**THE INTERACTION BETWEEN CANNABINOID RECEPTOR INTERACTING PROTEINS AND DOPAMINE RECEPTORS**

Therapeutic uses of cannabinoid neurotransmitter proteins have been observed to include appetite management, pain relief, and enhanced motor control. The purpose of this study was to investigate the in vitro interactions between cannabinoid receptor interacting proteins (CRIP1a) and dopamine receptor proteins (D1) as determined by interactions on glutathione s-transferase columns (GST columns). We hypothesized that we would find no interaction between CRIP1a and a control GST column after washing the column with a low concentration of phosphate buffered saline (PBS), but that we would find strong interactions between CRIP1a and both D1 and cannabinoid membrane receptor proteins (hCB1). E. coli bacteria were induced to produce D1 proteins, hCB1 proteins, and CRIP1a proteins. After these proteins were applied to the GST columns, the columns were washed with PBS to determine if the wash produced a good negative control condition in which CRIP1a, engineered without a GST tag for binding the protein to the column, did not stick to the column in the absence of the GST tagged proteins D1 and hCB1. In fact, we found that CRIP1a bound both to columns containing D1 or hCB1 and to an empty column. Thus, our hypothesis that the PBS wash prevents false positive CRIP1a sticking to the empty column was incorrect. We were unable to obtain a good negative control and believe that possible reasons for our findings include using an antibody for Western blot analysis that was not specific enough, as well as the method of producing the protein-protein interaction that we used.

**ALCOHOL INDUCED ANXIOLYSIS IN B-ENDORPHIN KNOCKOUT MICE**

Endorphins mediate the perception of pleasure and the inhibition of pain perception. Specifically, Beta endorphin is thought to mediate stress responses as well; therefore lowered levels of beta endorphin may increase stress response and anxiety. The current study considered the anxiolytic effects of alcohol in beta endorphin knockout mice. Anxiety was determined for male and female heterozygous, wild type, and beta endorphin knockout mice through the use of plus maze and light-dark box behavioral paradigms. Subsequent tests compared behavior between subjects after administration of intraperitoneal injections of saline or 1.5 mg/kg of 20% ethanol. Mice deficient in Beta endorphin demonstrated significantly greater anxiolysis after alcohol administration than heterozygous and wild type mice, despite the fact that in tests with no injections saline administration beta endorphin knockouts demonstrated the most anxious behavior. Thus, alcohol may act in the same anxiolytic manner as and as a substitute for beta endorphin. Perhaps alcoholism is correlated with low beta endorphin levels and is therefore a manner of self-medication for said deficit.

**DISTRIBUTION OF PERIOD 1 PROTEIN IN THE CIRCADIAN CLOCK OF BEHAVIORALLY "SPLIT" HAMSTERS**

The circadian clock in the suprachiasmatic nucleus of the mammalian brain regulates daily cycles in physiological and behavioral processes. The molecular components of the clock mechanism have been identified and undergo a daily rhythm in gene and protein abundance. In nocturnal rodents, one critical clock protein, Period 1, peaks at dusk and is correlated with the animal’s activity onset. Animals housed in a light:dark cycle or in constant darkness typically have one activity bout per day. Under constant lighting conditions, however, hamsters display a phenomenon called “splitting” which is characterized by two activity bouts within a 24-hour period. Based on this phenomenon, it has been theorized that the clock is comprised of two independent oscillators coupled together. The present study was designed to elucidate the molecular mechanisms underlying the splitting phenomenon. Using a combination of activity, which can also reset the clock, and unique lighting regimen, we were able to induce ‘splitting’ in hamsters. Using immunohistochemical techniques, we assayed the levels of Period 1 protein in the suprachiasmatic nuclei from “split” and “unsplit” hamsters. Preliminary data indicate that in hamsters with “split” activity rhythms there are two peaks of Period 1 protein which are correlated with the two activity bouts. In addition, the total number of cells immunopositive for Period 1 in the brains of the “split” hamsters were approximately half that of the “unsplit” controls. These data suggest that each independent oscillator is represented by a subset of cells within the suprachiasmatic nucleus that gives rise to each activity bout in the “split” condition.

