SYNAPSE 2012 – Abstracts: updated 12-March-2012

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Hayes, T. & Schirillo, J.
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Do Pupils Dilate to Pleasant Images and Constrict to Unpleasant Images?
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Eckhard Hess claimed that pupils dilate to pleasant images and constrict to unpleasant images. However, his work was confounded since his images luminances and contrasts across conditions were inconsistent. We overcome this limitation and suggest a new, promising methodology for research in this area. We presented rightward or leftward facing male and female portraits by twelve artists to observers in either their original or mirror-reversed position. Simultaneously, we measured observers pupil size while asking them to report how (dis)pleasing they found each image. We found that only in viewing male portraits, pupil diameter was a function of arousal. That is, larger pupil diameter occurred for images rated both low and high in pleasantness. We discuss these findings in regard to the perceived dominance of males and how emotional expressions may be driven by hemispheric laterality.

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PI3-Kinase Mediates Zinc-Induced Mitophagy in Neuronal Cells
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Zinc in neural tissue is normally highly regulated, however, evidence suggests that the accumulation of free zinc ions (Zn2+) in response to injurious stimuli can induce neuronal death. The imbalance of cellular Zn2+ is implicated in pathologies such as stroke, epileptic seizures and Alzheimer's disease. Although many targets are associated with Zn2+-induced neuronal death, recent studies have suggested that Zn2+ can give rise to and accumulate in autophagic lysosomes. Autophagy is a programmed intracellular mechanism used to remove dead or non-functional organelles. Our previous findings suggest that mitochondria may be an important intracellular target for Zn2+, which could contribute to overall energy failure of the neuron. Despite progress in understanding Zn2+- induced pathologies, the mechanism(s) of mitochondrial autophagy (mitophagy) remains unclear. Here, we hypothesize that Zn2+ induces mitophagy in cultured neuronal cells. After exposing cells for two hours to Zn2+, we visualized mitochondria and autophagic lysosomes using fluorescent microscopy and measured the degree of co-localization between signals. Our results show that Zn2+-induced mitophagy and cell death occur at similar concentrations. Other studies provide evidence that autophagy is regulated by many diverse signaling pathways, including phosphatidylinositol 3-kinase (PI3-kinase) and mitogen-activated protein kinase (MEK). To determine a downstream mechanism, we used protein kinase inhibitors to demonstrate that Zn2+-induced mitophagy is mediated through activation of PI3-kinase, but not through MEK. These findings provide new insight into the mechanism of Zn2+-induced neurotoxicity.

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Toxoplasma gondii in Cats and Humans
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Toxoplasma gondii is a cosmopolitan parasite that may infect any warm-blooded animal. The natural life cycle of T. gondii is between a definitive host, any member of the family Felidae, and an intermediate host, a small rodent or bird. Humans may serve as an intermediate host for T. gondii, and risk infection from contact with cats and/or any intermediate host in the form of undercooked meat. In humans Toxoplasma gondii causes the clinical disease Toxoplasmosis, which is lethal to any immuno-suppressed individual but asymptomatic to healthy adults. Toxoplasma gondii has an affinity for neural tissue, and may specifically affect the amygdala and limbic system. Some disorders thought to be associated with neural toxoplasmosis include schizophrenia, OCD, autism, and suicidal thoughts. The purpose of this study was to determine if any correlation existed between sero-positive felines and infection rates in humans. The human sample population for this study consisted of students and faculty of Presbyterian College during the summer. Blood was obtained via finger prick and centrifuged to obtain the serum. The cat sample population consisted of feral cats living on the Presbyterian College campus and cats collected from the upstate of South Carolina. ELISA was used to determine a relative population of sero-positive individuals in the sample populations of humans and cats. Of the cats tested, 27% were positive for T. gondii, while 6% of the human population was positive. All of the sero-positive cats were either feral or semi-feral, while all of the sero-postive humans indicated close contact with cats on their survey. This research suggests that human contraction of Toxoplasma may occur via transmission from feral cats.

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The Effects of Down-Regulating SOD1 on a Drosophila Model of MJD
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The goal of this research has been to determine the extent to which the down-regulation of Super Oxide Dismutase 1 (SOD1) affects the progression of Machado-Joseph Disease (MJD). MJD, also known as spinocerebellar ataxia type 3 (SCA3), is a form of dominantly-inherited ataxia caused by an unstable CAG repeat on chromosome 14q32.1 The average age of onset is 35 to 40 years of age, and the core features are progressive ataxia, dysarthria, postural instability, nystagmus, eyelid retraction, facial fasciculations, and often dystonia in younger patients (Sudarsky and Coutinho, 2005). The mutant CAG, or polyglutamine, repeat tends to be 62-84 units long, as opposed to the normal 12-37 CAG units, with a strong correlation between repeat size and age of onset and severity of symptoms (Maceil, et al, 1995). We investigate the disease with a Drosophila transgenic model that closely replicate the characteristics of polyglutamine disease in humans. The focus lies specifically on the abnormal protein formations observed in the disease pathology (Marsh et al, 2003) as well as neurodegeneration, late onset, and early death (Warrick,et al, 2005). SOD1 binds copper and zinc ions and is one of two isozymes responsible for destroying free superoxide radicals in the body. The encoded isozyme is a soluble cytoplasmic protein, acting as a homodimer to convert naturally-occurring but harmful superoxide radicals to molecular oxygen and hydrogen peroxide, the down-regulation of which has been associated with increased neuronal apoptosis and DNA degredation (Troy and Shelansky, 1994). Interestingly, preliminary results suggest that knocking down the SOD1 gene prevents the disease from progressing rapidly in the most severe forms of the disease while not having a major effect on the normal form.

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The Relationship Between Exercise Duration and the Positive Reinforcing Effects of Cocaine

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Previous studies have reported that voluntary wheel running decreases cocaine self-administration in laboratory rats; however, it is less clear whether greater degrees of running are associated with greater reductions in cocaine self-administration. The purpose of the present study was to determine whether greater amounts of running in a forced exercise procedure are associated with greater reductions in cocaine self-administration. To this end, male rats were obtained at weaning and trained to run on a treadmill for 0 min/day (sedentary), 30 min/day (low output), or 60 min/day (high output) at 13.4 m/min (0.5 mph). After 6 weeks, rats were implanted with intravenous catheters and trained to self-administer cocaine under positive reinforcement contingencies. Cocaine self-administration was similar between rats in the sedentary and low output conditions; however, cocaine self-administration was numerically reduced in rats in the high output condition. Although preliminary, these data suggest that a large amount of exercise (i.e., > 30 min/day) may be required to produce protective effects on measures of drug self-administration.

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Identification of Dual-Target Drugs for Alzheimer's disease
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Alzheimer's disease (AD) is the most common form of debilitating dementia; it produces symptoms of long-term memory loss, linguistic incapability, and chronic cognitive failure. Biochemical studies have shown that the amyloid-beta protein (Aβ) forms aggregates that deposits across the brain and these aggregates are fundamental to the disease symptoms. Aggregation of monomeric Aβ begins with a lag phase following by rapid growth during which soluble aggregates are created. These aggregate intermediates eventually form fibrils, which deposit as Aβ plaques in AD patient brains. In addition, AD brains exhibit deficits in the neurotransmitter acetylcholine. Currently, FDA approved drugs reduce Acetylcholinesterase (AChE) activity in order to improve AD symptoms. However, AChE inhibitors could additionally have the potential to stop Aβ aggregation. This project evaluates the inhibitory potential of several derivatives of a known AChE inhibitor on Aβ aggregation. Aβ monomer was aggregated in the presence of NaCl, to promote nucleation, and excess molar quantities of AChE inhibitors. The effect of inhibitors upon both nucleation, indicated by an extension of the lag time to aggregate appearance, and the extent of aggregation was determined. Effective inhibitors were identified as those that delayed nucleation or curtailed the extent of fibril formation.

