SYNAPSE 2011 Abstracts (Listed in the order that they were received)

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Dissociating the Roles of the Cerebellum and Motor Cortex During Adaptive Learning
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Adaptation to a novel visuomotor transformation has revealed important principles regarding learning and memory. Computational and behavioral studies have suggested that acquisition and retention of a new visuomotor transformation are distinct processes. However, this dissociation has never been clearly shown. Here, participants made fast reaching movements while unexpectedly a 30-degree visuomotor transformation was introduced. During visuomotor adaptation, subjects received cerebellar, primary motor cortex (M1) or sham anodal transcranial direct current stimulation (tDCS), a noninvasive form of brain stimulation known to increase excitability. We found that cerebellar tDCS caused faster adaptation to the visuomotor transformation, as shown by a rapid reduction of movement errors. These findings were not present with similar modulation of visual cortex excitability. In contrast, tDCS over M1 did not affect adaptation, but resulted in a marked increase in retention of the newly learned visuomotor transformation. These results show a clear dissociation in the processes of acquisition and retention during adaptive motor learning and demonstrate that the cerebellum and primary motor cortex have distinct functional roles. Furthermore, they show that it is possible to enhance cerebellar function using tDCS.

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Relational Memory for Manipulated Scenes in Rhesus macaques
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Relational memory is the ability to associate multiple distinct elements into a coherent representation. Previous studies have shown that human subjects demonstrate relational memory for items in a complex scene by preferentially viewing objects in repeated, altered scenes in comparison to those in unmanipulated scenes. However, amnesic patients who had sustained damage to the hippocampus did not demonstrate this type of memory, indicating that this is hippocampal-dependent. In this study, we examined the ability of Rhesus macaques to demonstrate relational memory by viewing a manipulated region of a repeated scene, compared with a repeated unmanipulated scene. Four head-fixed monkeys performed a free-viewing task where they were presented complex scenes and were allowed to freely view these images for 10 seconds on a 19in CRT monitor. Each trial included two images, a novel image then followed by a repeated scene with or without manipulation. Manipulated images were defined by movement or replacement of an object to a new location or with a novel object, respectively. Eye movements were recorded with an infrared eye-tracking system. We found that monkeys spent more time viewing the manipulated objects than when they were unmanipulated, both when moved and replaced by a new object ($p<.001$). This effect was significant within 1s following stimulus onset ($p<.01$), indicating quick recognition of the manipulation. These data demonstrate that monkeys are able to form memories for relational aspects of visual scenes. We are currently

investigating the neural signals within the hippocampus that may support relational memory encoding and retrieval.

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Effect of Crude Kava Root Extract on Helstead Category Test Performance in Young Adults
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The objective of this study was to assess if a single 200 mg oral dose of a standardized 30% kava (Piper methysticum) extract produced impairments in executive function, as measured by a computerized version of the Russell Revised Short Form of the Halstead Category Test (RCat). The study was designed as a randomized, double blind, placebo controlled trial and took place at a private university in the United States. Participants included 54 undergraduate students (21 males and 33 females) (age 19.9 ± 1.3 years). Participants were randomized to either a placebo or kava group, and following a 3 h fast, ingested one clear, unmarked gelatin capsule that contained either 648 mg powdered gelatin (placebo) or 200 mg of a standardized 30% kava extract. A computerized version of the RCat was administered to each participant 60 minutes after capsule ingestion. Executive function was measured using the number of errors committed and average response time for each of the 6 su btests of the RCat. Results showed no difference in performance on the RCat between the placebo and kava groups. For both outcomes, the main effect of treatment and interactions between treatment and subtest, sex, and body weight were not statistically significant (all p's > .15). It was concluded that a single oral dose of 200 mg of a standardized 30% kava extract had no effect on performance on the RCat in young adults. This supports prior research suggesting that unlike other anxiolytics, kava extract does not appear to induce cognitive dysfunction at clinically relevant doses. However, additional research is needed to determine if impairments in executive function are produced by higher doses.

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Can the 8 Coil Shakti alter subjective emotional experience? A randomized, placebo controlled study.
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The Shakti is a commercially available transcranical magnetic stimulation (TMS) device that produces complex, low intensity fields and is marketed for use in promoting altered states of consciousness. Research involving the Shakti has typically not incorporated a randomized double blind design, and the publicized effects of the Shakti may result from participant expectancies or experimenter suggestion. This study measured the effect of 30 minutes of exposure to the Shakti's Emotional stimulus train on response to static images, using a randomized placebo controlled design. After 30 minutes of Shakti exposure given while wearing earplugs and sitting blindfolded in a dark room (device electronics were turned off for placebo group), participants (11 males, 26 females) viewed a randomly ordered slide show of 54 images chosen from the International Affective Picture System (IAPS). For each image, participants responded to questions of emotional valence, type of emotion, and effect strength using visual analog scales. The emotional valence of the image significantly affected responses to the image

(all p's < .0001), demonstrating that viewing the IAPS images resulted in varying emotive states. Neither the main effect of treatment (Shakti on or off), nor the interaction between treatment and image valance (positive, negative, neutral) were significant (all p's > .09). Because the study followed parameters set by the device manufacturer, previous reports of alterations in emotional perception caused by the Shakti are likely due to participant expectancies or experimenter suggestion and should not be attributed to the direct effects of the Shakti TMS stimuli on temporal lobe activity.

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Modafinil Reverses Methamphetamine-Induced Memory Deficits in the Object to Place Task
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Chronic methamphetamine leads to persistent cognitive deficits in humans and animals. We showed that both contingent and non-contingent meth impairs memory on the object to place task. Further, we assessed whether modafinil would reverse this cognitive impairment. In the first experiment, male Long-Evans rats received either saline or acute meth injections (4 x 4 mg/kg, 2 hr intervals). In a second experiment, rats self-administered I.V. meth (0.02 mg/infusion) on an FR1 schedule of reinforcement (7 days for 1 hr/day, followed by 14 days for 6 hr/day), or received yoked saline infusions. Following one week of withdrawal, rats were tested in the object to place task. Rats were allowed to interact with four objects for 5 minutes in a closed test chamber. Following familiarization, rats received either vehicle or modafinil (100 mg/kg) injections. Ninety minutes later, the location of two objects was switched, and total time spent with each object was recorded.

Our results showed that saline-treated rats spent more time interacting with objects in the changed locations. In contrast, rats with a history of meth spent similar amounts of time at all objects, indicating a memory impairment. Modafinil-treated meth rats spent more time interacting with objects in the changed locations, indicating a reversal of memory impairment. These findings demonstrate both meth-induced cognitive deficits on the object to place task, and the reversal of these deficits by modafinil. Meth-induced dysregulation of monoamine transporters and their modulation by modafinil may account for these results, therefore comparisons of monoamine transporter levels and markers of meth toxicity will also be presented.

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Effects of Social Housing on Cellular Proliferation and Estrogen Receptor Alpha in a Monogamous, Biparental Rodent: The California Mouse: (Peromyscus californicus).
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Social environment, particularly isolation, has a significant impact on neuroendocrine responses, neurogenesis and social behaviors. Steroid hormones, including estrogens, have been shown to influence rates of neurogenesis. We determined if social environment affects cell proliferation and estrogen receptor alpha (ERα) distribution in established neurogenic regions, such as the

dentate gyrus (DG), as well as regions of the limbic system associated with social behavior. Using a monogamous and bi-parental rodent species, the California mouse (Peromyscus californicus), we housed adult mice with a same sex conspecific or in isolation for 4 days. Cell proliferation was measured using injections of 5-bromo-2'-deoxyuridine (BrdU). Cell phenotype was determined using double labeled immunofluorescence for BrdU and ERα. Preliminary data demonstrate co-localization of BrdU and ERα in the DG. Additional data collection will determine differences across groups in ERα concentration and double labeled cells.

