:
Traumatic brain injury (TBI) is a serious health care problem in the United States with an estimated 1.7 million injuries annually at an estimated yearly cost of greater than 76.5 billion dollars. However, there are currently no pharmacological treatments approved for the clinical treatment of this condition. One of the issues with therapeutic studies in TBI is many mechanisms involved in the injury process have not been elucidated. The fundamental concept underlying this study is that TBI-induced excitotoxicity increases mitochondrial Ca2+ cycling/overload and reactive oxygen species (ROS) production, ultimately leading to mitochondrial dysfunction and subsequent neuronal cell death. Compelling experimental data demonstrates that mitochondrial dysfunction is a pivotal link in the neuropathological sequelae of brain injury. We have shown here that Pioglitazone, a drug known for its PPAR agonistic properties, can increase mitochondrial bioenergetics and cortical sparing following TBI but these effects do not appear to be dependent upon PPAR interaction. We hypothesize that this drug's neuroprotective mechanism is directly related to interactions with the novel mitochondrial protein, mitoNEET. In support of this hypothesis we show that pioglitazone is not protective in mitoNEET knockout animals and that treatment with a specific mitoNEET ligand (NL1), is neuroprotective following TBI. Therefore, we believe Pioglitazone to be a novel mitochondrial targeting drug which is able to alter mitochondria bioenergetics following TBI. The results of these studies will help to shed light on the fundamental processes involved in TBI neuropathology and may pinpoint potential novel interventions and targets for the treatment of TBI.

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71 **Abstract Title:** **Sox4 Expression During Zebrafish Development**

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Abstract:
Sox4 is a member of the group C of SRY-related HMG box-containing transcription factors. During zebrafish development, it is expressed throughout the central nervous system. sox4 knock-out mice die of severe heart malformation as well as defects in B lymphocyte differentiation and reduced numbers of insulin-producing beta cells. Over-expression of sox4 in mice causes impaired oligodendrocyte differentiation. These data suggest that sox4 promotes the differentiation of multiple cell lineages during development. Sox11 is another group C Sox protein. sox4 and sox11 share sequence homology and display similar expression patterns during embryonic development. Neither sox4 nor sox11 null mutation in mice shows CNS malformation, but absence of both leads to severe hypoplasia of the spinal cord, which suggests a functional redundancy of sox4 and sox11 proteins in CNS development. Morpholino knock-down of sox11 in zebrafish causes micropthalmia, coloboma, reduced numbers of photoreceptors and defects in lens. We want to investigate the role of sox4 during retinal development and to determine if there is functional redundancy between sox4 and sox11. The goal of this study was to determine the expression pattern of zebrafish sox4 during retinal development, and in a transgenic zebrafish model of chronic rod photoreceptor degeneration and regeneration.

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72 **Abstract Title:** **Acyclic lobelane analogs as novel inhibitors of vesicular monoamine transporter-2 function**

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Abstract:
Monitoring electrophysiological and neurochemical signals in the CNS with precise spatial targeting and rapid temporal resolution is needed to improve patient outcomes while minimizing the invasiveness of procedures. Presently, electrophysiological recordings are limited in the number of channels and the spatial area covered. Meanwhile, direct neurochemical monitoring with microdialysis is fraught with problems of limited spatial and temporal resolution. Our two pronged approach in developing both a ceramic-based tapered site microelectrode array (MEA) and modifying an existing clinically approved depth electrode, with up to 65 micro-contacts, will serve to advance the state of the art for intraoperative electrophysiological and neurochemical recordings. The MEA is designed for use in aiding in a more precise placement of Deep Brain Stimulation (DBS) electrodes with fewer invasive insertions than the single wire electrodes routinely used. Current DBS surgeries may require 2-3 invasive procedures which could affect patient health. The tapered conformal MEA can provide targeting information simultaneously in 2 axes for more efficient DBS probe placement. Meanwhile, the micro-contact probe can be custom fabricated creating recording sites around the surface of the cylindrical probe, thus, simultaneously yielding a 360° recording field at various depths. Preliminary recordings using the micro-contact probe in rats showed dynamic responses to in vivo glutamate challenges and resting glutamate levels of 1.8 µM under isoflurane anesthesia that decreased by 78% to 0.4 µM with urethane anesthesia. These two electrode designs provide minimally invasive techniques for potentially measuring electrophysiological and neurochemical signals in the human CNS.