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Comparative Study of Stimulus Velocity Encoding by Wind-sensitive Interneurons in *Periplaneta americana* and *Tenodera aridifolia*
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Many insects possess a wind-detecting sensory system. This wind-sensitive system varies in the size and number of wind-sensitive interneurons that transmit information from the rear of the animal to the thoracic motor centers and head ganglia. Some insects, such as the American cockroach *Periplaneta americana*, use wind generated by predators to elicit escape responses. Other insects, like the Chinese praying mantis *Tenodera aridifolia*, also possess this sensory system, but wind does not evoke escape responses. Differences in wind-evoked behavior between these species may be due to how well wind

stimulates wind-sensitive interneurons that activate pre-motor and motor neurons in the thorax. To determine whether such differences exist between these two species, we measured neural responses to 300 ms wind puffs with velocities between 0-250 cm/s (eight different velocities tested). Spike counts were measured during the first 100 ms (dominated by wind acceleration) and the second 150 ms (dominated by constant wind velocity). We generated a stimulus-response curve (stimulus = wind puff velocity; response = spike counts) for each species and compared them. During the first 100 ms, wind elicited more spikes in *P. americana* than *T. aridifolia*. During the second 150 ms, the two species exhibited similar spike counts across the velocity range tested. Previous studies demonstrated that wind acceleration, not velocity, is the important component for initiating escape responses. The observed differences in the neural response during the first 100 ms between the two species could contribute to their distinct wind-evoked behaviors.

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The Differential Roles of the Entorhinal Cortex and Fimbria Fornix in Delay Non-Matching to Sample in Rats
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Because of the prevalence in the elderly of Alzheimer's disease, which impairs memory, efforts to understand the underlying neural mechanisms contributing to memory and cognitive function are important. Here we aim to discern the roles of key structures associated with learning and memory impairment. The fimbria-fornix and entorhinal cortex are two separately important structures to the proper function of the hippocampal system - a system implicated in learning and memory. The fimbria fornix is the primary fiber bundle connecting the hippocampus to midbrain structures. The entorhinal cortex gives rise to the perforant pathway that relays signals from cortical structures into the hippocampus. To test the role of these structures in spatial and working memory male Sprague-Dawley rats were trained on a Delayed-Non-Matching-to-Sample (DNMTS) paradigm in an operant box. After reaching criterion the rats received bilateral entorhinal cortex lesion (BECX), bilateral fimbria-fornix transection (BFFX), or sham craniotomy treatment. Rats were given a 5-12 day recovery period before resuming behavioral testing. Post-operative behavioral testing began with 14 consecutive days of testing followed by ten weeks of testing on weekdays only. In both experimental cases, behavioral impairment on the DNMTS occurs early in pre-operative testing. However, data analysis suggests that BECX rats recover to preoperative function around the fourth week of post-operative testing whereas the impairment of BFFX is persistent throughout the postoperative testing. In addition analysis of histological samples from each experimental condition suggests lesion induced cholinergic sprouting in response to BECX where the same area in BFFX samples show a high level of cholinergic deafferentation. These differences in behavioral and histological results suggest that the entorhinal cortex and fimbria fornix differentially signal the hippocampus and contribute to working memory in DNMTS.

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The role of apoptosis in diabetic retinopathy
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Diabetic Retinopathy (DR), a devastating microvascular disease and sensory neuropathy, is the leading cause of blindness in adults ages 20-74 in Western countries. Similar to the liver, the retina depends on an insulin signaling system, and any malfunctions severely impair the metabolic functions necessary for

structural and functional maintenance of retinal cells. Pro-survival signaling through the Akt pathway seems to play a prominent role in the pathologies of DR, but the exact mechanism is uncertain. In this study, we sought to investigate the role of hyperglycemia and cytokines on neurotrophin-mediated pathways leading to uncontrolled retinal neuronal apoptosis. Experiments were conducted in vitro using neuronal-like R-28 cells examined in both 10% FBS media as well as serum-deprived media. Cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-1β were added to demonstrate the damaging effects of cytokines in hyperglycemic conditions. Insulin and insulin-like growth factors (IGF)-1 and 2 were used to counteract apoptotic signaling. We found that TNF-α and IL-1β did cause a significant increase in cell death in serum-deprived cells and that insulin and IGF-1 seemed to provide a protective effect, promoting cell survival. These discoveries are consistent with previous research, demonstrating a pro-apoptotic effect of cytokines in the absence of growth factors and a protective effect by neurotrophins acting on the pro-survival cell pathway.

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Characterizing Slitrk Expression in the Developing Zebrafish Central Nervous System with In Situ Hybridization
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Slitrks are a family of six transmembrane proteins expressed throughout the central nervous system, associated with neurite outgrowth, inhibitory synapse development, and neuropsychiatric diseases (Aruga & Mikoshiba 2003; Takahashi et al. 2012; Beaubien & Cloutier 2009; Pocena et al. 2011). Published research examining Slitrks has focused on mainly behavioral studies and has been conducted in mice. In order to study the molecular mechanisms of Slitrks, zebrafish are an excellent alternative vertebrate model system because their embryos are transparent, plentiful, and develop rapidly. To understand the roles that Slitrks play in establishing the nervous system, we must first characterize the spatiotemporal expression patterns of each Slitrk mRNA in the brain through in situ hybridization. Thus far we have examined Slitrk1-5 mRNAs on cryostat sections of embryos at 72 hours and 5 days post fertilization. Preliminary results reveal intriguing differences in the expression patterns of Slitrk1-5 in the midbrain, hindbrain, spinal cord, and eye. When all six Slitrk expression patterns are characterized, Slitrk expression will be knocked down to observe how the nervous system develops in the absence of specific Slitrks. These future studies will help us to understand how Slitrks influence neuron morphology and connectivity. This work is supported by HHMI, Davidson College, and a NSF Research at Undergraduate Institutions Award.

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The Effects of the D3 Antagonist S33138-1 on EtOH Consumption by C57 Mice
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Millions of Americans are negatively impacted by binge drinking and addiction to alcohol. Alcoholism results, in part, from the positive reinforcement produced by the mesolimbic dopamine reward pathway. This study seeks to explore the D3 receptor's role in this pathway and alcoholism. Forty C57BL/6 mice were trained to binge drink ethanol and thirty of the mice were given varying doses of the D3 receptor antagonist, S33138-1. Our results indicate that the ethanol consumption decreased in a dose dependent manner, with the medium dose of 0.64mg/kg causing the largest decrease in

consumption. These findings imply that a D3 receptor antagonist could be used to eliminate the rewarding effects of alcohol and provide a potential drug treatment for alcohol addiction.

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Human Interpretations of Facial Expression and Attraction
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The purpose of this study was to establish a subset of attractive faces displaying happy or neutral facial expressions to see if happiness mediates attractiveness. Images were obtained from the FERET Facial Stimuli Database from the Defense Advanced Research Products Agency. The images were black and white frontal views of faces presented on a neutral gray background. The images were of multiracial males ranging from 19 to 70 years of age. Female participants (N=30) rated faces electronically on a Likert scale of attractiveness ranging from 1 to 9, with one being Very Unattractive and nine being Very Attractive. The same images were also rated on a Likert scale of happiness ranging from 1 to 9, with one being Very Unhappy and 9 being Very Happy. The top twenty highest rated attractive faces and lowest twenty attractively rated faces were chosen for data analysis based on their average rating across participants. There was a significant interaction observed between attractiveness level and smiling facial expressions. Paired samples t-tests indicated the most attractive faces were rated significantly different in happiness and attractiveness when smiling rather than exhibiting a neutral expression. Unattractive faces displaying a smile or a neutral expression were rated significantly different on the happiness scale, but not on the attractiveness scale. Per results of the study, a total of 40 images (20 Attractive, Smiling and 20 Unattractive, Smiling) will be used in a mismatch negativity study in which smiling attractive and smiling unattractive faces alternate as the standard and deviant stimuli.

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Investigating Sensitization to the Discriminative Stimulus Properties of Methylphenidate
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Despite widespread use, abuse, and intense research on psychostimulants, little is known about sensitization to the discriminative stimulus properties of these drugs. The current study investigated sensitization to the discriminative stimulus of methylphenidate (MPH) using a C57BL/6J mouse model. All mice were first trained to press a lever for sucrose reinforcement (5% w/v; 0.1 ml vol). Then, in a regimen designed to produce sensitization to the locomotor activating effects of MPH, the mice were either injected daily with 8 mg/kg MPH (SENS, n=10) or with saline (CTL, n=10). At the conclusion of the regimen, mice were challenged with a low dose of MPH (2 mg/kg) and the SENS group showed greater activity than the CTL mice. Mice returned to the discrimination procedure and were trained to discriminate 4 mg/kg MPH from saline. Mice in the SENS group had more sessions demonstrating correct lever choice, consistent with the development of sensitization to the discriminative cue. Finally, a separate group of mice were injected bilaterally in the nucleus accumbens (NAc) with the selective reuptake blockers nisoxetine and nomifensine for norepinephrine and dopamine, respectively. These blockers produced similar results for the discriminative stimulus when mice were challenged with subthreshold doses of MPH. This data emphasizes the importance of the NAc in processing the discriminative stimulus, and the involvement of norepinephrine and dopamine in the discriminative cue. We found that prior experience with a psychostimulant can sensitize the subject to the drugâ€™s

discriminative stimulus effects. Future research should focus on the role of the NAc in processing discriminative stimuli.