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Ontogenetic Comparison of the Retina of the *Ambystoma tigrinum* at Distinct Developmental Stages
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The amphibian brain can be seen as a prototype for vertebrate nervous systems. Many neuroscience studies have examined central nervous system structures of amphibians, especially the salamander, including sensory structures. In this study, the in situ brain of the tiger salamander (Ambystoma tigrinum) was compared at distinct aquatic stages of development, with focus primarily on the visual system. Specifically, we compared retinal development at the early aquatic stage (Harrison stage 40) of ontogeny to the retina at the late aquatic stage of development. The late aquatic stage occurred at approximately 100 days and was indicated by retention of gills and aquatic life style. Differences in the visual system, specifically the retina, were observed due to maturation of the salamander towards a terrestrial lifestyle. The most notable difference between the different stages is the localization of the retina around the lens at the early aquatic stage and retraction towards the optic cup at the late aquatic stage. It is also suggested that cell morphology occurs during maturation. These results suggest development from an aquatic lifestyle towards a terrestrial lifestyle, yet the largest morphological change may be once the animal takes on a land-dwelling lifestyle.

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A Genetic Screen to Identify a Role for Palmitoylation in Drosophila Circadian Behavior
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Infantile neuronal ceroid lipofuscinosis (INCL) is caused by mutations at the CLN1 locus. INCL is characterized by neurodegeneration and a diversity of symptoms including sensory and motor dysfunction, seizures, and disrupted circadian rhythms. These abnormal conditions stem from the elimination of palmitoyl-protein thioesterase 1 (PPT1) expression, a thioesterase enzyme encoded by CLN1. PPT1 is involved in removing palmitate, a post-translational protein modification on cysteine residues. Palmitate is added by a family of transferase proteins that are characterized by a DHHC (Asp-His-His-Cys) active-site motif during palmitoylation. Palmitoylation is implicated in regulating a variety of neural activities, including synaptic vesicle fusion and directing protein association with lipid domains on the cell membrane. Recent work in our laboratory has suggested that PPT1 mutations in Drosophila melanogaster produce alterations in circadian rhythms. To investigate wheth er circadian rhythms are influenced by the

reduction of DHHC transferase expression, we performed a genetic screen using RNA interference (RNAi) lines specific for individual DHHC homologs in the Drosophila model system. We used the Gal4 driver/UAS system to knock-down DHHC expression in the adult nervous system. Drosophila were placed in activity monitors and circadian rhythms on both light/dark and dark/dark cycles were measured. Beyond our behavioral assay, we also utilized RT-PCR to measure the extent to which RNAi knocks down DHHC gene expression. Our screen seeks to determine whether or not palmitoylation is required for the robust expression of circadian rhythms in the Drosophila. This study will help characterize the link between alterations in palmitoylation activity and the disruptions observed in NCL patients.

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Extinction Training during Reconsolidation Window: Key to Attenuation of Cocaine Memories
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Previous studies have shown that memories, when recalled, enter a labile state during which they are susceptible to disruption. During this reconsolidation period, it is possible to influence how well memories are encoded for future control of behavior. While this is well-established in the study of fear and stress, it is unclear how drug-seeking-related memories might be influenced by manipulations of reconsolidation. In this study, we disrupted cocaine-seeking memories by extinguishing cocaine-related memories during the reconsolidation phase. Animals underwent cocaine conditioning using conditioned place preference. Following conditioning, the cocaine memory was retrieved, or not, by exposure to the cocaine-associated context, with the goal of disrupting the association in one group and not in the other. We found that starting extinction training one hour after the cocaine contextual memory was retrieved (during the reconsolidation window) significantly blocked cocaine-primed reinstatement of a cocaine preference: $p < 0.01$. There was no significant difference in the expression of cocaine preference or extinction between the two groups. We also found no difference in the number of extinction sessions between the retrieval and no retrieval groups. In the final analysis, we believe that the results of these studies will contribute significantly towards the development of treatments for drug abuse and addiction.

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The Role of Glutamate Receptors in Extinction Learning
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Drug associated cues that operate under classical conditioning are thought to maintain addiction and relapse. Extinguishing these associations could help in providing a remedial apparatus for those individuals suffering from addiction. Since extinction learning is now viewed as novel learning, interventions can be formulated to facilitate the extinction process by manipulating the systems that are responsible for learning, which would entail manipulating the glutamatergic mechanisms that underlie learning and memory. The substrates of interest are the N-methyl-D-aspartate (NMDA) receptor, type 5 metabotropic glutamate receptors (mGluR5), and 3-cyano-N-

(1,3-diphenyl-1H-pyrazol-5-yl) benzamide (CDPPB). By manipulating these neurochemical structures, our aim is to show enhanced extinction of alcohol-seeking behavior as indicated by an increase in neuronal plasticity.

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Changes in CRF Peptide Following Chronic Intermittent Ethanol Exposure in C57BL/6J Mice
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Alcohol dependence is influenced by many biological and environmental factors, resulting in changes within regions of the brain that regulate reward and stress mechanisms. Over time, these changes may enhance vulnerability to stress-induced relapse behavior. A neuropeptide called corticotropin-releasing factor (CRF) is the primary regulator of stress systems in the brain, and it is essential for producing the physiological and behavioral responses to stress both within and outside of the hypothalamic-pituitary-adrenal (HPA) axis. CRF also plays a role in influencing the brain's reward circuits, including the processing of rewarding stimuli, such as alcohol. In this research project, chronic intermittent ethanol (CIE) exposure/withdrawal was employed to examine regional changes in CRF activity in the brains of adult male C57BL/6J mice. Mice were sacrificed after either two or five cycles of CIE exposure at different time intervals following final ethanol exposure. B rain regions examined included the amygdala, BNST, and the paraventricular nucleus of the hypothalamus, all of which are essential to reward and stress modulation. Results indicated that stress associated with repeated CIE exposure and withdrawal produced changes in CRF peptide levels in the amygdala, and BNST, but no changes were seen in the paraventricular nucleus.

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Palmitoylation and Retinal Axon Guidance
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A primary focus of the neuroscience community is to understand the most basic structure of our nervous system, neuronal connections. This basic structure is composed of billions of synapses which are formed continually over time from the initial development of the embryonic nervous system. How a developing axon travels long distances through the embryo to find its correct target cell among millions of alternatives is a primary question that is variable within the developing nervous system but crucial to our understanding of the foundation of our nervous system. Recent research has shown that the palmitoylation of proteins is important for normal development. Palmitoylation alters a protein's activity by attaching a small fatty-acid called palmitate. We hypothesize that palmitoylation may be crucial for the proper development of retinal axon pathways that enter and connect to the brain. The fruit fly has a well-characterized nervous system making it a good model to study how the connections of the brain are made. We have eliminated the function of the enzymes that add palmitate in fly retinal cells to investigate the consequences of altering palmitoylation on the developing connections between the eye and brain.

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Pharmacological effects of TBOA on extracellular glutamate in the nucleus acumbens
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This study investigates the effects of microinjecting DL-threo-β-Benzyloxyaspartic acid, a non-selective glutamate reuptake blocker, into the nucleus accumbens (NAc) on extracellular glutamate (GLU) concentration. It has been demonstrated in a mouse model of ethanol dependence, that ethanol dependent mice drink more ethanol than non-dependent mice. Furthermore ethanol dependent mice exhibit higher levels of extracellular GLU in the NAc than non-dependent mice. Microinjections of TBOA into the NAc produces increases in ethanol drinking, thus strengthening the correlation between increased glutamate in the NAc and excessive ethanol drinking. This study will directly examine the effects of microinjection TBOA on extracellular GLU in the NAc by simultaneously conduction microdialysis to the NAc. In this way, we will be able to quantify the extent of the GLU increase with doses of TBOA known to significantly increase ethanol drinking.

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The Effect of Repeated Ethanol Withdrawal on Cognitive Performance in a Mouse Model of Ethanol Dependence
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Cognitive deficits arise from functional and structural changes in the frontal cortices of the brain that emerge over repeated withdrawals from chronic alcohol use. The aim of this study is to determine if a rodent model of alcohol dependence results in persistent cognitive deficits following withdrawal. Mice were made dependent via exposure to 4 cycles of vapor treatment with each cycle consisting of one binge exposure (defined as 4 bouts each consisting of 16 hours exposure to ethanol and 8 hours in the home cage) plus a 72 hour withdrawal period. Following the fourth cycle, mice rest in their home cages for an additional four days: an acute withdrawal phase. Seven days following their last exposure to ethanol, mice began training in the water radial arm maze. The maze consists of a central pool with four arms, all of which contain submerged platforms that serve as an escape. Each session consists of 4 trials each lasting for 3 minutes or until a platform is found. Once the platform is found, it is then removed, and the mouse is allowed to rest for thirty seconds; the procedure is then repeated 3 more times. Performance is quantified by working memory correct (WMC) errors, which occur when a mouse enters a previously baited arm. We hypothesized that alcohol dependent mice will exhibit slower learning than the non-dependent ones. That is, dependent mice will display more WMC errors than control. Results showed that dependent mice exhibited significantly more WMC errors than the non-dependent mice.