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73 **Abstract Title:** **Experimental and Computational Analysis of Mouse Sleep-Wake Dynamics**

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Abstract:
Genetic and behavioral screening of mice play important roles in sleep research, but the need for invasive electrophysiological (EEG/EMG) measurements for determination of sleep-wake and behavioral state limits the scope and rate of experimentation. In this study we explore the utility of a noninvasive method based on the signal from a piezoelectric sensor on the cage floor for scoring sleep-wake behavior in mice. It was previously demonstrated that the piezo signal can accurately discriminate sleep from wake activity; however, this was verified mostly by visual observation. Here we perform a more objective validation by correlating piezo measurements with EMG activity, which is dramatically suppressed during sleep. Furthermore, the piezo sensor is sensitive to respiration-related thoracic movements. Since breathing is relatively irregular in REM sleep compared to non-REM, we extract piezo features that reflect breathing regularity to try to distinguish between these sleep states. We validate our methods against simultaneous video/EEG/EMG measurement, which constitute the gold standard for scoring sleep. But rather than rely on subjective visual scoring to determine state, we use an unsupervised probabilistic model, the hidden Markov model (HMM), to automatically partition time series of extracted EEG/EMG features into REM, non-REM and wake states. A similar HMM, estimated exclusively from piezo features of instantaneous energy and breathing regularity, displayed dynamical stages with a similarity to REM/non-REM sleep, transient arousal, and wakefulness. These preliminary results suggest that a combination of piezoelectric measurements and computational modeling could yield a novel noninvasive method for analysis of sleep and sleep-related disorders.

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74 **Abstract Title:** **Endocannabinoids Mediate Neurotoxicity and Neuroprotection in an In Vitro Model of Ethanol-Induced Neurotoxicity**

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Abstract:
Excessive alcohol consumption produces neurodegeneration which may lead to cognitive deficits. Understanding the mechanisms that lead to neurodegeneration may open new therapeutic avenues to treat alcohol use disorders. We hypothesized that targeting the endocannabinoid system by inhibiting anandamide catabolism would attenuate neurodegeneration induced by ethanol withdrawal (EWD). To test this hypothesis, organotypic hippocampal slice cultures were exposed to either control or 50 mM ethanol (EtOH) media for 10 days. Cultures were withdrawn for 24hr with 5 µM NMDA, respective drug treatment and 2.5 µg/mL Propidium Iodide (to assess cell damage). EtOH significantly potentiated NMDA toxicity in CA1 and CA3 during EWD. Application of 50.0 nM URB597, a fatty acid amide hydrolase (FAAH) inhibitor, completely reversed EWD potentiation of NMDA toxicity, but had no effect on NMDA treatment alone. To determine if CB1 receptor activation was involved in the neuroprotective effect of URB597, we applied the CB1 antagonist SR141716 (0.1 to 100.0 µM). SR141716 also attenuated EWD potentiation of NMDA neuronal damage. After these unexpected results, we tested the hypothesis that CB1 antagonism was mediated though increased GABA neurotransmission, as GABA tone is, in part, under control of CB1 receptors. 10 µM Bicuculine was applied with 10µM SR141716 to determine the involvement of GABAaR. Our results showed that elevation of GABAaR activity is not necessary for the observed neuroprotection by SR141716 as antagonism did not prevent SR141716 mediate neuroprotection. In conclusion, we have shown that the endocannabinoid system is sensitive to ethanol exposure in hippocampal slice cultures and that manipulation of this system affords neuroprotection against EWD-induced excitotoxicity.