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Microcircuitry of Cholinergic Interneurons in the Monkey Putamen
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Recent electrophysiological studies in the non-human primate have demonstrated that striatal Tonically Active Neurons (TAN's) display long-latency (multisynaptic) inhibition upon CM stimulation. Previous studies indicate that GABAergic interneurons and axon collaterals may lead to the decrease in TAN firing rate upon CM/PF activation. Preliminary data indicates that 30% of the total symmetric, GABAergic (intrinsic) inputs onto SCI's are from Substance P-ir and Enkephalin-ir MSN collaterals, however, surprisingly, 70% of GABAergic inputs onto SCIs remain unknown. In light of the unknown GABAergic inputs, we investigated whether PV-ir boutons are a source of GABAergic innervation upon SCIs in the non-human primate putamen. Double label immuncytochemical localization of ChAT and Parvalbumin was undertaken on the electron microscopic level on tissue from two rhesus monkeys. The current study found that approximately 50% of the total PV inputs preferentially target SCIs, 84% of which form symmetric synapses onto SCIs. Although symmetric PV inputs onto ChAT dendrites were the focus of our study, we also sought to better characterize the general synaptology of PV boutons in the monkey putamen, Although the role of the thalamostriatal system in relation to the basal ganglia is still largely unknown, further research on the topic could provide valuable insight into debilitating movement disorders.

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Acid Ceramidase and Cortisol Responding in H295R Adrenal Carcinoma Cells
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The enzyme acid ceramidase (AC) is a regulator of ceramide, a basic building block of the sphingolipids, which directs such important processes as cell proliferation, differentiation, and survival, as well as participating in protective inflammatory processes and programmed cell death. AC is up-regulated in multiple cancer cell lines and many primary tumors. One of us has previously linked AC over-expression to the physiological stress response cancer cells (Turner, Cheng, Beckham, Keane, Norris, & Liu, 2010). Intriguingly, AC also figures in at least one other process that contributes to the formation of cancer, namely the stress response. For example, critical processes leading to cortisol production, the bodyâ€™s primary stress hormone, and the transcription of multiple steroid-modulating genes involve AC. As an upstream regulator of cortisol synthesis in adrenal cortex cells, it is possible that AC may play a role in psychological stress responses, much like it participates in physiological stress responses, such as the mobilization of stress kinases, immune cell trafficking, and the initiation of cell signaling pathways of mitogenic activity. It has been established that the cascade of hormones that follow psychological stress (i.e., the binding of ACTH to adrenal cells that leads to cortisol release) involves changes in sphingolipid expression and also that addition of sphingolipids to cultured cells results in increased expression of cortisol. However, no research to date has examined how over-expression of AC in adrenal cortex cells affects stress-related steroid release. Our research is aimed at elucidating the effect of acid ceramidase expression on cortisol production in the adrenal cortex cell line H295R.

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Left and right amygdalar differences in the number of parvalbumin-positive neurons
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The amygdala is a centrally located brain region and is important in emotional regulation such as the management of fear and stress. Past studies have shown that the right-hemisphere amygdala is more sensitive to the onset of pain and emotional stress than the left. Parvalbumin is a phenotypic marker for an inhibitory interneuronal population. Parvalbumin-positive neurons in the basolateral complex inhibit Ca2+/calmodulin-dependant protein kinase II (CAMKII) positive neurons which is an excitatory projection neuronal population. This study sought to estimate the number of parvalbumin-positive neurons in the left and right basolateral complex (BLC) within the rat amygdala. After anesthesia, animals were perfused with 4% paraformaldehyde and brains were harvested. Every third section throughout the amygdala was immunohistochemically-stained for parvalbumin. The optical fractionator method was then performed using the Microbrightfield Stereologer system to obtain an unbiased estimate of the number of parvalbumin-positive neurons in subdivisions through the entire BLC. The lateral amygdala was divided into dorsolateral, ventrolateral and ventromedial divisions. The basolateral amygdala was divided into posterior, anterior and ventral divisions. The results indicate that there are significantly more parvalbumin-positive neurons in the left basolateral amygdala compared to the right basolateral amygdala. Future directions include investigating differences in CAMKII-positive neurons as well as other interneuronal populations, which contain calretinin, calbindin, and somatostatin in the left and right amygdala and total number of neurons in these amygdala regions. The parvalbumin data suggests that there is more inhibitory control of CAMKII projection neurons in the left basolateral amygdala than in the right basolateral amygdala.

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IMPULSE: The benefits and challenges of running a reviewer training site as a student organization
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In response to the Boyer Commission report 15 years ago, R1 institutions were exhorted to include undergraduates in primary research outside the classroom. At the University of South Carolina (USC), undergraduates themselves have created a student organization that offers a scientific publishing experience by forming a reviewer-training site for IMPULSE. IMPULSE is the first online, undergraduate, international journal for research publications in neuroscience. The student organization is one of seven reviewer-training sites for IMPULSE located around the world. The IMPULSE chapter at USC has a President, Vice President, Secretary, and Treasurer with approximately 50 members that span freshmen to seniors. A few of the benefits of this organization format include, maximizing student participation in research-related activities while minimizing faculty time commitments, accessibility of financial resources available to student organizations, increased leadership training opportunities, use of campus facilities, increased presence on campus through participation in student organization fairs, and an increase in student member diversity across disciplines, colleges, and class. At the same time some of these benefits come with additional challenges which include: balancing scientific rigor with different levels of student ability, increased bureaucracy for student organizations, effective transfer of responsibilities and leadership and, the greatest challenge, recruiting new members and making scientific publishing appealing to a diverse undergraduate population. The student organization format

not only provides a large number of undergraduates a way to be involved in the research process, but requires that they confront all the problems with organizing a research body as well.

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IMPULSE and Social Networking: extending the global reach
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IMPULSE is an online neuroscience journal for undergraduates, providing an outlet for students to publish their work and an opportunity to learn about scientific publishing by serving as authentic peer reviewers. One challenge for the journal has been extending this opportunity to more students around the world. Currently, the international review team comprises students from over 15 universities, but most are in the US and affiliated with Reviewer Training Sites, where they receive formal training on the process. All but one of these sites (University of the Free State, SA) is also in the US. In order to address this issue, IMPULSE has created a new position in the Publicity Editorial staff: Social Networking Editor. This individual is tasked with maintaining the Facebook presence, using a new Twitter account, and exploring other options to increase the visibility of IMPULSE for non-US, and particularly non-Anglophone, neuroscience, pre-graduate students. One of the major problems in recruiting more international participation is that many countries follow a traditional educational model for pre-graduate students that does not include research as not part of their curriculum. Thus, the students have no research experience and little understanding of the value of either scientific publishing or reviewing. While the European Union Bologna 2010 Report states that "the latest research findings shall inform and drive teaching and learning at European universities", the needed cultural shift in post-secondary education has not yet occurred on a large scale. The traditional, course-based approach to science education is even more evident in other regions. In India, recognition of the need for change is addressed in a report from India's National Science Academy and Academy of Sciences, which states that "leading postgraduate teaching Universities and IITs should be encouraged to impart undergraduate science education" - it is clear that both in terms of numbers and quality, a vast expansion and intensification of higher level education embedded in research is essential. Part of the IMPULSE mission is to help students around the world engage in primary research reporting and reviewing, and the social networking tools available today are being harnessed to make that happen.