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The Effects of Ethanol on the Working Memory in Mice
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It is well known that chronic consumption of alcohol can have adverse effects on cognitive functions. Therefore, we investigated how long-term exposure of ethanol affects the working memory in mice. The subjects were made dependent to this drug of abuse by placing them in vapor inhalation chambers for repeated cycles where they were exposed to volatilized alcohol. This treatment was followed by a period of withdrawal and the mice subsequently underwent cognitive behavioral tests in plus-maze based learning models. We found that a seven day withdrawal period resulted in no difference between the control mice and the ethanol dependent mice in memory function, whereas significant differences in learning abilities were seen with a three day withdrawal period. The control mice followed the same treatment paradigm, but were placed in vapor inhalation chambers without being exposed to the volatilized alcohol. Currently, we are conducting studies that are designed to confirm that the substrate affects the prefrontal cortex through lesioning this part of the brain via stereotaxic microinjection of the neurotoxin N-Methyl-D-aspartic acid (NMDA). The mice will undergo the same plus-maze based learning procedure as in the first two experiments to explore how this neurotoxin affects their cognitive abilities compared to the control mice that were injected with saline. Thereafter, immunohistochemistry will be conducted to confirm that the microinjection of NMDA was performed in the desired area of the brain.

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Specificity of Chemical Irritant Tolerance in Birds
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The paradox of the Capsicum pepper- that capsaicin (CAP), a trigeminal irritant inherent to the chili fruits, discourages consumption by mammalian seed predators without repelling seed dispersing birds- leads to an inquiry of whether birds also have a tolerance for other trigeminal irritants. We used allyl isothiocyanate (AITC), which stimulates the Transient Receptor Potential A1 (TRP-A1) channel of the trigeminal system and capsaicin (CAP), which stimulates the Transient Receptor Potential V1 (TRP-V1), to assess the specificity of the evolved response of the bird trigeminal system. While the avian tolerance of CAP is well documented, no information exists about the ability of birds to detect AITC. In the present study, house sparrows (Passer domesticus) were used to address the question of chemical irritant specificity in the bird trigeminal system. We experimentally compared the birds' food consumption and aversive response to bird chow coated in 100mM AITC solution, 100mM CAP solution, 100mM methyl anthranilate (MA) solution, and 100mM phenethyl alcohol (PEA) solution versus un-enhanced bird chow. Our results show that the birds' food consumption may have been slightly altered by the addition of CAP and MA but unaffected by the addition of AITC and PEA. Marginally significant differences were detected in number of head shaking events between MA and control, and significant differences were observed for food rejection events between MA and control.

These data suggest that birds can detect CAP and MA but not AITC and PEA; however, the effects of CAP on aversive response remain unclear.

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The Degree of Potentiation and Selectivity of Magnolol and Honokiol on the Subunits of the GABA(A) Receptor
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The National Center for Complementary and Alternative Medicine (NCCAM) estimates that nearly 40% of adults in the United States use some form of alternative medicine. It is important to determine their mechanism of action so that they can be used safely and appropriately. Extracts from Magnolia tree bark have been used for centuries in traditional Chinese medicine to treat a variety of neurological diseases, including anxiety disorders. The active ingredients in the extract have been identified as magnolol and honokiol, and these isolated compounds have been shown to be modulators of the $GABA_A$ receptor. These receptors are ligand-gated ion channels which are activated by gamma-aminobutyric acid (GABA), the primary inhibitory neurotransmitter in the CNS. The subunit composition of neuronal $GABA_A$ receptor is heterogeneous, and the pentameric receptor can contain subunits from seven different families with multiple subtypes. The goal of these studies was to determine whether modulation by magnolol and honokiol was dependent upon the subunit composition of the receptor. We used patch clamp recordings from transiently transfected cells to measure the response of receptors containing each of the six different alpha subunits. Both magnolol and honokiol significantly enhanced the activity of all the receptors, regardless of the alpha subunit. Future studies will examine the effect of the beta, gamma, and delta subunits and will use point mutations to determine the site of action of these modulators within the receptor. Our data indicate that these two compounds are effective positive modulators of $GABA_A$ receptors and would be expected to reduce neuronal activity, reducing anxiety and seizure activity while producing sedation. They may also interact with other medications with similar actions, including alcohol.

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Working Memory Errors in Alcohol Dependent Mice
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The negative reinforcing effects of withdrawal produce a range of behavioral and physiological consequences in both humans and animal models of alcohol dependence. For the latter, ethanol vapor inhalation chambers are an efficient way to induce dependence using a chronic intermittent schedule of exposure. Cognitive deficits in attention and executive function have been experimentally demonstrated in alcohol-dependent humans. Comparatively, we hypothesized that similar cognitive deficits could be observed in an animal model. To test this, alcohol dependent mice were produced through a 4-week chronic intermittent regimen in an ethanol vapor chamber. Alcohol-treated mice and control mice were then subjected to 14 days of experimentation using the radial arm water maze task. Performance was measured as a function of working memory errors made per day throughout the experiment. We will present the results

of the behavioral trials and compare the performance of our control and alcohol-dependent animals.

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Comparison of Ion-Based Periodic Spiking Models Using Phase Response Curves
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Neural cells use action potentials, abrupt changes in the electrical potential across a cell's membrane, to communicate with other cells. Action potentials are generated and sustained by ionic currents flowing through cell's membrane. Among the most involved are sodium influx that ensures cell depolarization and potassium efflux that ensures cell repolaroization. The ionic balance is quickly re-established by a sodium-potassium exchanger. However, in a closely packed bundle of neuronal cells with fast diffusion of ionic species in the extracellular space an action potential generates in one axon can impact the excitability of nearby neurons. Previous studies have considered the role of potassium in seizures by employing a computational model of potassium diffusion between periodically firing neurons. However, absent from most of these studies on diffusion coupling is the potential effect of sodium dynamics on this form of neuronal coupling. This study employs a Hodgkin-Huxley model of the axon modified, based on previously developed models, to produce a periodic spiking potential. The model accounts for the dynamics of major ionic species, as well as certain aspects of the extracellular environment. This model is compared to "reduced" models, in which the dynamics of only a specific ion are considered, through analysis of periodic response curves produced by a simulated injected current into the neuron. These data are then used to provide evidence for whether potassium in isolation is sufficient to describe the behavior of such dynamic coupling systems, or if a more detailed model is necessary.

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Identification of hormone receptors that mediate metabolic homeostasis in Drosophila
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The development of RNAi as a genetic research tool has been one of the most profound revolutions in the biological sciences. With RNAi it is possible to selectively inhibit the expression of a single gene from the entire genome of an organism. When techniques using RNAi are applied to the fruit fly, Drosophila melanogaster, they prove to be especially illuminating due to extensive investigation of the fruit fly genome. In Drosophila it is possible, using RNAi, to target not only specific genes, but specific genes within specific cells, a feat unprecedented in genetics. Using this tool I undertook a screen of membrane receptors on adipokinetic hormone (AKH) cells. AKH cells are integral to the stress response pathway of Drosophila. By selectively inhibiting the expression of specific cell membrane receptors I uncovered key hormones—indirectly through the associated receptor—that are involved in the pathway leading to a stress response. The process involved first the crossing of transgenic flies, AKH::GAL4, to various mutants containing RNAi for a single receptor. The progeny of these crosses were then collected and starved; median survivorship curves were created based the time at which fifty percent of the flies had died. The results clearly demonstrated hormones involved

in the inhibition of stress response as well as others that were factors in the initiation of the response. As the stress pathway is conserved throughout metazoans, this screen and resulting experimentation are crucial for the understanding of our own physiology as well as prevention of stress related illness.