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75	Abstract Title:	**Administration of the Nrf2-ARE Activators Sulforaphane and Carnosic Acid Attenuate 4-hydroxy-2-nonenal Induced Mitochondrial Dysfunction Ex Vivo**

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Abstract:
Traumatic brain injury (TBI) currently signifies a substantial health and socioeconomic dilemma in the United States with roughly 50,000 cases resulting in death each year. The pathophysiological importance of oxidative damage after TBI has been extensively demonstrated. The transcription factor Nrf2 mediates transcription of antioxidant/cytoprotective genes by binding to the antioxidant response element (ARE) within DNA. Upregulation of these genes constitutes a pleiotropic cytoprotective-defense pathway. Previously, we demonstrated the in vivo post-injury time-course of Nrf2-ARE mediated gene expression in the cortex and hippocampus of male CF-1 mice utilizing a unilateral controlled cortical impact (CCI) injury model. Interestingly, increased Nrf2-ARE mediated expression was not observed until 24 hours, whereas prior work showed oxidative damage occurring 1-12 hours post-TBI. As neuronal mitochondria have previously been shown to be susceptible to oxidative damage, we sought to mechanistically investigate whether Nrf2-ARE activation in vivo could protect mitochondria under conditions of oxidative stress ex vivo. Young adult male CF-1 mice were administered one of two known Nrf2-ARE activators I.P. – sulforaphane (5.0mg/kg) or carnosic acid (1.0mg/kg) – or their respective vehicle 48 hours prior to Ficoll isolation of cortical mitochondria. Purified mitochondria were then exposed in vitro to 30uM of 4-hydroxy-2-nonenal (4-HNE) for 15 minutes at 37 degrees Celsius. Mitochondrial bioenergetics was then assayed on the XF-24 Bioanalyzer (Seahorse Bioscience, USA). The administration of sulforaphane (SFN) and carnosic acid (CA) significantly ($p < 0.05$) attenuated 4-HNE induced inhibition of mitochondrial respiration for both Complex I and II. Furthermore, CA and SFN both significantly ($p < 0.05$) reduced 4-HNE bound mitochondria protein as determined by Western blot. These results demonstrate the capability of Nrf2-ARE induction in vivo to protect from 4-HNE toxicity to cortical mitochondria ex vivo. Ongoing studies will determine the therapeutic efficacy of Nrf2-ARE activators to attenuate post-TBI pathophysiology.

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76	Abstract Title:	**Intranasal Delivery of the Synthetic Peptide DNSP-11 in a Parkinson's Disease Rat Model**

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Abstract:
Dopamine neuron stimulating peptide-11 (DNSP-11) is a synthetic, 11 amino acid-amidated peptide sequence derived from the pro-region of GDNF, which has been shown to provide anti-parkinsonian actions, including neuroprotection and neurorestoration in a severe lesion rat model of PD following intracranial infusion. However, due to DNSP-11's small size and protective properties, it is a strong candidate for less-invasive delivery methods including intranasal-administration. In our pilot studies, we tracked fluorescently (FITC) labeled DNSP-11 after intranasal delivery and DNSP-11's ability to protect against moderate 6-OHDA lesioning after intranasal-administration in 4 to 6 month old male Fisher 344 rats. Tissue analysis showed fluorescently (FITC) labeled DNSP-11 entering the CNS at thirty (30) minutes and increased labeling in the hippocampus at two (2) hours post intranasal administration. An ELISA assay is now being developed to quantify intranasally-administered DNSP-11 in the olfactory bulb, pre-frontal cortex, striatum and hippocampus. Additionally, intranasally-administered DNSP-11 was found to increase dopamine turnover in the striatum of normal rats determined by HPLC-EC analysis. Finally, following a moderate 6-OHDA lesion of the substantia nigra, animals that received intranasally administered DNSP-11 for three (3) weeks were found to be significantly protected from dopamine loss in the striatum through HPLC-EC analysis. These results support the hypothesis that intranasally administered DNSP-11 protects against dopamine loss in our PD rat models.