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Effect of Social Isolation on Adult Neurogenesis in the California Mouse
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Rates of adult neurogenesis in mammals are known to be affected by various extrinsic factors including social environment. Generally, enriched environments increase, whereas stressful environments decrease rates of adult neurogenesis. The California mouse is a highly social rodent species that displays monogamy and bi-parental care. Consequently, this species may be particularly sensitive to manipulations of its social environment. In the present experiment, subjects were housed either with a same-sex age-matched conspecific, or singly housed (isolation) for four days. In order to quantify neurogenesis, mice were given two injections of BrdU (a cellular marker for newly divided cells) on day three of the housing conditions and brain tissue was collected on day four. Immunofluorescence was used to visualize BrdU positive cells. We are in the preliminary stages of quantifying the number of BrdU positive cells in the dentate gyrus of the hippocampus (an established proliferative zone). In addition,

we plan to quantify expression in other limbic nuclei including the hypothalamus and amygdala. We predict there will be fewer BrdU positive cells in the brains of isolated animals. These results could offer a better understanding of the neural mechanisms that underlie the behavioral deficits associated social deprivation.

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Methamphetamine-Induced Memory Deficits and Reversal by Modafinil: Role of Glutamate Receptor Expression
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In humans and animals, chronic methamphetamine (METH) exposure causes long term cognitive deficits in executive function, information processing, and episodic memory. Here, we demonstrate in rats that chronic self-administered METH impairs memory on an object-in-place task (OIP) and we evaluate whether modafinil (a cognitive enhancing drug) reversed this memory impairment. Rats self-administered intravenous METH on a fixed-ratio 1 schedule of reinforcement (i.e. after each lever press the rat received an infusion of METH), or received saline for 21 days and then entered a withdrawal period. After one week of withdrawal, rats were tested for OIP recognition memory. Rats explored four objects for five minutes in a closed test chamber. Ninety minutes later, the location of two objects was switched in order to assess memory for object location and the total time spent at each object was recorded. Half the rats received either vehicle or modafinil (100 mg/kg) immediately after familiarization. Saline-treated rats spent more time interacting with the objects in changed locations, while METH-treated rats did not show object preference, indicating a memory deficit. METH-treated rats that received modafinil showed a reversal in this deficit. In order to evaluate neurobiological modifications, we compared glutamate NMDA receptor levels in brain areas involved in memory tasks (e.g. the perirhinal cortex, and hippocampus). Western Blotting revealed that METH self-administration had no effect on glutamate NMDA2B receptor expression levels in the hippocampus but decreased NMDA2B levels in the perirhinal cortex. Further examination of NMDA2B receptor levels in the prefrontal cortex may reveal more about the mechanisms that underlie METH-induced OIP deficits and its subsequent alteration by modafinil.

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Effects of Ethanol Dependence on BDNF Expression in Specific Brain Regions
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Brain derived neurotrophic factor (BDNF) is an essential component of the nervous system because it plays an important role in numerous neural functions including neurodevelopment, neuroprotection and synaptic plasticity. Additionally, BDNF is thought to be involved in the neurobiological mechanisms underlying alcohol and drug dependence, including neuroadaptations resulting from chronic alcohol exposure in animals and humans. Recent studies suggest that BDNF levels are reduced in prefrontal cortex of ethanol-dependent mice. The main goal of the present study was to determine whether ethanol dependence produces brain-regional as well as time-dependent changes in BDNF expression. To accomplish this, a mouse model of ethanol dependence that involves repeated cycles of chronic intermittent ethanol exposure was used. Adult male C57BL/6J mice were separated into two treatment groups. One group received chronic intermittent ethanol vapor exposure in inhalation chambers (16 hr/day x 4 days) for 4 weekly cycles while the remaining mice served as controls, similarly

handled but exposed to plain air in control chambers. At various time points following final exposure (0-Hr, 8-Hr, 48-Hr, 72-Hr, or 7-Days) mice were sacrificed and brains were extracted and dissected to yield samples of 5 distinct brain regions: prefrontal cortex, hippocampus, amygdala, dorsolateral striatum, and nucleus accumbens. The brain samples were flash-frozen and then prepared for analysis of BDNF peptide levels by using an ELISA assay procedure. Preliminary data indicate that repeated cycles of chronic intermittent ethanol exposure produce significant reductions in BDNF peptide levels in prefrontal cortex, and this effect extends to later time points after withdrawal. Analysis is currently underway for the additional brain regions. It is anticipated that this study, when completed, will provide new and valuable information regarding the role of BDNF in neuroadaptations that result from ethanol dependence. Supported by grants from NIH and the Department of Veterans Affairs Medical Research.

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Construction of siRNA retroviral vectors against LPA receptors for analysis in the chick visual system
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During the process of development, a fertilized egg cell divides and differentiates into tissues and organs that form a functional body. The process is extremely precise and organized. For the chick visual system, the Retina Ganglion Cells (RGCs) in the eye extend their axons to reach the connection sites in the tectum. We are interested in how these growth cones find their way to form these connections with eventual hope of understanding the development of human visual system. The theory is that there are guidance molecules in the brain telling the growth cones where to go. We are interested in what molecules are involved in this process. Recent studies revealed that lysophospholipids, especially lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P), are important signaling molecules. In vitro experiments have shown that LPA and S1P can cause RGC growth cones to collapse. There are currently six known LPA receptors, known as LPA1 to LPA5 and p2y5. We are constructing retroviral vectors containing siRNA for the infection of chicken embryos to investigate which LPA receptor is responsible for the LPA induced collapse. Thus far we have constructed siRNA hairpins against lpar1, lpar2, lpar3 and lpar4, inserted them into a chicken microRNA expression vector, and verified the vector by sequencing. We have also made a new variant of the RCASB retroviral vector with a GFP gene (Green Fluorescent Protein). We are working on cloning the siRNA cassette into the viral vector and testing the knock down effect via quantitative RT-PCR in DF-1 cells, a chicken fibroblast cell line. Once we verify the knock down effect of the siRNA containing virus, we will inject the virus into chick embryos and investigate the role of each LPA receptor in the development of visual system.

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Circadian Gene Expression in the Starlet Sea Anemone, Nematostella vectensis
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An organism's daily physiological and behavioral patterns are controlled by an endogenous circadian clock, which is synchronized to rhythmic environmental stimuli. We have been studying the circadian rhythms of the Cnidarian anthozoan *Nematostella vectensis*, the Starlet Sea Anemone. Holding a unique phylogenetic position as a member of the outgroup to Bilateria, *N. vectensis* allows us to study the evolution of the circadian clock. Previously, we demonstrated these animals undergo a daily endogenous rhythm in locomotor behavior which can be synchronized to the external photoperiod. In order to elucidate the molecular mechanism of the circadian clock in this species, and consequently

provide insight into its evolution, we have localized the gene expression of two circadian clock components, crypotochrome1a (cry1a) and clock (clk) in *N. vectensis*. Cry1a and Clk are molecular components of the transcription/translation feedback loop regulating circadian rhythms and have been extensively studied in mice and insects. In *N. vectensis*, these core clock genes have been previously isolated and shown to oscillate rhythmically. Using in situ hybridization, we found clk and cry1a gene expression restricted primarily to portions of the oral disk surrounding the mouth and the tips of the tentacles. These neuron-dense regions receive maximal exposure to natural sunlight. Based on this evidence, we believe the master clock is an organized structure within the nervous system of *N. vectensis*. By characterizing the temporal and spatial expression of these putative circadian genes, we can begin to provide insight into how the use of these genes has been modified throughout evolution.

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Oxytocin Receptor Distribution in the Extended Amygdala in Response to Cocaine Withdrawal
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Relapse is a major obstacle in the treatment of cocaine addiction and the anxiety during withdrawal from cocaine is a chief contributor to this relapse. The widely studied HPA axis, or hypothalamic pituitary adrenal axis, uses hormones such as CRF (cortiotropin releasing factor) and NE (norepinephrine) to explain the stress feedback loop. Although multiple systems influence this phenomenon, oxytocin has emerged as a potential contributor to withdrawal mediated by anxiety. Oxytocin is a neuropeptide whose receptors in the central nervous system are less widely expressed than CRF and NE; therefore, it could potentially lead to better pharmacological treatments for anxiety, depression, and addiction to drugs of abuse. To better understand the role of oxytocin in withdrawal from drugs of abuse resulting in anxiety, we used an animal model of cocaine addiction, in which rats self-administered cocaine during daily, six hour sessions for 14 days; a group of saline animals served as controls. Following self-administration and two days of abstinence, we assessed anxiety levels and found animals that self-administered cocaine exhibited higher levels of anxiety-like behavior using the elevated plus maze and the defensive burying test. To identify potential changes in oxytocin receptor levels in response to cocaine self-administration, we isolated the extended amygdala of these animals and quantified protein expression using Western Blotting. Evaluation of oxytocin receptor expression is ongoing and will provide a neurochemical correlate to the anxiety-like behavior changes reported during withdrawal from cocaine.