**A DROSOPHILA GENETIC MODIFIER SCREEN APPROACH TO UNDERSTANDING THE MOLECULAR BASIS OF INFANTILE NEURONAL CEROID LIPOFUSCINOSIS**

Infantile Neuronal Ceroid Lipofuscinosis is caused by mutations in the cln1 gene which encodes palmitoyl-protein thioesterase 1 (Ppt1), suggesting that there is an important role for the regulation of palmitoylation in normal neuronal function. Loss of Ppt1
function in patients produces granular osmiophilic deposits (GRODS) in all cells and massive neurodegeneration (1). Consistent with an important role for PPT1 in neurons, the protein is found with synaptic vesicles and synaptosomes in neuronal cell culture (2,3). What remains unclear is why the loss of Ppt1 results in the observed disease pathophysiology in neuronal cells and resulting neurodegeneration. Is the substrate accumulation the primary cause of the disorder or rather a secondary symptom of the disease? (4) The loss of the Ppt1 protein may have a primary effect on neuronal cellular processes such as cellular trafficking or signal transduction, which on their own, or through secondary effects, leads to the disease pathology. We have previously shown that targeted over-expression of DmPpt1 in the developing Drosophila visual system using GMR-Gal4 leads to the loss of cells, including neurons, through apoptotic cell death (5). We have performed a gain-of-function modifier screen in which we have used ~2000 EP and EY transgenic overexpression lines to suppress or enhance the degeneration produced by DmPpt1 overexpression. Several of the modifier genes we have identified have been previously shown to be important for neuronal function confirming an important role for DmPpt1 in neuronal signaling and synaptic development. Further analysis of the genes identified in our gain-of-function approach will facilitate the identification of in vivo substrates and signaling pathways that DmPpt1 may modulate thus shedding light on the molecular etiology of INCL.

Department of Psychology, University of Evansville
DIETARY PHYTOESTROGEN EXPOSURE DURING PERINATAL PERIOD EFFECT ON ULTRASONIC VOCALIZATIONS, OPEN FIELD, PLAY BEHAVIOR ACTIVITY IN RATS
We recently reported that perinatal exposure to phytoestrogens (genistein and diadzein) alters ultrasonic vocalizations (USV) emitted from neonatal rats during a 30-minute isolation from the dam and home cage (Becker, et al., 2005). These results suggest that removal of phytoestrogens from the diet may induce an anxiogenic effect that is reversed by dietary replacement of phytoestrogens. In the present study, pregnant rats received a free feeding diet of either rat chow that contains low amounts of soy protein (genistein 0.62 µg/g), normal rat chow (genistein 9.47 µg/g), or rat chow free of soy protein and given a phytoestrogen supplement (genistein 48.42 µg/g) from the second week of pregnancy to weaning (day 21). USV emissions were measured on postnatal days 10 and 15 during a 10-min isolation test in 22%#61616; C, 32%#61616; C, or 35%#61616; C testing environments. There were significant differences in USV emissions due to phytoestrogen exposure, testing temperature and subject age. Furthermore, ongoing experiments involve measuring rat behavior in an open field (30 min) and during social interaction with another same-age rat (15 min) after weaning (day 22-24) and again at puberty (day 43-45). These studies suggest that there may be subtle but significant effects on thermoregulation, exploratory behavior and social interactions that are dependent upon exposure to plant estrogens during early development. (UEXPLORE grant to LMB, JJP, LAB & MMB, LAB)
axons form the optic nerve and are the only retinal axons that leave the retina. Here we used confocal microscopy to characterize the developmental expression pattern of neurofilament associated antigen recognized by the 3A10 monoclonal antibody during the stages when RGC axons navigate from the retina to their target, the optic tectum. We observed that the RGCs began to express the antigen at stage 33/34 of development, when RGCs have just crossed the optic chiasm. The antigen is expressed more robustly with maturation through stage 45. Thus, 3A10 immunostaining can be used to identify RGCs in retinal neuron cultures.