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77 **Abstract Title:** **Assessment of Central Infusion of Insulin-Like Growth Factor-1 following Traumatic Brain Injury in Mice**

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Abstract:
Traumatic brain injury (TBI) produces neuronal dysfunction and loss, which can culminate in lasting motor and cognitive impairment. Insulin-like growth factor-1 (IGF-1) is a potent neurotrophic factor capable of mediating both neuroprotective and neuroreparative mechanisms. We hypothesized that elevating brain levels of hIGF-1 would attenuate behavioral dysfunction, reduce cell loss and promote neurogenesis after severe controlled cortical impact (CCI) brain injury. To measure hIGF-1 levels in the brain, C57BL/6 mice were subjected to 0.9mm CCI and treated with either 10ug/d hIGF-1 (n=4) or vehicle (n=2) for 3d via intraventricular (ICV) infusion initiated at 15 minutes post-injury. IGF-1-treated mice had measurable brain hIGF-1 levels and significantly increased levels of phosphorylated Akt, consistent with biological activity of IGF-1. In order to evaluate the efficacy of IGF-1 treatment, mice received 0.9mm CCI or sham injury and hIGF-1 or vehicle (n=19 CCI/treatment, n=10 sham/treatment) for 7d via ICV infusion initiated 15 minutes post-injury. Compared to vehicle-treated mice, IGF-1 treated mice exhibited significantly improved motor function over the 7d period ($p<0.05$), as well as improved cognitive function assessed by a novel object recognition task ($p<0.06$). Although IGF-1 appeared to be associated with a substantial increase in numbers of newborn hippocampal neurons, a subset of mice showed a marked exacerbation of brain swelling. Therefore a subsequent cohort of mice received delayed IGF-1 (n=5) or vehicle (n=3) treatment by implanting a non-primed pump after 0.9mm CCI. Administration of hIGF-1 delayed approximately 6h after TBI alleviated the previously observed increase in brain swelling, and replicated the increase in numbers of newborn neurons at 7d post-injury. Future experiments will quantitatively evaluate the efficacy of delayed IGF-1 infusion in attenuating behavioral dysfunction, reducing cell loss and promoting neuroreparative processes after severe brain injury.

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78 **Abstract Title:** **The Effects of Methylphenidate on Prefrontal Cortex Glutamate Signaling in Awake Freely Moving Rats**

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Abstract:
The medial prefrontal cortex (mPFC) is an area of the brain that is necessary for executive function, and is also implicated in neuropathologies including drug addiction. The mPFC both sends and receives glutamatergic input, and the projection from the mPFC to the nucleus accumbens is hypothesized to be critically involved in the rewarding properties associated with drug addiction. To measure glutamate in the mPFC, we have used our microelectrode array (MEA) technology. Our ceramic based MEAs detect glutamate on platinum recording sites through the use of the enzyme glutamate oxidase. A unique feature of the MEA is the ability to subtract the background current on sentinel sites from the glutamate current on enzyme coated sites, thus isolating a self-referenced glutamate signal that reflects in vivo concentrations. We examined the effects of methylphenidate (MPH) on glutamate levels in the infralimbic (IL) mPFC of awake, freely moving rats, and found decreased tonic resting levels of glutamate in the MPH treated animals ($n = 5$) in the IL compared to saline controls ($n = 6$; $p < 0.001$, significant interaction of time and MPH administration). We also observed significantly increased motor activity in MPH treated animals (MPH, $n = 6$; SAL, $n = 7$; $p < 0.001$, significant effect of treatment). We intend to further examine the relationship between MPH treatment and glutamate signaling in rats performing an operant task. We anticipate that our findings will help contribute to better targeted therapeutic strategies for treating stimulant drug abuse.

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79 **Abstract Title:** **Blast Induced Brain Injury: Influence of Shockwave Components**