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Auxiliary Subunits Alter the Pharmacology of Recombinant Kainate Receptors
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Normal neuronal activity requires an appropriate balance between excitation and inhibition. Most of the fast excitatory neurotransmission in the brain is mediated by ionotropic glutamate receptors. There are three types of these receptors, named AMPA, NMDA and kainate. The kainate receptors are highly expressed in the hippocampus, a brain region in the temporal lobe critical for learning and memory. Overactivation of kainate receptors can be associated with schizophrenia, Huntingtonâ€™s disease, and epilepsy. The kainate receptor is tetrameric, with up to five different subunits (GluK1-GluK5) which can combine to form homomeric or heteromeric receptors. In addition, the auxiliary subunits Neto1 and Neto2 also assemble with kainate receptors in neurons and alter their functional

properties. The goal of this work was to examine whether co-assembly of kainate receptors with Neto 1 altered the pharmacological response to subunit-selective antagonists. We used patch clamp recordings from transiently transfected cells to measure the response of recombinant receptors to the antagonist ACET. In the absence of Neto1, ACET only inhibits receptors that contain the GluK1 subunit. However, when co-expressed with Neto1, we found that heteromeric GluK2/GluK4 receptors became sensitive to ACET inhibition. These results show that the auxiliary subunits can dramatically alter the characteristics of ionotropic glutamate receptors. These findings are important because they can help to predict the response of neuronal kainate receptors in brain regions that express the auxiliary proteins and may aid in the development of new drugs that can regulate excitatory neurotransmission.

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Using a two-bottle preference assay to demonstrate aversion of Drosophila melanogaster to insect defense chemicals
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abstract.: As a defensive mechanism, insects release a wide variety of chemicals. Many of these chemicals are known to irritate mammals by eliciting chemesthesis, or a sense of irritation mediated by the somatosensory system. We designed this experiment to determine if insects, particularly Drosophila melanogaster, were also behaviorally averse to these insect defense compounds. Furthermore, we investigated whether the known fruit fly irritant receptors dTRPA1 and painless helped mediate the behavioral aversion to these chemicals. Wild type (Canton S) and two mutant fly lines (dTRPA1 and painless) were tested using a two bottle preference test. In this assay, flies were given access to one capillary tube containing 1% sucrose and another capillary tube containing 1% sucrose plus the chemical of interest. After three hours, the amount of liquid consumed from each tube was noted and used to calculate a preference ratio. Results indicate that wild type fruit flies do not avoid two of the defense chemicals (trans-2-hexenal and trans-2-hexen-1-ol) but are behaviorally averse to others (benzaldehyde and benzoquinone). Preliminary analysis suggests that both dtrpA1 and painless channels play a role in mediating the response of fruit flies to benzoquinone and benzaldehyde. In the future, we will test additional insect defense compounds.

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A Role for Beta-endorphin in Ethanol-Stress Interactions
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The opioid peptide Beta-endorphin is synthesized and released in response to stressful stimuli as well as acute alcohol administration. Its release following exposure to an inescapable aversive situation may mediate behaviors that contribute to allostasis of the stress response. The present study examines the effects of Beta-endorphin on immobility in assays involving inescapable stress, both under basal conditions and after acute administration of EtOH. Female and male transgenic mice with varying capacities to translate Beta-endorphin were subjected to either the forced swim test (FST, Experiment 1) or the tail suspension test (TST, Experiment 2). In Experiment 3, mice were divided into three groups by hormonal status (male, female-estrous, female-nonestrous) and injected with either 1 g/kg EtOH or equivolume saline 14 minutes prior to the TST. Experiments 1 and 2 demonstrated a direct relationship between Beta-endorphin levels and immobility. Additionally, males displayed more immobility than females. A main effect of genotype in Experiment 3 replicated findings in Experiments 1 and 2. There

was also an effect of EtOH (increasing immobility) and a significant interaction reflecting particularly robust effects of the drug in mice with low Beta-endorphin. In addition, there were interactions between Beta-endorphin, EtOH effects, and hormonal status. These findings support the contention that Beta-endorphin moderates behavioral responses to stressful stimuli and suggest a role for this peptide in coping behavior. Furthermore, the effects of EtOH on the response to stress may be mediated by Beta-endorphin. Sex differences in this influence may contribute to sex differences in disease susceptibility and expression.

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Behavioral Aversion Assay to Assess Predator Responses to Insect Chemical Defense Compounds
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As a form of resistance towards predation, a variety of insect species release irritating defensive chemicals. We hypothesize that these defensive chemicals activate the trigeminal nerve in mammalian predators such as the rat. The trigeminal nerve innervates the mouth, nose and eyes and is a main contributor towards chemesthesis. Chemesthesis is the stimulation of the somatosensory system (including pain receptors) with chemicals. Stimulation of the nose with allyl isothiocyante (AITC), the active ingredient of wasabi, is an example of chemesthesis. We are using a behavioral aversion assay to determine whether rats avoid 8 insect chemical defense compounds (benzaldehyde, benzoquinone, formic acid, 2-heptanone, 6-methyl-5-hepten-2-one, trans-2-hexenal, trans-2-hexen-1-ol, and tetradecane). Rats are placed into a large Plexiglas box with a petri dish in each corner, one dish containing the stimulus randomly place in one corner and the others containing deionized water in the remaining 3 corners. The rat's behavior is videotaped for 10 minutes. The video is later played back and analyzed with tracking software (Noldus Ethovision) to track the rat's movements and time spent in each corner, most notably the corner with the irritant. Preliminary results suggest that, as hypothesized, rats spend less time in the corner with the irritant than the other three corners. Once we have established that these irritants are avoided we will begin determining which sensory receptors they may be activating using molecular biological techniques.

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Hydrodynamic forces reduce growth cone extension rates in vitro
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Proper nervous system development requires that axons synapse with targets that are often long distances from the neuron's soma. Growing axon terminals accomplish this wiring task by extending growth cones to traverse these distances. Growth cones are dynamic structures that extend and rapidly modify their morphology by actively restructuring cytoskeletal proteins in response to molecular guidance cues. Previous work in our lab indicated that fibroblast growth factor-2 (FGF-2), one such guidance cue, does not influence Xenopus RGC growth cone extension rates when applied to growth cones extending in stagnant culture media (Healey et al., 2006). Yet, when FGF-2 was applied in flowing media, FGF-2 appeared to enhance growth cone extension rates (McFarlane et al., 1996). To determine if hydrodynamic forces influence growth cone extension rates, we compared Xenopus laevis retinal growth cone extension rates in stagnant and flowing conditions using time-lapse microscopy. Growth cones in flowing conditions extended only 57% as rapidly as growth cones imaged in stagnant conditions ($p<0.01$). In addition to the differences observed between growth cone extension rates in flowing

versus stagnant conditions, extension rate consistency was also examined. During 90 min. of time-lapse imaging, growth cones in flowing culture media exhibited slower extension rates in their final 60 min. of extension, ~59% of the rate observed in the first 30 min. ($p<0.05$). Conversely, growth cones exhibited no significant extension rate changes over the 90 min. of imaging in stagnant culture media. Finally, an inversely proportional relationship was observed between media flow rate and growth cone extension rate. These data indicate that hydrodynamic forces are a potential confounding variable for in vitro growth cone studies and should be carefully considered in experimental design.

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A Developmental Rat Brain Atlas Using Diffusion Tensor Magnetic Resonance Imaging
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Diffusion Tensor Imaging (DTI) is a Magnetic Resonance Imaging technique based on the diffusion properties of water in biological tissues. The directional restriction of water diffusion between and within axons allows for detailed visualization of white matter connectivity in the brain. This technology is being used at the Duke University Center for In Vivo Microscopy to establish a three-dimensional atlas of white matter development in the rat brain, one of the leading model systems for human neurological diseases. Critical areas of the brain involved with vision and perception were segmented from the DTI data, including the optic nerve, optic chiasm, optic tract, lateral geniculate nucleus, and primary visual cortex, across multiple time points to follow the development of the visual system in the rat. The software involved in the analysis of the Magnetic Resonance Imaging data, Avizo and TrackVis, can be used to gather quantitative metrics of white matter integrity throughout development. These statistics elucidate the normal course of white matter development in the rat visual system, and allow quantitative comparisons to human disease models involving damage to the visual pathways. By mapping specific structures and projections as the rat brain develops, the developmental rat brain atlas will provide a highly detailed, three-dimensional atlas of white matter to complement existing histology-based atlases.