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EFFECTS OF A SEROTONIN RECEPTOR SUBTYPE 3 AGONIST AND ANTAGONIST ON INTER-MALE AGGRESSION IN MUS MUSCULUS

Research has revealed an inverse relationship between serotonin (5-HT) levels in the brain and aggressive behavior. However, effects on aggression at the level of the receptor have yet to be elucidated for many 5-HT receptor subtypes. This study examined the effects of the serotonin receptor subtype 3 (5-HT3) agonist m-chlorophenylbiguanide and antagonist ondansetron on inter-male aggression in mice. Using a resident-intruder paradigm designed to assess both offensive and defensive aggression, male C57BL/6J mice received 1 mg/kg i.p. injections of either m-chlorophenylbiguanide, ondansetron, or an inactive vehicle and were subsequently exposed to male AKR/J mice for a period of 10 minutes. Attack latency and the proportion of time engaged in a range of behaviors, such as grooming, inactivity, defensive, and offensive, were recorded. Subject C57BL/6J mice were then immediately run in an open field test for an additional 10 minutes to examine any anxiolytic or sedative effects of the drugs. Preliminary results show no significant differences between drug groups with respect to the open field test. Data analysis regarding attack latency and the behavioral profiles of the aggressive encounters are currently being examined. In conclusion, the radical difference between the 5-HT3 receptor, a ligand-gated ion channel, and the remaining G-protein coupled 5-HT receptor subtypes may serve as an explanation for any observed or absent effects of the 5-HT3 receptor agents on inter-male aggression.

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THE BED NUCLEUS OF THE ACCESSORY OLFACTOR TRACT IS NOT FEMINIZED BY PRENATAL STRESS

Prenatal stress prevents full masculinization of several nuclei along the vomeronasal pathway (VNP) to the spinal cord. The present study compared the adult volume of the bed nucleus of the accessory olfactory tract (BAOT) in prenatally stressed males with those of non-stressed males and females. Previous studies show the BAOT to be sexually dimorphic (male > female) and to be altered by postnatal surges and reductions in testosterone (T) in both males and females. The sexual dimorphism in the BAOT was confirmed, but prenatal stress however, had no effect on the size of the BAOT in males. Prenatal stress alters the surge in T on embryonic days 18 and 19 in male rats. This late prenatal surge masculinates certain regions in the brain, like the sexually dimorphic nuclei of the medial preoptic area (SDN-MPOA), the spinal nucleus bulbocavernosus (SNB), and dorsolateral nucleus (DLN), but does not affect others. This may be attributed to the different periods of neuronal development and sexual differentiation when certain nuclei are sensitized to T and its metabolites.

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THE EFFECT OF AN INACCURATE CLOCK ON HUMAN TIME PERCEPTION

Although many studies have demonstrated the ability of humans to accurately judge brief time duration, there has yet to be a study that directly considers the effect of a clock on human time perception and that studies human time perception at relatively long intervals. The present study investigated the impact of an incorrect clock on time perception of long intervals. The study included a no-clock group, a correct-clock group, and two incorrect-clock groups (the clock showed that 30 min had passed in either 15 or 45 actual minutes). Participants were asked to work on logic and math problems until they believed that 30 min had elapsed. Differences in stop times and the absolute difference of stop times from 30 min were compared across groups and correlated with impulsivity and locus of control scores. The no-clock and correct-clock groups both averaged stop times of approximately 31 min, whereas fast and slow-clock groups averaged stop times of approximately 26 and 36 min, respectively. These results indicate that most people can detect a clock that is 50% inaccurate; however, the inaccurate clock causes their time perception to be in error by about 30% in the direction of the inaccuracy. Generally, participants with a more external locus of control showed more accurate time estimation. Impulsivity had no effect when a clock was present, but accuracy decreased as impulsivity increased in the no-clock group. The results have implications for studies on proposed long-interval and attentional neural clock mechanisms (frontal lobes, anterior cingulate cortex, thalamus, hippocampus).