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Prepulse Inhibition of Startle Varies With Attention Task Difficulty
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Prepulse inhibition of startle (PPI) is due to an inhibitory mechanism that attenuates interruption of prepulse processing by a subsequent startle stimulus, and has been shown to vary with directed attention. Startle eyeblink EMG was measured from 57 participants, divided into three groups, with all groups receiving an identical initial block of trials, and then given different instructions: Detect (press a button whenever they heard a tone); Discriminate (press one button when they heard a low frequency tone and a different button when they heard a high frequency tone); and Control (no task instructions). Task accuracy was lower and reaction time was longer for the Discriminate group than for the Detect group. Compared to the Control group, PPI increased from Block 1 to Block 2 in the Discriminate group but not the Detect group, suggesting that adding discrimination as a higher level task to simple detection causes increased protection of prepulse processing (as seen in increased PPI). Implications for the use of PPI to investigate attention will be discussed.

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Pop-Out as a Function of Strobe Flash
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This psycho-physical study explored whether twenty experimentally naive subjects could reliable perceive whether a number of gray ellipses reversed direction; i.e., ‘popped-out’. The experiment used a total of 24 stimuli (with either a 0.4, 0.6, or 0.8 gradient, with either 0, 0, 1, 2, 4, 8, 12 or 17 flipped ellipses). Each stimuli figure was illuminated by flashing a white LED for either 1.12 ms (short duration) or 2.20 ms (long duration). Percent correct was plotted for each condition. Unexpectedly, with zero flips and short duration as the gradation got stronger (from 0.4 to 0.8) the percent correct decreased. This effect went away with long durations. As expected, for both durations, as the number of flips increased from 1 to 2 to 4, the percent correct increased. Likewise, as the gradation got stronger (from 0.4 to 0.8) the percent correct increased. These effects were near maximal when there were a large numbers of flip (8, 12, or 17). This is interesting given that with stronger gradations zero flips showed a decline in performance at short durations. A correlation between short and long durations show that at lower percent correct long durations outperformed short durations. Thus, as gradients get stronger it’s easier to detect ‘pop-out’. It is also easier to detect ‘pop out’ as the number of flipped ellipses increase. These results are discussed in terms of brief stimulus presentations which do not allow for eye movements.

Jones LS, Bhatia R, Biyun C, Boozell T, Cook M, Eisenhofer J,Flores K, Francis TC, He J, Kitson K, Kusper M, Minton B, McClellan K, Nazir A, Newsom K, Pathak A, Robinson E, Rogers C, Rambo R, Redfearn, C, Shapiro, L, Sweitzer SM2, Sybert K, Wozniak, J, Young R
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IMPULSE Reviewer Training Sites: Involving Student Groups in the Research Enterprise

In response to the Boyer Commission report, R1 institutions were exhorted to include undergraduates in primary research. Many PUIs do this, but the challenge for R1s is how to extend this opportunity to large numbers of students. One option is for faculty to become Faculty Advisors with IMPULSE and to mentor and train neuroscience (and related) majors as reviewers for IMPULSE. This online journal has been publishing undergraduate neuroscience articles since 2003, offering students worldwide the opportunity to learn about scientific publishing by serving as peer reviewers.

Currently, the international reviewers come from over 15 universities. Most reviewers are affiliated with Reviewer Training Sites where they receive formal training, but the particulars of training vary among sites, creating multiple possible models. At ASU the students take a 1-3 credit course, Scientific Publishing, and follow a published curriculum (JUNE Spring 2006 Vol. 4, Issue 2 http://www.funjournal.org/previous-issues/2006-vol-4-issue-2). At Middlebury, St. Olaf, Furman, and Salve Regina the Faculty Advisors work with students from their lab groups and classes, using the manuscripts to teach about reviewing. At USC the student reviewers started a university IMPULSE club, and their FA, a medical school neuroscientist, serves as their club advisor, mentoring for manuscript reviewing and club events, like Brain Awareness Week. Another medical school example comes from the University of the Free State, South Africa, where a small group of neuro-interested undergrads work with a faculty mentor to learn how to read and review the original submissions. Faculty are encouraged to consider ways they might enhance the research experience of their undergraduates by hosting a Reviewer Training Site at their institution.

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Brain Awareness Week 2010: Small Group Based Elementary School Activities
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Neuroscience education can provide practical tools with which a student can better understand the way in which he/she interacts with the world. The pedagogical tools to aid this understanding are largely not in place before secondary and tertiary educational levels. However, Brain Awareness Week provides a perfect opportunity to be a guest educator at all levels. Brain Awareness Week 2010 was celebrated in five days of activities designed for K-5th grades which promoted a functional understanding of neuroscience. Standard and novel pedagogical tools were developed and employed to show the relevance of neuroscience as more than a pedantic exercise. These activities took place at The Center for Knowledge, a public magnet elementary school in Columbia, South Carolina. Every day began with an introductory lecture, followed by small group hands-on activities led by undergraduate and graduate students.

The day was concluded with a review of the information, a presentation of collected data and explanation of trends, or the reading of a children's book related to the activities. Kindergartners explored the comparative anatomy of skulls from carnivores, herbivores, and omnivores and reinforced healthy eating for strong bones and helmet safety. First grade examined the critical role that the brain plays in understanding the perception of our five senses. Second grade did comparative neuroanatomy by measuring brain weights and sizes from different animals. Third grade built on their knowledge of neuroanatomy by learning about neurons, neurotransmission, and neural circuits as well as completing a ruler drop experiment to calculate nerve conduction velocity. Fourth grade went on to explore the effect of neurotoxins from spiders, puffer fish, and snakes in a hands-on demonstration of neurotransmission. Fifth grade was introduced to the hippocampus, learning and memory, and how to use tricks to improve memory. The specific topics for each grade were chosen to integrate into the Core Knowledge Curriculum that is taught at The Center for Knowledge.

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The Effects of Neuropsin on Oligodendrocyte Apoptosis
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Neuropsin, a serine protease, has been implicated in the pathological increase of neural cell apoptosis. Damaging amounts of apoptosis, or programmed cell death, are particularly detrimental to oligodendrocytes, glial cells that myelinate the axons of neurons of the central nervous system, as these cells are post-mitotic and myelinate several axons simultaneously. The current study aimed to explore the effects of neuropsin on oligodendrocyte survival. This was accomplished by treating oligodendrocytes derived from the spinal cords of chick embryos with a range of neuropsin and measuring oligodendrocyte survival 24 hours and 48 hours after treatment using MTT assay. The study demonstrated a significant increase in oligodendrocyte survival in the 48-hour assay compared to the 24-hour assay in the 1 nM, 21.25 nM, 127.5 nM, and 148.75 nM treatment groups. Because this significant increase was also observed in the control group, this does not directly implicate neuropsin in this effect. These results may reinforce the established importance of extracellular matrix molecules as substrates for neuropsin's proteolytic function.

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Evaluation of the Significance of Bmi-1 as a Target of MicroRNA-128 in Medulloblastoma Cells
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Medulloblastoma is an aggressive primary brain tumor and is one of the most common pediatric brain tumors. Medulloblastomas have a dismal prognosis and a reliance on conventional, cytotoxic therapies often results in neurological impairments, especially in young children. Recently, improvements in therapeutic techniques have been made through the discovery of microRNAs. MicroRNAs are small, non-coding RNA molecules that regulate gene expression. These microRNA molecules have the ability to interact with oncogenes and suppressor genes. Previous research has found that microRNA-128 targets and interacts with the oncogene, Bmi-1

in glioblastoma cell lines. Bmi-1 is essential to the development of the central nervous system but can be detrimental if it facilitates the proliferation of abnormal cells. As found in previous research with glioblastoma multiforme cell lines, we hypothesized that over-expression of Bmi-1 can be suppressed or down regulated with miRNA-128 in medulloblastoma cell lines. Results from this study suggest that, although both medulloblastoma and glioblastoma tumors have Bmi-1 over-expression, these tumors have distinct microRNA profiles. This understanding contributes to the fairly new field of microRNA profiling. MicroRNA profiling and knowledge of expression patterns can perhaps enhance the future treatment and prognosis of medulloblastoma.