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Bilateral Entorhinal Cortex Lesions Impair Acquisition of Working Memory Task in Rats
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One of the first and most prominent clinical symptoms of Alzheimer's disease, a devastating neurodegenerative disorder, is memory impairment. The display of this cognitive deficit is preceded by the formation of amyloid plaques and neurofibrillary tangles in the entorhinal cortex, ultimately leading to neuronal death of this structure that plays a fundamental role in relaying sensory information between the neocortex and the hippocampus. To mimic this pathology, rats received bilateral entorhinal cortex lesion operations, and both this experimental group and a control group that received sham operations were trained to acquire a spatial working memory task on an eight-arm radial maze. Additionally, because bilateral entorhinal cortex injury induces reorganization of a number of heterotypic inputs via axonal sprouting to the hippocampus, the sprouting response of the septodentate pathway and the commissural and associational fiber plexus were quantitatively analyzed. The rats in the sham group reached criterion (seven out of eight correct responses for five out of six consecutive days) within about three weeks of post-operative testing, which was significantly faster than the rats in the lesion group. However, the rats in the lesion group reached criterion in about five weeks and were

making similar numbers of errors as compared to the control group by the end of the 12-week testing period. This recovery may be supported by the heterotypic sprouting response of the septodentate pathway and the expansion of the commissural and associational fiber plexus.

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Developing optogenetic techniques to probe the role of orexin/hypocretin in reward and addiction
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The orexins (also known as hypocretins) are a pair of neuropeptides expressed exclusively in the lateral hypothalamus (LH) and have been shown to play important roles in wakefulness, food-seeking, and drug-seeking reward behavior. We are currently developing ways to manipulate the activity of LH orexin neurons in in vivo rat models using optogenetic techniques in order to better understand the impact that orexin neuron activation has on reward seeking behavior. Genes for the light-activated cation channel channelrhodopsin-2 (ChR2) along with the mCherry reporter protein were packaged into lentiviral vectors. Expression of construct was controlled by the prepro-orexin promoter, producing selective expression of ChR2-mCherry in orexin neurons. Lentiviral vectors were injected into the lateral hypothalamus unilaterally. Following 6-8 weeks of viral incubation, rats were perfused, and brain sections containing the lateral hypothalamus were subjected to immunohistochemical processing. We used immunohistochemical techniques to identify the co-expression of mCherry and orexin to verify that ChR2 expression was selective to orexin neurons. Preliminary data show that orexin neurons in the injection area strongly express mCherry and ChR2. In addition, we observed a small subset of neurons outside the LH orexin region that exhibited weak mCherry expression. We are currently in the process of determining the source of this signal. One possibility is that these neurons express a low level of orexin not normally seen using standard immunohistochemical techniques. Another possibility is that neurons damaged by viral injection exhibit low levels of nonspecific immunohistochemical labeling. A final option is that the orexin promoter used in our construct produces low levels of non-selective expression. Currently, we are developing and applying additional immunohistochemical procedures to further investigate these possibilities.

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Depolarization Stimulates Neurite Development In Vitro
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Neurons are the fundamental cells of the nervous system and action potentials are the primary mechanism by which neurons propagate information. Communication between neurons occurs across specialized connections called synapses. Well before synapses are formed, developing neurons produce spontaneous action potentials. This spontaneous activity is thought to be critical to the proper development of neurons as well as axons and dendrites, neuronal projections collectively termed neurites. The purpose of this study was to determine if spontaneous action potentials play a role in shaping neuronal morphology. Dissociated stage 34 Xenopus retinal neurons were cultured for 48 hrs in an environment of increased extracellular K+ to depolarize the neurons and stimulate increased spontaneous action potential firing rates. After 48 hrs, neurons were fixed then treated with Hoechst to label nuclei and beta-tubulin immunostaining to delineate neurites. Neuronal morphologies in control and depolarizing (high K+) conditions were analyzed by examining primary neurites, total neurite lengths, and extents of neurite branching from isolated neurons. Results revealed that increased activity

leads to increases in the total length of the neurites, primary neurites, neurite branches, and varicosities. Thus, a neuron's activity appears to influence its morphological development. Because an increased firing rate has a significant effect on neurite development, subsequent experiments will determine how silencing action potentials influences neuronal morphology in vitro.
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Expression of LPA and S1P Receptors in Retina Through RT-PCR and Identification of GPCR Intracellular Signaling Pathways Involved in Growth Cone Collapse
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Our research focuses on the development of the visual system in chicken embryos. What we are specifically interested in are the molecules involved in axon guidance. Although there have been several axon guidance molecules identified, the full complexity of how growth cones connect topographically to the brain still remains unknown. We have shown that lysophospholipids, lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P), induce growth cone collapse in chick retinal axons in vitro, therefore, leading us to hypothesize these molecules are potential axon guidance molecules providing inhibitory cues to growth cones. LPA and S1P bind specifically to G-protein coupled receptors (GPCRs), which activate one of four possible intracellular pathways (Gi, G12/13, Gq, Gs). Currently, there are five LPA and S1P receptors, LPA1-5 and S1P1-5 respectively, in mammals (although only 3 S1P receptors in chick). We investigated which intracellular pathways LPA and S1P mediate by using pharmacological inhibitors to block intracellular pathways, measuring growth cone collapse induced by LPA or S1P. The inhibitors used were Pertussis Toxin (PTX), which blocks the Gi pathway, Y-27632, a Rho Kinase inhibitor that blocks the G12/13 pathway, and U-73122 a Phospholipase C (PLC) inhibitor that blocks the Gq pathway. We found that the Rho Kinase 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 found to partially reduce the level of growth cone collapse, indicating that the Gi pathway contributes to growth cone collapse as well. However, inhibiting PLC showed no effect of reducing growth cone collapse in the presence of LPA or S1P, suggesting the Gq pathway is not involved in growth cone collapse. We are currently investigating the expression of LPA and S1P receptors in the retina through RT-PCR. We have previously examined retinal expression of LPA receptors 1-5, and now we have identified S1P receptor 1 and 3 expression in the retina. In the future, in order to determine if LPA and S1P act as axon guidance molecules, we will knock down the expression of these lysophospholipid receptors through siRNA and analyze if there are any mapping errors. This research will allow us to gain a further understanding of how the visual system develops and all the mechanisms involved.

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Effects of Color on Psychophysiological Response and Ratings of Picture Valance and Arousal
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Color psychology refers to the study of color and its effect on arousal and behavior. The present experiment examines the impact of color on physiological response and the rating of valence and arousal during presentation of IAPS photos. Subjects are assigned to one of six experimental groups formed from combinations of high valence or low valence pictures and three picture border colors of red, blue and grey. All subjects also rated the same set of neutral pictures in their respective conditions.

Subjects rate their valence and arousal in response to each picture on an ordinal scale from 1 (low) to 9 (high) while their skin conductance (SC) and facial electromyography (EMG) are recorded. Results will be analyzed to determine whether colors have an effect on valence and arousal ratings and are reflected in peripheral nervous system responses representing valence (corrugator vs zygomaticus facial EMG) and arousal (SC).

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Anxiotypic Behavior Following Withdrawal from Cocaine Self-Administration
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Drug addiction is a chronic relapsing disorder associated with compulsive drug taking behavior and the subsequent impairment of social and occupational functioning. Relapse is hypothesized to be motivated by an aversive affective state that increases the probability that an addict will seek out substances of abuse in order to temporarily remove unpleasant feelings associated with withdrawal. In an effort to validate a rodent model of drug relapse, we studied anxiotypic behavior following acute withdrawal from chronic cocaine self-administration in Sprague Dawley rats (n=16). The transition to chronic cocaine abuse was modeled using a 14-day extended-access cocaine self-administration regimen. Rats were intravenously administered 0.2ml of cocaine hydrochloride dissolved in physiological saline (4mg/ml) contingent upon a lever press in a self-administration chamber (30 x 20 x 20 cm). Subjects exhibited an escalation in drug taking behavior as trials progressed. Following two days of abstinence, anxiotypic behavior was measured using the elevated plus maze (EPM) and defensive burying test (DBT). In rats undergoing cocaine withdrawal, we found a statistically significant increase in anxiety-like behavior on both the EPM ($t(6)=2.073$, $p<.05$) and the DBT ($t(10)=2.161$, $p<.05$) compared to control subjects. Escalation in drug intake and withdrawal induced anxiety are characteristic of human substance abuse disorders and are believed to be mediated by allostatic deviation of the reward system and hypothalamic pituitary adrenal axis. This drug relapse regimen provides a pre-clinical model of the neural mechanisms of anxiety motivated relapse. Future studies will examine the expression of neuropeptides in the extended amygdala following withdrawal using this behavioral model.