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THE EFFECTS OF GBH AND FLUNITRAZEPAM ON MOTOR PERFORMANCE

Gamma-hydroxybutyrate (GBH) and flunitrazepam (Rohypnol) are two pharmacological agents that have been implicated in some instances of alleged sexual assault, presumably due to their motor impairing effects. The purpose of the present study was to...
examine the interactions between GHB and flunitrazepam in an animal model of motor performance. Ten male rats were trained to walk on a rotordod apparatus rotating at 16 rpm, and the latency to fall from the apparatus was recorded. When administered alone, GHB produced dose-dependent decreases in motor performance as revealed by reductions in the latency to fall from the apparatus. In drug combination tests, flunitrazepam dose-dependently enhanced the effects of GHB in a generally additive manner. Collectively, these data indicate that GHB and flunitrazepam may produce potentially dangerous interactions under conditions in which recreational drug use is common.

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STABILITY OF THE TAIL WITHDRAWAL REFLEX IN RATS
Previous results in rats (Cleland and Bauer J. Neurosci. Rapid Comm. 22:5265-5270, 2002) revealed that pinpoint heat stimuli applied to the tail evoked withdrawal movements whose direction depended on the location of the stimulus. However, because movements were characterized only by the initial direction and speed of movement, the complete time course of the response could not be determined. The goal of these experiments is to fully quantify the 3-dimensional trajectory of the tail during withdrawal movements from pinpoint heat stimuli. As a first step we sought to determine whether any characteristics of the movement showed habituation or sensitization over repeated trials. Rats were restrained vertically and 25 pinpoint (0.5mm) heat stimuli were delivered one every 5 minutes with a laser to the right side of the tail (midway along length). The resulting movement was captured using two high speed (250 frames/s) cameras. The tail trajectory projected onto the horizontal plane was plotted by automatically tracking in software the location of the tail (marked in black) at the level of the stimulated spot. Our preliminary results suggest that most key features of the movement: direction, speed, latency, curvature and distance traveled over 380 msec do not exhibit systematic changes over time. Consequently, the current model appears to provide a stable response suitable for further investigation of the dependence of stimulus location on direction and speed of movement.

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AGE-SPECIFIC ROLE OF PROTEIN KINASE C IN A RODENT MODEL OF SICKLE CELL PAIN
Endothelin-1 (ET-1) is a potent vasoconstrictive peptide that contributes to the pain associated with sickle cell disease, cardiovascular disease, and cancer. Our laboratory has recently shown that ET-1 produces increased pain associated behaviors (nociception) in younger animals than in older ones. Also, in the younger animals, ET-1 produces more of these behaviors in males than in females. Protein kinase C (PKC), a family of developmentally regulated enzymes, has been shown to drive nociceptive responses. We hypothesize that PKC mediates sex- and age-specific ET-1 induced nociception. This hypothesis was tested by administering a non-specific PKC agonist (PMA) or a PKC antagonist (Chlereythrine) followed by ET-1 subcutaneous in the left plantar hind paw in postnatal day 7 and 21 Sprague Dawley rats. Animal behaviors were recorded for 75 minutes and later analyzed for paw flinching and licking by a researcher blinded to animal treatment. Our results showed that in P7 rats, PMA alone did not produce nociceptive behaviors. However, PMA increased ET-1 induced behaviors from 50-75 minutes. In P21 rats, PMA alone produced behaviors at all times. PMA injected along with ET-1 produced more behaviors from 0-20 minutes. In P21 rats, CH injected with ET-1 decreased behaviors from 50-75 minutes. We can conclude from these results that PKC mediates age-specific responses to Endothelin-1 in neonatal rats. These findings have implications for pain therapies that specifically target infants, children, or adults.

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UP-REGULATION OF RNA BINDING PROTEINS IN RAT DORSAL ROOT GANGLION AFTER CONDITIONING AXOTOMY
Robust regeneration into the spinal lesion site is seen after conditioning axotomy of the peripheral branch of the rat dorsal root ganglion and subsequent spinal axotomy, indicating a cell body response to injury. The response is hypothesized to include up-regulation of RNA binding proteins in order to facilitate the transport of RNA into the extending growth cone. In-situ hybridization on lesioned L4 and L5 ganglia screen for the up-regulation of five RNA-binding proteins after conditioning axotomy.