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An Electrophysiological Investigation of Long Term Recovery from Progressive Unilateral Entorhinal Cortex Lesion in Rats.
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Already the leading cause of dementia, Alzheimer's disease, a devastating progressive neurodegenerative disorder, is becoming more prevalent every year. The entorhinal cortex (EC) is the primary source of innervation to an area of the hippocampus called the dentate gyrus through the ipsilateral perforant pathway. It undergoes significant degeneration during Alzheimer's disease. In studies using rats given unilateral EC lesions, the crossed temporodentate pathway (CTD), a less profound source of innervation to the dentate gyrus arising from the contralateral EC, compensates for perforant pathway deterioration by sprouting and thereby increasing its synaptic efficacy. Research demonstrates that experimentally produced progressive lesions of the EC, done over multiple surgeries, produce a more robust sprouting response than those done in one stage. Some research indicates that the sprouted CTD is capable of supporting Long Term Potentiation (LTP), a strengthening of neural connections at a synaptic level, and that LTP underlies the putative lesion induced improvements in the CTD's synaptic efficacy. This project is an electrophysiological investigation of the sprouted CTD's ability to induce LTP in rats 90 days after they receive unilateral EC lesions. Rats were given either progressive or one-stage unilateral EC lesions and 90 days to recover upon which time the ability of the sprouted CTD to support LTP was measured. Research in our lab using shorter time points indicates that the sprouted CTD does not consistently support LTP. These data from 90 day cases corroborate these findings.

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Age at time of sound-induced seizure bout affects neuron loss in the dorsal nucleus of lateral lemniscus
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Long-Evans rats can be made susceptible to sound-induced or audiogenic seizures (AGS) through two developmental events exposing animals to loud sound: upon ear canal opening and again two weeks later. In this model of acquired, reflex epilepsy, neuronal excitation in the inferior colliculus (IC) initiates activity that results in generalized seizures as anomalous excitation spreads to neurons of brainstem and forebrain structures. Intrinsic and extrinsic inhibitory connections modify excitatory responses within the IC and thus contribute to the

initiation of AGS. GABA neurons in the dorsal nucleus of the lateral lemniscus (DNLL) are the primary source of extrinsic inhibition for the IC and may be damaged with repeated AGS activity. In this study, we examined the effect of age of repeated seizures on neuron density in the DNLL. Rats primed for AGS on postnatal day (pnd) 18 (120-dB tone pips) and pnd 32 (120-dB noise) were exposed to seizure-inducing 120-dB noise 10 times in 5 days as juveniles (n=6, pnd 35-39), adolescents (n=6, pnd 58-62), or adults (n=6, pnd 155-159). Juvenile (n=4), adolescent (n=4) and adult (n=4) control groups, not primed for AGS, were also exposed to sound 10 times in 5 days. Normal control rats (n=4) were available. Rats were sacrificed 100 days after the last sound exposure, and GABAergic neurons of DNLL were identified with immunohistochemistry. Alternate sections were stained with thionin. Neuron counts were made based on morphological criteria using a Plan 40 objective, digital microscopy, and stereological technique. DNLL neuron densities were compared across AGS and control groups. Rats primed for AGS averaged lower neuron density (M=7.3, SEM=0.2) than control rats (M=10.6, SEM=0.2) ($p<.0001$) indicating cell loss dependent on AGS activity. Among AGS groups, DNLL neuron densities depended on age when seizures occurred and were least for adult rats (M=6.3, SEM=0.3), greater for adolescent rats (M=7.5, SEM=0.3), and greatest for juvenile rats (M=8.1, SEM=0.3) ($p<.0001$). The data suggest that neuron loss in the DNLL, including inhibitory GABAergic neurons, results from seizure activity and not simply exposure to loud sound. It seems that seizures caused damage in the DNLL, which could contribute to a loss of extrinsic inhibition of the IC where abnormal neural activity in the developmentally primed AGS-prone rat begins. In AGS-prone animals, the age at which seizures occur was important for the extent of cell loss, which was less pronounced for juvenile than adolescent seizures and greatest after a seizure bout in adult rats. As is often the case with brain injury, it is better to be young when having sound-induced seizures.

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Environmental Enrichment Affects Severity and Intensity of Audiogenic Seizures in Rats
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In the brain, a generalized seizure is widespread over-excitation of neurons. As multiple seizures occur, excessive excitation produces brain damage through mechanisms including glutamate toxicity. Changes in neural circuits lead to increased severity and intensity of seizure behavior. In rats, sound-induced or audiogenic seizure (AGS) behavior includes wild running followed by clonic convulsions. Because brain areas that are susceptible to seizure (e.g., amygdala, cortex) also are affected by environmental enrichment (EE), we examined whether EE would mitigate the severity and intensity of AGSs. On postnatal day (pnd) 18 and 32, Long-Evans rats (N=36) were primed and tested for AGSs using loud tones (120-dB, 10 kHz pips, 8-min) and noise (120 dB, 2 min), respectively, which damage and change the auditory pathway resulting in lifelong reflex seizures with exposure to loud noise. From pnd 35 to 62, 10 seizures were induced at a rate of one every 3 days with 19 rats being enriched on non-induction days. Enrichment included 1.5 hours interaction with familiar and unfamiliar same-sex rats in a cage with objects, ramps, and platforms. Seizure severity was assessed using latency to wild running and clonus data, and the duration of clonus reflected seizure intensity. Data points from across inductions were compared. As the number of seizures increased, the severity of seizures also increased for all

rats. Latency to wild running decreased by 29.7% in unenriched rats and 24.5% in enriched rats from initial to the last inductions, and latency to clonus decreased by 18.8% and 6.2% in unenriched and enriched rats, respectively. Thus, enrichment only mitigated the severity increase across inductions mildly. In contrast, clonus duration was almost constant for unenriched rats throughout AGS inductions (3.3% decrease) while the intensity of seizures for enriched rats decreased by 38.8% ($p<.05$). Unlike the onset of AGS activity (i.e., latency to running and clonus), which is dependent on brainstem structures, the duration of seizures may depend more on over-excitation of forebrain areas like the amygdala and cortex. These are regions in which enrichment is known to stimulate neural plasticity, which may have helped mitigate seizure intensity in our rats.

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Spectrofluorimetric Determination of the Affinity of Leadmium Green for Lead, Cadmium and Zinc
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Increased intracellular free levels of certain metal ions, such as zinc and cadmium, can be pathophysiological. As a convenient way to study metal ions in cells, we use fluorescent probes, which are chemically engineered molecules that fluoresce upon binding to free metal ions. The dissociation constant (Kd) is useful in determining the binding strength between probes and heavy metals. This parameter is the concentration at which fifty percent of the receptors (fluorescent probe) are bound to ligand (free metal ion). Some fluorescent probes are specific to certain metals (i.e., FluoZin-3 and zinc) and will show a lower Kd value when compared with other metals. Invitrogen™ designed a fluorescent probe called Leadmium Green, which is advertised as a detector of free intracellular lead and cadmium. However, very little information is available about this probe, including its Kd. Using the cell-impermeant version of Leadmium Green, we determined in vitro affinities for zinc, lead, and cadmium. Using the cell-impermeant form of the better-documented probe FluoZin-3, we corroborated the affinity of this dye for zinc and cadmium. While Leadmium Green's binding kinetics are complicated, we conclude that it binds zinc with an affinity as strong or stronger than its affinity for cadmium.

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A Characterization of Leadmium Green's Response to Intracellular Free Metals
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Cadmium and lead are toxic metals that collect in living systems as a result of environmental exposure. However elucidating their effects at the level of the single cell has been difficult due to inadequate methods for detecting and monitoring them. Invitrogen™ has developed a new fluorescent indicator called Leadmium Green, which the company purports will detect cadmium and lead. However, our previous in vitro characterization showed that Leadmium Green is also relatively sensitive to zinc. Here we used fluorescence live-cell microscopy to test Leadmium Green's response to varying concentrations of zinc and cadmium in the neuronal cell line, HT-22. We further compared Leadmium Green to other commercial indicators, including fura-2 and

FluoZin-3. Our results suggest potential problems with interpreting Leadmium Green responses in vivo, and question its utility as a live-cell indicator.