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Abstract:
Blast-induced traumatic brain injury (bTBI) has been referred to as the defining injury of Operations Enduring and Iraqi Freedom (OEF/OIF). Additionally, civilians are increasingly at risk from terrorism and industrial accidents. Little is known about which components of the blast contribute to injury. We designed and built a multi-mode shock tube (McMillan Blast Device - MBD) similar to the compressed air-driven shock tube at Walter Reed Army Institute of Research. This new shock tube is able to use four driving modes (compressed air- or compressed helium-driven membrane rupture, a 2:1 mixture of H2 and O2 - oxyhydrogen, and RDX – the primary explosive component of C-4 plastic explosives) to generate the blast wave. Analysis of the blast waves produced by the MBD showed that compressed air-driven membrane rupture produced shockwaves that differed substantially from those produced by compressed helium-driven membrane rupture as well as those produced by chemical explosives (oxyhydrogen and RDX) with respect to the pressure-time profiles of the shockwave. Furthermore, the brains of rats exposed to compressed air-driven blasts showed more evidence of blood-brain barrier breakdown, reactive astrocytosis and microglial activation than those of rats exposed to oxyhydrogen-driven blasts. These data suggest that compressed air-driven membrane rupture produces fundamentally different shockwaves than those produced by chemical explosives and that these differences in shockwave components may contribute to different pathologies, thus providing a foundation for the use of the MBD in bTBI research.

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80 **Abstract Title:** **Toward a Model of Drug Discrimination in Male Japanese Quail: Operant versus Classical Conditioning Techniques**

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Abstract:
The discriminative stimulus (SD) effects of psychostimulants in Japanese quail are not well understood. Studies of drug discrimination (DD) in quail may provide valuable information about how interoceptive drug cues may contribute to drug addiction and relapse. The purpose of the current research was to develop a model of DD to investigate the SD effects of methamphetamine (METH) in quail. In Experiment 1, three (N = 3) adult male quail were used to develop a model of DD using operant conditioning techniques. Quail were trained to discriminate 3.0 mg/kg METH (ip) versus saline with a two-choice operant DD task. The results indicated that quail were unable to acquire DD. In Experiment 2, two (N = 2) adult male quail served as subjects in a Pavlovian model of DD where 3.0 mg/kg METH (ip) served as a feature positive SD for visual access to a female quail and saline served as a feature negative SD. Following an injection of METH or saline, quail received multiple intermittent presentations of a stimulus light followed by brief visual access to a side chamber through a narrow window. A female was present in the side chamber during METH sessions but not during saline sessions. Preliminary results indicate that quail spent more time looking through the window following METH administration compared to saline. Furthermore, preliminary test data indicate that cocaine may dose-dependently substitute for the METH SD. The results suggest that METH functions as a SD and that cocaine produced SD effects similar to those produced by METH. Furthermore, Pavlovian DD techniques appear to be better suited for studies of DD in quail compared to operant DD techniques.

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81 **Abstract Title:** **Differential Rates of Recovery from Sport-Related Concussion: Electrophysiologic, Symptomatic, and Neurocognitive Indices**

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Abstract:
Objective: To compare motor evoked potentials (MEPs), self-reported symptoms and neurocognitive test scores following acute sport-related concussion. Subjects: Nine Division I collegiate athletes (3 females, 6 males, age 20.0±0.87 years). Interventions: Transcranial magnetic stimulation (TMS) was applied over the motor cortex and MEPs were recorded from the contralateral upper extremity. Post-concussion symptoms were evaluated using the Head Injury Scale (HIS); the Concussion Resolution Index (CRI) test battery was administered to assess cognitive functions. All measures were evaluated 1, 3, 5 and 10 days post-concussion. Separate one-way repeated measures MANOVAs were used for between-day comparisons of the dependent variables. Results: Scores on the HIS and CRI improved during the initial 1 to 10 days following concussion but MEPs did not. Concussed athletes reported more frequent and greater severity of symptoms on the first day following injury ($F_{3,24} = 18.2$, $P<.001$). Processing speed on the CRI was slower on the first day post-concussion ($t_8 = 4.6$, $P=.002$) and demonstrated a steady improvement (faster PS) over the testing period. Median MEP latencies were significantly longer (slower) on testing day 10 (27.2±2.3 msec/m) compared to day 1 (25.4±1.4, $t_8=-2.69$, $P=.03$). Ulnar MEP amplitudes were significantly smaller on day 3 (.27±.10 µV) compared to day 5 (.41± 16, $t_8 = -3.48$, $P=.008$). Conclusions: In the initial 10 days following concussion, symptoms and neurocognitive test performance improved but MEP changes persisted. The pattern of impairments suggests different neurophysiologic mechanisms may be responsible for the MEP changes compared to post-concussion symptoms and neurocognitive deficits.