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THROMBIN ACTIVITY AND LEVELS DURING THE PERIOD OF MOTONEURON PROGRAMMED CELL DEATH IN THE CHICKEN EMBRYO
Evidence has shown that thrombin is not only a principal component of the blood coagulation cascade, but plays an integral role in nervous system growth and maintenance. As part of the serine protease family, thrombin mediates cellular responses through a G protein-coupled, cell surface protease activated receptor-1 (PAR-1). Once activated, thrombin alters astrocyte stellate morphology, stimulates axonal growth cone collapse, and prevents neurite outgrowth. In the nervous system, PAR-1 activation by thrombin can also induce the expression of specific cell death genes that ultimately lead to cell death. The understanding of apoptotic regulation may lead to further scientific advances in certain neurodegenerative disorders. The aim of this study is to ascertain the days during chick embryonic programmed cell death (PCD) that thrombin is active and whether thrombin levels coincide with the expected decrease in motoneuron survival. This preliminary study indicates that thrombin activity during embryonic days (E)3-10 peaks on E4 with activity approximately 1.5 x 10-3 absorbance units per unit of protein and decreases to approximately 4.7 x 10-4 absorbance units per unit of protein on E8. Overall, it is important to understand the functions and related roles of serine proteases on the nervous system so that we can better treat and prevent cell death after traumatic injury and neurodegenerative disease.

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IS THE OSCILLATION OF PHOSPHOLIPASE C BETA 4 IN THE MOUSE REGULATED BY A CIRCADIAN CLOCK?
An organism’s circadian, or daily, rhythms in physiology and behavior are regulated by an endogenous clock. In mammals, the central circadian clock is located in the hypothalamic suprachiasmatic nucleus (SCN). In other body tissues, peripheral clocks are responsible for local oscillations. While considerable progress has been made in understanding clock cell function, much remains to be elucidated regarding specific intracellular signaling pathways used in to communicate timing information throughout the body. A well known signal transduction enzyme, phospholipase C (PLC), has been implicated in the circadian system in several species and is the focus of our research. Specifically, we are interested in the PLCβ4 isomorph, as PLCβ4 knockout mice exhibit a circadian phenotype. The precise role for this enzyme in the circadian system, however, has not been adequately addressed. To gain further insight into the role of PLCβ4 in the mammalian circadian system, we initiated experiments investigating the possibility that PLCβ4 protein

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abundance cycles over the course of the day. In mice housed on a light:dark photoperiod, we found that PLCß4 levels oscillate in the mouse SCN and liver with a peak in the early night (Jenkins et al, Soc. Neurosci. Abs, 2005). The present study was undertaken to address whether this rhythm is generated by the endogenous circadian clock or driven by the external photoperiod. Specifically, we assayed the abundance of PLCß4 protein in the SCN and the liver of mice housed in constant darkness. Preliminary data indicate that in the SCN, PLCß4 protein levels are not rhythmic, suggesting the cycle previously observed is regulated by the photoperiod. Analysis of PLCß4 protein levels in the liver is currently underway.

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THE ROLE OF PLCBETA IN RETINAL SIGNALING TO THE MOUSE CIRCADIAN CLOCK
The central biological clock of the mammalian brain is located in the hypothalamic suprachiasmatic nucleus (SCN), and functions to govern the timing of an organism's physiology and behavior. The SCN is synchronized to the environment through sensory input, the most predominant signal being light. Light provides a daily timing cue by resetting the clock mechanism. While many aspects of the resetting mechanism are known, the intracellular pathways mediating photic input in the SCN are not well understood. In light of recent studies, our laboratory has been investigating a potential role for the signal transduction enzyme phospholipase C ß4 (PLCß4) in the mammalian circadian system. We hypothesize that a possible role for PLCß4 is to mediate the light signal in the SCN, in part, because it has been shown to be associated with receptors known to be involved in this response. To address this, we selectively blocked the activity of PLCß in an SCN in vitro slice preparation. We were able to completely inhibit the ability of pituitary adenylate cyclase-activating polypeptide (PACAP), a neuropeptide released onto the SCN from projections of the retina, to reset the daily rhythm of SCN firing frequency. In order to identify the particular PLCß isoform mediating this response, we are assaying the levels of PLCß1, 2, 3 and 4 in the SCN in response to a nighttime light pulse. Thus far, we observe a significant increase in PLCß4 immunoreactivity in the ventral (light-receptive region) SCN and are currently analyzing data from the three other PLCß isoforms. While our data clearly indicate PACAP resets the clock in a PLCß-dependent manner, and that PLCß4 may be a constituent of this pathway, we have not yet determined the extent to which the other PLCß isozymes are contributing to this response.