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Fluorescence Visualization of Cadmium Import in Mitochondria Isolated from Mouse Brain
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The intracellular accumulation of cadmium and can be detrimental to energy-intensive tissues, such as brain and heart. Although these metal ions disrupt mitochondrial function, the exact mechanism is unclear. Here, we characterize metal effects on mitochondria using a novel paradigm. We isolated mitochondria from mouse brain and adhered individual organelles to microscopy glass. We used the newly developed fluorophore, Leadmium Green to visualize cadmium accumulation in isolated mitochondria. Our data show that the dye responds to cadmium in a concentration-dependent manner. Mitochondrial inhibitors were used to manipulate cadmium import. Both FCCP, a mitochondrial uncoupler, and ruthenium red, an inhibitor of the calcium uniporter, enhanced cadmium uptake in mitochondria in response to cadmium. Our results suggest that cadmium uptake into mitochondria occurs regardless of membrane potential and through a pathway not shared with calcium.

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The Role of Low-Threshold Calcium Channels in Alcohol-Mediated Sleep Disruption
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Connections between thalamus and hippocampus are critical in the generation and maintenance of sleep rhythms, including the theta rhythm. Burst firing, mediated by the T-type low threshold calcium channel, is essential for these rhythms. Though the exact mechanisms are unknown, behavioral, physiological, and molecular data suggest that ethanol modifies sleep rhythms and burst firing through actions on T-type calcium channels, including the Cav3.2 subtype. We hypothesized that ethanol actuates changes in T-current through upregulation of Cav3.2 expression within thalamus and hippocampus. Mice were subjected to 16hrs of ethanol exposure, through vapor chambers, followed by acute ethanol withdrawal over an 8-hour period. Following 4 days of repeated exposure and withdraw, mice were sacrificed and brains were sectioned and stained immunohistochemically using a polyclonal antibody for the Cav3.2 protein. Antibody labeling was examined within thalamus, specifically reuniens nucleus (RE) and thalamic reticular nucleus (TRN), and the hippocampal CA1 and DG domains using confocal microscopy. Quantitative region of intensity (ROI) analysis was utilized to examine changes in fluorescent intensity. In controls, Cav3.2 expression was observed in RE, TRN, CA1, and DG. Following ethanol exposure, ROI analysis demonstrated no significant change in Cav3.2 protein expression. These results suggest that the known ethanol-induced alterations in T-current are not mediated by an increased amount of T-channel proteins. Current efforts are examining whether ethanol induces changes in the sub-cellular distribution of Cav3.2 channels; a shift from distal to proximal dendritic sites could facilitate the observed changes in T-current.

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Gender Differences in Risky Behavior Diminish Among Both Low and High Risk Takers
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Although it is a long-standing finding in risk taking research that men are more likely than women to exhibit risky behaviors, women increasingly engage in risky behaviors (Weden & Zadin, 2005). A current perspective on risk taking is that sensitivity to punishment is supported by frontal brain function and that risk takers may be compromised in this aspect of cognition (Bechara, Damasio, & Damasio, 2000). The Iowa Gambling Task (IGT: Bechara, et al, 2000) was designed to measure this sensitivity, showing that most people settle for smaller rewards to avoid severe punishment. Conversely, poor performance on the IGT has been shown by alcoholics and violent offenders (Fishbein, 2000; Grant, Contoreggi, & London, 2000). We compared the risky behaviors (i.e., truancy, substance abuse, reckless driving, school and work procrastination, public "acting out" and unprotected sexual encounters) of 155 undergraduates, hypothesizing those low and medium in their reporting of risky behaviors would show the traditional gender difference, and that those reporting high numbers of risky behaviors would be more similar. We also hypothesized that the high risk group would score lower on the IGT. Partially supporting our hypotheses, we found that men reporting medium levels of risky behaviors differed significantly from women, $F(1, 153) = 26.71$, $p < .05$. Men and women of low and high risk did not show significant gender differences on risky behavior. As hypothesized, the low and medium risk groups were higher on IGT scores that the high risk group, $F(2, 135) = 3.86$, $p < .05$.

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LPA and S1P induce retinal growth cone collapse via activating GPCR intracellular pathways Gi and G12/13
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Our research focuses on the molecules involved in axon guidance during the development of the visual system in chicken embryos. Although there have been several axon guidance molecules studied and identified that contribute during the development of the visual system, the full complexity still remains unknown. The axon guidance molecules we are particularly interested in are two lysophospholipids: lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P). We have shown that LPA and S1P induce growth cone collapse in chick retinal axons in vitro. LPA and S1P bind specifically to G-protein coupled receptors (GPCRs), which activate one of four possible intracellular pathways (Gi, G12/13, Gq, Gs). We are investigating exactly which intracellular pathways LPA and S1P mediate. We are using pharmacological inhibitors to block individual intracellular pathways, measuring growth cone collapse by LPA or S1P. The inhibitors used were Pertussis Toxin (PTX), which blocks the Gi pathway, and Y-27632, a "ROCK" inhibitor that blocks the G12/13 pathway. We have found that the "ROCK" inhibitor Y-27632 prevents growth cone collapse in both LPA and S1P treatments, indicating that the G12/13 pathway is involved in growth cone collapse. PTX, an inhibitor of Gi, was also found to partially reduce the level of growth cone collapse, indicating that the Gi pathway also contributes

to growth cone collapse. We are also analyzing the effects of the Gq pathway by using a Phospholipase C inhibitor. This research is allowing us to identify the intracellular GPCR pathways involved in growth cone collapse with LPA or S1P.

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Expression of Choline Acetyltransferase in the Rat Medial Septum During Lesion-Induced Axonal Sprouting of the Septodentate Pathway
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Reactive synaptogenesis occurs when healthy neurons innervate an area that has been deafferented due to injury. This phenomenon has been observed in the dentate gyrus of the hippocampus following ipsilateral entorhinal cortex damage. A substantial portion of this reactive synaptogenesis arises from the medial septal nucleus; the septodentate pathway proliferates in response to entorhinal cortex damage. Because the septodentate projections are cholinergic, the increased presence of acetylcholinesterase (AChE) in the dentate gyrus is considered evidence of septodentate sprouting. Many past studies have observed lesion-induced septodentate proliferation through the increased levels of proteins such as AChE and choline acetyltransferase (ChAT) in the hippocampus. However, no study has found evidence for this type of growth in the medial septal nucleus cells that are sprouting. The current study aimed to examine changes in the expression of the ChAT gene in the septal cells of adult male Sprague-Dawley rats. ChAT is an enzyme responsible for the production of the neurotransmitter acetylcholine. It is produced in the cell bodies of neurons and then transferred to their axon terminals where it forms acetylcholine by transferring an acetate ion to a choline molecule. This study examined ChAT expression levels in septal cells 2, 7, and 21 days following entorhinal cortex lesions. Although evidence of septodentate sprouting was found in the dentate gyrus of each subject via AChE staining, increased ChAT expression in septal cells was not found in the 2-day post-lesion group. As of now, data for the 7 and 21-day post-lesion groups have not been collected.

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Limiting Central Nervous System Side Effects in Common Medications
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Many current medications are notorious for their side effects of sedation and effects on motor coordination, both of which are mediated by effects in the central nervous system. In this project we investigated whether or not current medications can be manipulated in order to keep them from crossing the blood brain barrier and in effect limit their effects on the central nervous system. A common medication has been chemically modified so as to retain the chemical structure of the medication but limit the ability of the medication to cross the blood brain barrier. The hypothesis was that the modified compounds that did not cross the blood brain barrier would not cause motor incoordination. To asses effects on motor coordination we measured gait, stride, interstep distance and interlimb coordination. Thus far in the project the compound modifications appear to decrease medication induced motor incoordination. This suggests common medications

can be chemically modified to limit penetration of the compounds into the central nervous system and prevent centrally mediated motor incoordination.