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82 **Abstract Title:** **Introduction of a new neurophysiology laboratory for students at the University of Kentucky**

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Abstract:
The new experimental laboratories and obtained infrastructure for the Animal Physiology (Bio 350) with electrophysiological equipment, we are now able to piggy back a new course specifically on neurophysiology. This course is to be 1st taught in the spring 2013 and will be listed as a Bio 500 level course. Both advanced undergraduates and graduate students will be able to take this course. The prerequisite will be to have completed Bio350 or its equivalent with some electrophysiological understanding. The students will learn the following topics: (1) Electrophysiological equipment (extracellular & intracellular amps, microscope use, electrode production); (2) Membrane potentials in crayfish abdomen muscles and influence of [K]o on membrane potential ; (3) To stimulate motor nerves and record EPSPs/IPSPs; (4) Measure facilitation and depression in tonic and phasic neuromuscular junctions; (5) Learn to record from proprioceptors (extracellular) in the crab leg and relate to joint positions; (6) Learn to record from tension receptors in the crab leg related to muscle length; (7) Learn how to forward fill neurons from the crab leg proprioceptors (CoCl2, 4-Di-2 ASP) as well as stain with methylene blue; (8) Learn how to dissect the leech ventral nerve cord and obtain intracellular recordings from identified neurons. They will use current injections and measure threshold. Potentially, they will learn to use two intracellular electrodes to record in situ synaptic connections. Thus, they will investigate the ionic currents making up various types of action potentials; (9) Mapping skin receptive fields on the leech while recording from neurons; (10) Learn how to remove and culture leech neurons for forming synapses in culture; (11) Vision: crayfish & fruit fly eyes and caudal photo receptor in crayfish; (12) Quantal analysis of synaptic transmission; and (13) computational simulations as well as some pharmacological applications. These new hands-on laboratories will be videoed for the different techniques and posted on the course website for it to be freely accessible to students in the course as well as world wide. Also we plan to publish them at Journal of Online Visual Experimentation (JoVE). In addition, students learned how to collect and interpret the data to develop critical thinking skills. They will also be required to write a lab report as if it were for peer review for publication to a scientific journal.

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83	**Abstract Title:**	**Relationship of Neuroprotective Effects of Lipid Peroxyl Scavenger U-83836E to Early Motor Functional Recovery and Tissue Damage After Traumatic Brain Injury**

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Abstract:
The free radical scavenger and lipid peroxidation inhibitor U-83836E has previously been shown to improve cortical mitochondrial respiratory function and preserve Ca2+ buffering capacity following severe controlled cortical impact traumatic brain injury (CCITBI) in mice. Consequently, restoration of mitochondrial bioenergetics by U-83836E lead to a reduction in post-traumatic calpain-mediated cytoskeletal damage. The established potent biochemical protective efficacy of U-83836E shows promise of its therapeutic potential, but warrants further investigation into posttraumatic effects on neuronal damage and functional recovery. We examined the ability of U-83836E to reduce peak neurodegeneration occurring at 48 hr using de Olmos aminocupric silver staining and to improve behavioral recovery measured by neuroscore at 24 hr and 48 hr after severe CCI-TBI in mice. Administration of a repeated 24 hr dosing paradigm of 30 mg/kg U-83836E did not improve overall motor deficits, but did suggest improved hindlimb motor function at both time points. The same dosing regimen also reduced the volume of hemispheric neurodegeneration at 48 hr post-injury by approximately 50% ($p<0.05$ vs vehicle-treated brains). These data demonstrate that U-83836E-induced mitochondrial and cytoskeletal protection occurred in the remaining post-injury brain tissue. Collectively, the data thus far supports the idea that targeted inhibition of posttraumatic lipid peroxidation restores the cellular homeostatic functioning of surviving neurons, which could represent a long-term antioxidant treatment strategy, especially when combined with alternative therapeutic targets for TBI.

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