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Calpeptin Protects Against Ethanol Induced Toxicity in C6 Astroglial Cells
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Alcoholism is a disorder that impairs the CNS in a chronic progressive manner by altering the structural-functional integrity of diverse neural population. The present study investigated the potentially toxic effects of the psychoactive alcohol, ethanol on astrocytes, which are the most predominant cells in the CNS. C6 rat glioma cells were differentiated into astrocytes and then exposed to ethanol (25-100mM) for either 6 hours (acute exposure) or 24 hours (chronic exposure). A cell viability assay measuring mitochondrial reductase activity, showed significant cell death at a higher concentration of ethanol (70mM) as compared to lower doses (Fischer's LSD $p<0.001$). The effect of ethanol was also assayed at the molecular level where we found detrimental consequences for both glutamate transporter proteins, an indicator of astroglial integrity, and DNA structure. In order to provide insight into the mechanism of cell death, we assessed the effect of ethanol on the expression of cellular markers for intracellular and extracellular induced apoptotis. Morphological assessment revealed the presence of pro-apoptotic proteases in cells exposed to high doses of ethanol (50mM, 100mM), which was determined by the

upregulation of Bax:Bcl2 (intrinsic proteases) and an early apoptotic marker Annexin V (extrinsic protease), further suggesting cell death may be mediated by both of these pathways. These results suggest that administration of increased concentrations of ethanol correlates with the upregulation of extrinsic and intrinsic pro-apoptotic proteases and leads to DNA damage. To probe the upstream regulators of ethanol-induced apoptosis, western blot analysis was performed on cells pretreated with calpeptin, a known blocker of calpain dependent cell death. The data demonstrates that pre-treatment with 100nM calpeptin rendered significant protection against 100mM ethanol. Future studies will be conducted on studying calpeptin's post-treatment on slice cultures of brain and spinal cord from rat pups.

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Parcipation of Projections from the Lateral Habenula to the Ventral Tegmental Area and to the Rostromedial Tegmental Nucleus using Optogenetics and Behavioral tests
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abstract.: The pathways projecting from the lateral habenula (LHb) to the rostromedial tegmental nucleus (RMTg) and to the ventral tegmental area (VTA) have recently become regions of interest due to their role in both aversive and appetitive behavior. Further study of the projectionsâ€™ influence on these behaviors could provide insight into human drug abuse. Anatomically, the pathway from the LHb to the RMTg is denser, but has less myelination than the pathway from the LHb to the VTA. We compared both projections to address questions regarding the differences in density and projection targets. This study utilized optogenetics to selectively inhibit the efferent glutamatergic projections from the LHb to the RMTg or VTA during behavioral testing. Adeno-associated virus (AAV) containing the coding sequence for eNPHR with a synapsin promotor was injected into the LHb and allowed to incubate for 2-3 weeks. Upon successful incubation, rats underwent conditioned freezing tests. Results so far show successful manipulation of the pathway during open field locomotor testing.

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Stress Effects on Alcohol Consumption
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There is a complex relationship between stress and alcohol consumption. Alcohol is known to activate stress systems in the brain. Conversely, alcohol can have an anxiolytic effect and decrease stress, which in turn motivates drinking. In this study, we looked at whether there would be an effect on alcohol consumption in both alcohol dependent and non-dependent mice after undergoing one of three stress challenges: restraint, forced swim or social defeat. Additionally, we were interested in whether there would be an escalation in alcohol consumption in mice given 24-hour access compared to the limited 2-hour access paradigm often used in drinking studies. We trained male C57BL/6J mice to have a baseline level of ethanol consumption before starting a schedule of alternating weeks between chronic intermittent ethanol exposure and 24-hour continual access. In the fifth cycle of this alternating schedule, stress challenges were applied each day for five days before being given access to alcohol. Results showed the greatest increase in drinking following restraint stress in both alcohol dependent and non-dependent control mice. Total alcohol consumption was lower for the 2-hour readings compared to the limited 2-hour access paradigm but there was an overall greater escalation in alcohol consumption given 24-hour access than the limited 2-hour access. Continuing research will look into the

effect on consumption given delayed alcohol access compared to immediate alcohol access following exposure to stress.

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The role of neuropeptide Y in anxiety-related behaviors associated with cocaine abstinence
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The anxiety associated with drug abuse is a leading cause for drug addicts to relapse. The resumption of cocaine use often alleviates the symptoms associated with the many negative effects of cocaine withdrawal, including anxiety. Our goal is to elucidate the role neuropeptide systems and the changes that occur in the brain due to drug abuse, addiction, and relapse. Neuropeptide Y (NPY) is an endogenous 36 amino acid neuropeptide abundantly found in the CNS. NPY receptors are densely expressed in areas of the brain implicated in the anxiety associated with cocaine withdrawal. The focus of this study is to investigate the anxiolytic role of NPY in changes that occur in the brain during withdrawal from cocaine abuse. We used an animal model of cocaine addiction in which animals self-administered cocaine in daily 2-hr access sessions for two weeks and then experienced abstinence for two days. Following abstinence, we measured anxiety using the elevated plus-maze. Brains of the animals were removed and central levels of NPY and NPY receptors in the bed nucleus of the stria terminalis , paraventricular nucleus , and the central amygdala were assessed using an enzyme-linked immunosorbent assay (ELISA)). Animals exhibited increased levels of anxiety during abstinence, and preliminary data suggest that there is a negative correlation between anxiety and the levels of NPY found in the brain. Our results indicate that NPY may be a significant factor in the anxiety involved with drug addiction. Future directions involve testing other areas of the brain associated with anxiety, specifically, the nucleus accumbens.

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Startle Response in the Tiger and Spotted Salamander
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The ability of sensory systems to detect disturbances in the environment and select appropriate responses is central to the survival of the organism. Salamanders are a source of great interest to the field of evolutionary neuroscience because they undergo such drastic morphological change during ontogeny. Such change affords opportunity to evaluate the neurological substrates that mediate behavior. As aquatic larvae, salamanders are vulnerable due to their small size. We tracked developmental changes associated with the startle response in two Ambystomids, the tiger (A. tigrinum) and spotted salamander (A. maculatum), across the larval lifespan from the early to the late aquatic stage. To evaluate the startle response, larvae from each species were placed in cups on a tray and subjected to a brief vibrotactile stimulus. Responses were defined as any movement from 0 degrees at the rostral tip of the animal. Responses were recorded and were then evaluated using a Z-test. Comparisons between species indicate that in the legless early aquatic stage (10 days post hatching), A. tigrinum exhibited the startle response while A. maculatum remained immobile. In the quadrupedal early aquatic stage (23 days post hatching), A. tigrinum continued to exhibit the startle response. A. maculatum showed an increase in propensity to respond the stimulus compared to the early aquatic stage. These data indicate that despite being sympatric and of a similar ecological niche, the two species demonstrate different predator avoidance behavior. We attribute species differences in startle response

to their morphological and ontogenetic differences. A. tigrinum is larger, with a slower rate of ontogeny while A. maculatum is a smaller but exhibits comparatively rapid development to the terrestrial stage.

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Gene Transfer for Narcolepsy
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Narcolepsy is a neurodegenerative sleep disorder, characterized by the loss of orexin/hypocretin (HCRT) neurons in the brain that results in excessive daytime sleepiness, cataplexy, and sleep attacks. Gene transfer is a recently successful treatment for many diseases that enables to a viral vector to safely transfer and express a particular gene, or genes, into the genome of an affected individual. Gene transfer using the recombinant adeno-associated virus (rAAV) expressing orexin was injected into the lateral hypothalamus (LH) of the HCRT knockout mice and behavior was monitored three weeks later for 48 hours. The animals were subsequently sacrificed and the brains were removed, sliced and immunohistochemistry tests were performed on the slices to assess the expression of orexin. The rAAV vector expressing orexin has been shown to successfully express HCRT and greatly reduce the incidence of cataplexy and excessive daytime sleepiness. These results indicate that further research should be done using the LH as a target for further gene transfer in conjunction with other therapies.