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The Effects of Moderate Cardiovascular Activity on Cognition: Testing the Transient Hypofrontality Hypothesis Using Trail Making Tests A & B
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The results from previous studies regarding the effects of cardiovascular exercise on cognition vary greatly. The transient hypofrontality hypothesis (Dietrich, 2006) explains that, when exercising, blood flow to the brain is directed toward areas more pertinent to exercise such as the primary motor cortex and thus limited to areas such as the non-motor frontal lobe regions. Consequently, non-motor regions of the frontal lobe have less neuronal activity, leading to suppressed inhibition and attention. In the current study, we examined the effects of moderate intensity cardiovascular exercise on visual and task-switching attention using the Trail Making Test (TMT) A and B. Fourteen female college athletes (mean age 20.3 years) equally divided into two groups, exercise and non-exercise control, completed the TMT A and B. Results indicated no significant difference in mean completion times between groups on either task despite proposed discrepancies in frontal lobe perfusion between the groups. Issues with task sensitivity and/ or exercise intensity for the test population may explain TMT results.

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Construction of siRNA Retroviral Vectors Targeting LPA Receptors in the Chick Visual System
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While it is known that axons must grow to highly precise locations to achieve proper function, the guiding molecules that promote such accuracy are still being elucidated. It is thought that specific guidance molecules help steer axons to their final position through attractions and repulsions. Evidence has shown that Ephs and Ephrins are one such set of guidance molecules that affect retinal ganglion cells (RGC) in the embryonic chick visual system, but it is apparent that there are more ligand-receptor interactions at work. Another possible axon guidance molecule is lysophosphatidic acid (LPA), which promotes growth cone collapse by binding to its receptors, of which there are 5 known (LPA1-5). We are constructing siRNA vectors for each LPA receptor. Thus far we have cloned gene-reducing hairpins via PCR, inserted them into a chicken microRNA expression vector, verified the vector by sequencing, transfected the plasmid into a chicken fibroblast cell line, and used Real-Time PCR to verify the silencing effect. Ultimately, we will insert our construct into a virus and use it to infect embryonic chick retina; by observing the resulting effects on the developing RGCs, we intend to investigate the role of LPA in axon guidance.

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dfrm1 Mutations in Drosophila Melanogaster as a Model for Human Autism
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The prevalence of autism spectrum disorder is continually increasing in our society. In order to develop effective treatments and work towards a cure for this disorder, it is necessary to develop animal models of the disease on which possible treatments can be tested. The dfmr1 gene into Drosophila melanogaster, fruit flies, has been proposed as a possible model. Our behavioral analysis shows that mutations of the dfmr1 gene cause behavioral changes that are reminiscent of the traits of human autism and can be used to test potential treatments. Results show a significant increase in repeated grooming behaviors in the experimental group compared to the control.

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Paired-Pulse Stimulation of the Crossed Temporodentate and Septodentate Pathways Induces Granule Cell Discharge 15 Days after Unilateral Entorhinal Lesions in Rats
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The central nervous system exhibits a capacity for functional reorganization following injury. More specifically, within the hippocampal formation several of the projections to the dentate gyrus proliferate extensively following the destruction of the entorhinal cortex, the major projection to the dentate. While the majority of neural projections from the entorhinal cortex to the dentate gyrus project ipsilaterally to form the perforant pathway, a small number of fibers cross the midline and terminate in the contralateral dentate to form the crossed temporodentate (CTD) pathway. Following a unilateral lesion of the entorhinal cortex, synaptogenesis has been observed to occur in the CTD fibers as well as in those fibers arising from the septum that terminate in the dentate gyrus to form the septodentate pathway. With long term survival after entorhinal lesions, the extensive proliferation of the CTD renders it capable of discharging the granule cells of the dentate gyrus (which is uncharacteristic of the normal CTD) and enhances field excitatory postsynaptic potentials in the dentate. Short term survival periods of 15 days or fewer, however, have proven to be ineffective. The present study examined whether prior stimulation of the proliferated septodentate pathway would potentiate granule cell responses evoked by CTD stimulation in male Sprague-Dawley rats after a short term survival period of 15 days. Rats were either given unilateral entorhinal lesions or sham operations. In an acute anesthetized preparation, stimulating electrodes were placed in the medial septum and intact entorhinal cortex 15 days following surgery. Evoked potentials were recorded with a glass microelectrode placed in the granule cell layer of the dentate. A “conditioning pulse” elicited in septum was paired with subsequent “test pulse” elicited in entorhinal cortex at inter-pulse intervals of 10-500 ms. We assessed the dentate response to the test pulse either with or without stimulation by the conditioning pulse. Whereas stimulation of the CTD failed to evoke granule cell discharge, pairing the CTD with septodentate stimulation produced granule cell discharge. These findings suggest that the combination of these two afferent inputs enhanced granule cell responses, perhaps by direct excitation or disinhibition of inhibitory dentate interneurons.

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Beta-Actin Expression During Induced Sprouting in the Hippocampus
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Functional reorganization is one method the central nervous system utilizes in order to recover from neurological pathologies, and axonal sprouting is thought to be a central component. Beta-actin (ACTB) has been implicated in neurite outgrowth following injury, and mRNA ACTB levels were examined in the medial septum to better understand ACTB's role in CNS sprouting. To induce sprouting, entorhinal cortices of Rattus norvegicus were electrically lesioned and brains removed at 2, 7, and 21 days post-lesion. Hippocampi were stained for acetylcholinesterase (AChE) to confirm sprouting, and mRNA was isolated from the medial septa and analyzed via RT-PCR to quantify relative amounts of ACTB mRNA in control and lesion hemispheres with respect to GADPH. AChE histology suggested that sprouting had occured. However, no significant difference in relative ACTB mRNA quantities was found among any of the test groups--potentially due to error in RT-PCR analysis. Future studies may want to examine why no significant ACTB upregulation was found.

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The Effects of Caffeine on Locomotor Activity in Juvenile and Adult Zebrafish.
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Caffeine has become one of the most ubiquitous drugs used in the western world. Concerns about the amount of caffeine consumed on a daily basis alone or in combination with other drugs such as alcohol have recently received increased attention. Zebrafish have served as a model organism for pharmacology and toxicology in the context of neuroscience for well over a decade. In the present study, we examined the acute effects of caffeine on adult and juvenile zebrafish locomotor activity. Adult fish were exposed to 10 µg/L of caffeine or a control condition for 5 minutes. Animals were subsequently transferred to an observational arena and locomotor activity was assessed using the Noldus Ethovision system. Two dependent measures were collected for 10 minutes: total locomotor activity and velocity. In adults, caffeine exposure produced a significant decrease in total locomotor activity ($t(22)=2.1$, $p=0.03$). A similar experiment was conducted in juvenile zebrafish at 30 days of age. Animals were exposed to 10 µg/L of caffeine and transferred to a 6 well tissue culture plate and behavior was measured using the Ethovision XT system. However, this experiment indicated no significant difference between groups with respect to total activity ($t(64)=0.47$, $p=0.32$). In a follow up experiment, these same fish were exposed to caffeine in a conditioned place preference (CPP) paradigm. Fish were placed individually in a rectangular arena divided into a brown area and a white area over a 9 day period. After an initial assessment of preference (brown area preferred), animals were exposed to caffeine in the white area of the arena and de-chlorinated water in the brown area.

The results from this experiment indicated a significant increase in activity in the white portion of the arena where animals were exposed to caffeine ($t(27)=2.29$, $p=0.02$). Taken together, these experiments indicate important differences in the response to caffeine between adult and juvenile zebrafish. Data from the CPP experiment suggests that caffeine may be reinforcing for adult zebrafish since the fish spent more time in the white area (initially non-preferred) of the arena following exposure to caffeine.

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Perforant Path Transections Induce Hippocampal Synaptic Reorganization in C57BL/6 Mice **erfeeney@davidson.edu**

In patients with Alzheimer's disease (AD), the characteristic loss of layer II entorhinal cells deprives the hippocampus of its primary cortical input, the perforant pathway (PP) to the dentate gyrus. In rats, experimental lesions of the entorhinal cortex similarly denervate the dentate gyrus, yielding initial synaptic loss and impairment of spatial and working memory function. Subsequent functional and electrophysiological recovery corresponds with synaptic turnover in the dentate molecular layer, the regaining of synapses on account of reactive sprouting and synaptogenesis by proximal axon collaterals. To investigate the functional and therapeutic implications of sprouting in compensation for AD-related entorhinal degeneration, the application of entorhinal cortex lesions (ECLs) to transgenic mouse models has grown increasingly popular. However, characterization of synaptic turnover remains inconsistently quantified in the wild type mouse. In collaboration with Pfizer Inc., we assessed synaptic densities of the dentate molecular layer in adult C57BL/6 mice. Mice sustaining unilateral perforant path transections were sacrificed at 1, 4, 10, 18, and 28 days post-lesion, and hippocampal sections were subsequently stained for presynaptic terminal proteins SNAP-25, GAP-43, synapsin, and synaptophysin to assess reinnervation and synaptic turnover. Ipsilateral-to-contralateral staining densities and laminar widths were assessed with BIOQUANT Image Analysis software, and shrinkage-normalized ratios were compared to 28 day-matched sham cases. At 28 days post-lesion, significant depression of shrinkage-normalized synaptic densities was observed in the ipsilateral outer molecular layer of lesion cases, but qualitative stain analyses revealed overt changes in laminar organization indicative of synaptic reorganization.

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Expression of Class III Beta-tubulin in the Medial Septum During Lesion-Induced Axonal Sprouting of the Septodentate Pathway in Rattus norvegicus
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Axonal sprouting is one mechanism the brain may use to repair structure and function after injury. In the CNS, where neuron regeneration is inhibited, areas of the brain denervated by damage can be reinnervated by nearby undamaged neurons. Axonal sprouting has been shown in

the dentate gyrus of the hippocampus, where neurons originating from the medial septum sprout new terminals in response to lesioning of the entorhinal cortex, which also innervates the dentate gyrus. This study attempts to examine the possible role of class III beta-tubulin in septodentate neurons during sprouting. The entorhinal cortices of male Sprague-Dawley rats were lesioned on one side and the other side acted as a control. Rats were sacrificed 2, 7, and 21 days after lesioning. Acetylcholinesterase staining confirmed sprouting, but real time reverse transcriptase PCR showed no significant upregulation of the tubulin gene compared to a control gene at any time point.

Speaker Abstracts – SYNAPSE 2011

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Adult Neuroplasticity: Lessons from the Birds

Seasonally breeding songbirds exhibit dramatic changes in brain and behavior.

Photoperiod and other environmental cues regulate variation in gene expression in the hypothalamus responsible from seasonal changes in gonadal steroid hormone secretion. For example, the mRNA coding for the key neuropeptide gonadotropin-releasing hormone that connects the brain to reproductive endocrine system decreases markedly in seasonally breeding starlings when they are in a non-breeding state. Seasonal changes in the reproductive neuroendocrine system such as changes in steroid hormone secretion in turn mediate marked changes in brain organization and function of areas controlling birdsong. Testosterone and its metabolites as well as activity-dependent changes in neurotropic expression are components of the mechanism that regulate these changes in brain morphology. This seasonal brain re-modeling involves the recapitulation of developmental events including the incorporation of new neurons into the song circuit. Recent studies have revealed distinct patterns of seasonal gene expression in the song nuclei as compared to seasonally changing hypothalamic regions.

Calcium signaling in sensory neurons: a TRP to the CRAC house!
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Chronic pain is a debilitating complication associated with a variety of diseases such as diabetes, cancer, AIDS, and nerve injury. Patients with chronic pain use health services up to five times as frequently as the rest of the population. Current lack of reliable treatment for chronic pain is largely due to the fact that the mechanisms of pain are complex and not fully understood. Recent evidence suggests that alterations in intracellular calcium (Ca^{2+}) play crucial role in the pathogenesis of neuropathic pain. Elucidating the mechanisms of Ca^{2+} signaling and understanding its role in sensory neuronal functions is an essential to understanding the pathophysiology of pain and to develop new pain therapies. Neurons utilize two main mechanisms to regulate their intracellular Ca^{2+} levels: (**i**) extracellular Ca^{2+} entry via voltage and/or ligand-gated Ca^{2+} channels, and (**ii**) Ca^{2+} release from intracellular stores of endoplasmic reticulum (**ER**) via the Ca^{2+} release channels such as **ryanodine receptor (RyR)**. A sustained release

and/or depletion of the ER Ca^{2+} stores leads to activation of Ca^{2+} influx by a mechanism known as **store-operated Ca $^{2+}$ entry (SOCE)**, which helps to refill the depleted ER Ca^{2+} stores as well as to regulate cellular functions. Our preliminary experiments using sensory neurons of adult rat dorsal root ganglion (DRG) demonstrated that both pain-sensing (nociceptive) and mechanosensitive neurons show RyR-mediated ER Ca^{2+} release. Interestingly, only nociceptive neurons exhibit fast Ca^{2+} entry via the SOCE pathway, whereas mechanosensitive neurons utilize a slower Ca^{2+} entry pathway to refill their ER Ca^{2+} stores. These intriguing findings indicate that sensory neurons contain distinct Ca^{2+} transport pathways to maintain and regulate their intracellular Ca^{2+} homeostasis. Our ongoing research is aimed at understanding the differences in Ca^{2+} signaling mechanisms between nociceptive and mechanosensitive neurons. Understanding the interaction between intracellular (i.e. Ca^{2+} release) and plasma membranes (i.e. Ca^{2+} entry) will uncover mechanisms for the regulation of intracellular Ca^{2+} signaling in nociceptive neurons, and will lead to the identification of novel drug targets for new analgesics to treat acute and chronic pain.

Enhancing Cholinergic Signaling Reduces Secondary Spinal Motoneurons Without Affecting Cell Division in Zebrafish

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Development is a critical period for the nervous system, as nascent neurons rely on a variety of molecular cues to differentiate, migrate, and form appropriate synaptic connections. Studies suggest that cholinergic signaling plays a role in this process before synaptogenesis; therefore, disrupting this system early on may help resolve the role of acetylcholine in development. Malathion is an organophosphate pesticide that inhibits acetylcholinesterase's catalytic activity, thereby enhancing cholinergic signaling. We used *islet1*-GFP zebrafish (*Danio rerio*) that express GFP in a subset of secondary motoneurons to examine the effects of altered cholinergic signaling on the development of these neurons. Embryos were reared in malathion for 48, 72, or 96 hours post fertilization (hpf). We then used confocal microscopy to quantify the number of GFP+ motoneurons at each time point and found that malathion significantly reduced the number of GFP+ motoneurons approximately 20—30% at all three time points ($p<0.001$). Potential mechanisms of this reduction include limited neurogenesis, enhanced apoptosis and/or necrosis, and/or delayed differentiation. In order to determine if malathion altered cell division required for neurogenesis, we used BrdU to mark cells undergoing mitosis at 48 hpf. The number of BrdU+ nuclei in malathion-treated embryos did not significantly differ from controls. Consequently, our data suggest that malathion does not alter the number of dividing cells, and therefore compromises developing GFP+ motoneuron numbers through another mechanism. This project was funded by grants from NSF, HHMI, and the Davidson Research Initiative.

Retinal Ganglion Cells Exhibit Unbranched Bipolar Neurite Development *in vitro*

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Neurons are the fundamental cells of the nervous system, and action potentials are the primary mechanism of conveying information between these cells. Even before synapses are formed, developing neurons produce spontaneous action potentials that are critical to proper development. Synaptic inputs are received by dendrites, specialized neuronal projections that can vary from simple processes to elaborately branched arbors. Many factors, including genetics, environment, and neuronal activity, can influence dendritic morphology. If dendrites do not develop properly, a neuron's role in a network can be altered. This study is to determine whether spontaneous action potentials that occur prior to synaptogenesis play a role in shaping dendritic morphology. *Xenopus laevis* tadpole retinal neurons were dissociated and allowed to develop for 48 hrs *in vitro*. After 48 hrs, the cells were fixed and stained with Hoechst to label nuclei and a α-tubulin antibody to label the cytoskeleton and delineate neuronal morphology. Morphological features of neurites in control conditions were then analyzed by examining the number of primary neurites, total neurite length, and amount of neurite branching. Preliminary results indicate that dissociated *Xenopus* retinal neurons at 48 hrs in control conditions most frequently exhibit bipolar morphologies with unbranched neurites. This result was rather unexpected because previous experiments have shown dissociation around stage 34 leads to the development of dendrite-like neurites *in vitro.* Future experiments will alter the rate of spontaneous action potentials *in vitro* as well as distinguish dendrites from axons *in vitro*. This project is supported by Sigma Xi, NSF, and Davidson College.