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Ethanol Induced Regulation of Apoptotic Markers and Protection by Calpeptin in Ventral Spinal Cord Motor Neurons
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Alcoholism has devastating effects such as hepatic toxicity, impaired movement and coordination, and cognitive dysfunction. The biochemical and molecular processes responsible for its effects are important factors in understanding how alcohol is detrimental to the CNS. This study focused on the different pathways of neuronal, especially motor neuronal cell death caused by exposure to the psychoactive-ethanol. Hybrid ventral spinal cord (VSC) motor neuron cells differentiated into motor neuronal phenotype were exposed to different concentrations of ethanol that correspond to the levels found in Blood Alcohol Concentrations (BAC) after social and binge drinking. Morphological assessment of VSC motor neurons via In Situ wright staining showed significant cell death at ethanol concentrations >50 mM. Cell death was also found in prolonged exposure of ethanol at concentrations >12.5 mM. To investigate the cell death pathways, apoptotic markers were assessed by western blotting. Increasing ethanol concentrations showed up-regulation of intrinsic and extrinsic apoptotic proteases such as caspase-3, caspase-8, Annexin V, and elevated Bax/Bcl2 ratio. Calpain, a major protease upstream of the proteolytic cascade, was also up-regulated. Calpeptin, a major calpain inhibitor, induced resistance against the up-regulation of these proteases. Thus, calpeptin shows cytoprotective properties against apoptosis induced by exposure to ethanol.

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Vasopressin-1b receptor distribution in the CeA and BNST during withdrawal from cocaine self-administration
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Relapse, a major obstacle in the treatment of cocaine addiction, is commonly triggered through increased anxiety, associated with abstinence from the drug. Vasopressin, a neuropeptide associated with anxiety, modifies primary neurotransmitters in the major stress pathway and consequently, contributes to stress levels. We hypothesize that the central amygdala (CeA) and the bed nucleus of the stria terminalis (BNST) are key regions of AVP-1b-induced modulation of the stress axis-activating neurohormone, corticosterone-releasing factor (CRF). Changes in AVP-1b levels were measured using a rat model of cocaine addiction. After fourteen days of long access (6 hours/day) cocaine self-administration and acute (42 hours) abstinence, anxious behavior was measured on elevated plus maze and AVP-1b levels were quantified in each animal using western blotting. Results from behavioral analyses demonstrated heightened levels of anxiety in our cocaine-exposed animals, but results from the Western blots showed no statistically significant difference in AVP-1b receptor levels in the BNST and CeA between the cocaine animals and saline animals ($t= 0.09720$, $p>0.05$; $t=0.2939$, $p>0.05$, respectively). These findings suggests either an alternate source of CRF modulation, or that stress related changes in AVP-1b receptor levels, occurring elsewhere in the extended amygdala, may underlie these behavioral changes. Future research may investigate the changes in vasopressin-1b receptor levels within other stress-contributing brain structures, including the paraventricular nucleus (PVN), caudate nucleus (CN) and the pre-frontal cortex (PFC), in order to identify the source of addiction-induced CRF modulation.

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Connectivity in the Human Mirror Neuron System of Republicans and Democrats
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With the 2012 presidential election fast approaching, and in the wake of increasing political polarization, it has become critical to examine possible factors underlying political affiliation. Previous research suggests that one important difference between Democrats and Republicans involves empathy, the capacity to recognize and share feelings being felt by other individuals. Specifically, the research supports the idea that Democrats are more empathetic than Republicans. Recent evidence from brain imaging experiments has linked the characteristic of empathy to activity in a specific subset of brain areas referred to as the human mirror neuron system (hMNS). Based on this previous research, we hypothesized that Republicans and Democrats would show different patterns of brain activity within the hMNS. In order to address this hypothesis we used functional magnetic resonance imaging (fMRI) to examine resting state connectivity of brain regions in the bilateral hMNS (Inferior Frontal Gyrus [IFG], Inferior Parietal Lobule [SMG/ANG] and superior temporal sulcus [STS]) in college students that identified themselves as either Republican or Democrat. Consistent with our hypothesis, we found evidence of greater connectivity between the IFG and IPL in Democrats as compared to Republicans in both the left and right hemispheres [$t(23) = 3.63$, $p < 0.0005$ and $t(23) = 2.80$, $p < 0.005$ respectively]. The current results provide novel data regarding the neural implementation of political affiliation and might serve as a touchstone for future research in this area.

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Gene Regulation Effects on MJD Mechanism
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Machado Joseph Disease (MJD) is a dominantly inherited neurodegenerative disorder caused by an abnormal amount of CAG repeats in DNA that form misfolded, toxic polyglutamine ataxin-3 protein structures (Paulson 2007; Warrick et.al. 2005). The results of this built up protein are reactive oxygen species and free radicals, which are known to accelerate the aging process. In addition, the protein aggregates and pulls in various other proteins including those responsible for various cellular regulation. This project outlines genetic manipulations performed on 3 different areas of possible disease pathology. The first section looks at the effects of Super Oxide Dismutase I and II, which have been discovered to slow down age-related biochemical alterations and extend the lifespan in Drosophila (McCord, 1969) through the reduction in levels of oxidative free radicals. The second aim looks at increasing levels of proteins that could be getting pulled into protein aggregates. Particularly, we up-regulate HATS that are responsible for regulating circadian rhythm. Lastly, we test the effects of up-regulating chaperone proteins, which are known to aid in protein folding and localization. Creb binding protein is a transcriptional coactivator, histone acetyl transferase, that has been implicated in polyglutamine pathology. We see a slowing of disease progression in the second and third experiments and will discuss what this means for our understanding of the disease pathology.

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Behavioral mashup: Studies on the effects of aging, housing condition, bisphenol-A, and capsaicin on zebrafish activity
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Zebrafish are used extensively now for behavioral and pharmacological investigations in neuroscience research. In these experiments, we examined the effects of environmental and pharmacological manipulations on zebrafish behavior. We also examined the effects of aging on acute measures of zebrafish locomotor activity. Animals were placed individually in an observational arena and activity was measured using the Noldus EthoVision system for 10 minutes. Aged zebrafish (19 months of age) show no significant change in general locomotor activity levels in comparison to 3 months of age (although there is a general decline in activity levels). Housing conditions resulted in significant alterations in locomotor activity in adult fish. Initial experiments indicated that diminished visual input (from covering the exterior of the tank with black poster board) resulted in a significant decline in activity levels of adult fish. However, later experiments with tank enrichment also resulted in significant declines in activity levels. Exposure to bisphenol-A resulted in significantly less activity that subsequently normalized a year after exposure. In another experiment, the effects of capsaicin, a common irritant to mammals and a potential deterrent for drug abuse, was examined in zebrafish. Exposure to capsaicin (1 µg/L) had no significant effect on the total amount of locomotor activity observed.

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Rodent-based Model of Epigenetic Influence on Social Consumption of Alcohol
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Consumption of alcohol in a social setting is generally accepted among the American population as a way to mingle and celebrate with others. The rodent genus *Peromyscus*, in which species differ in social structure, was used as a model to observe the effects of social behavior on alcohol consumption. *Peromyscus maniculatus* (BW) has been observed to be reproductively promiscuous and uniparental, while *Peromyscus polionotus* (PO) have shown monogamy towards their mated partners and biparental care of offspring. It was hypothesized that these behavioral differences could result in a species-dependent difference in the amount of alcohol consumption, and that a hybrid (F1) of the two species (BW x PO) might show consumption behavior more closely resembling one species or the other. To determine this, twelve male *Peromyscus* from each species (BW, PO, and F1) (n=36) were housed in same-species pairs with constant access to food and water. Each pair was also given access to a 6% ethanol solution for 16 hours during the night cycle for five days per week over a six week period. The amount of ethanol consumed each day was measured and total consumption was analyzed using a mixed-design ANOVA. It was found that the PO's drank significantly more alcohol than the BW's but not the F1 hybrids. The F1 hybrids showed a similar drinking pattern to that of the PO's, possibly alluding to a more dominate PO behavioral expression.