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ISOLATING THE HIPPOCAMPUS MAY LEAD TO DEFICITS IN OPERANT LEARNING TASK
The hippocampus is part of a circuit that includes the fimbria/fornix and the entorhinal cortex. Ablating any of these structures, or pathways connecting them, interrupts signal transmission between neurons, slowing or ceasing information flow and possibly leading to learning and memory deficits (Olton, 1982). We examined how hippocampal disconnection from these other structures affects rats, spatial memory and ability to learn complex sequences. After adequately learning a DNMTS task, rats received surgery in which both hippocampi were isolated from the rest of the limbic circuit. A noticeable dip in performance in the same pre-operative DNMTS task occurs immediately after recovery from surgery. Rats appear to partially relearn the task around three to four weeks post-operatively. This suggests that the disconnection succeeded in hindering the rat's ability to perform the DNMTS task.

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CHARACTERIZING PALMITOYL-PROTEIN THIOESTERASE 2 FUNCTION IN DROSOPHILA
The infantile onset form of Neuronal Ceroid Lipofuscinoses (INCL) is the earliest and most severe form of NCL, with neurological symptoms that reflect massive neurodegeneration in the CNS and retina. INCL is due to recessively inherited mutations at the CLN1 locus. This locus encodes the evolutionarily conserved enzyme palmitoyl-protein thioesterase 1 (PPT1), indicating an essential role for protein palmitoylation in normal neuronal function. There is a second conserved enzyme, PPT2, which is twenty-six percent identical to PPT1. While there is no known human NCL due to the loss of PPT2 activity, Ppt2- mice also have NCL pathology although it is distinct from that seen in Ppt1- mice. The differential phenotype along with biochemical experiments suggests that there are distinct roles of PPT1 and PPT2 in regulating palmitoylation in neurons and other cell types. To better differentiate the function of the two enzymes, we are presenting our preliminary characterization of the Drosophila ptp2 locus. Our ultimate goal is to elucidate the function of ptp2 and compare its cellular role to that of Drosophila ptp1.

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PROTEIN SYNTHESIS IS NOT REQUIRED FOR EXTINCTION OF PAIRED-ASSOCIATE ODOR DISCRIMINATIONS
Much research has shown that protein synthesis is critical for the new learning of certain types of information (e.g., Nader, Schafe, and LeDoux, 2000). However, the results from a small number of studies examining extinction and protein synthesis have produced mixed results (Lattal & Abel, 2001; Suzuki, et al., 2004). The present study examined the effect of a protein synthesis inhibitor (cyclheximide, 1 mg/kg) on extinction of memories for a paired-associate digging task (Bunsey and Eichenbaum, 1996). Rats learned that when cued with a specific initial scented cup of sand (e.g., garlic or oregano) a reward would be buried in the choice cup of a certain scented cup of sand (e.g., cinnamon or nutmeg). Rats received three days of shaping, and one day of training on the two discriminations. One day following training, rats received an extinction trial for one of the two odor discriminations (rats received either a cyclheximide or a vehicle injection before the extinction trial). One day following extinction, rats were tested for their retention of the two paired-associate discriminations. Results showed that all rats learned to dig in the correct odor and showed a strong preference for that odor during the extinction trial. Additionally, all rats showed memory for the extinction trial when tested (i.e., cyclheximide did not disrupt memory for extinction), and in fact, rats generalized their lack of responding to not only the extinguished odor discrimination, but also the odor discrimination that was not extinguished. These results indicate that protein synthesis may not be critical for extinction of an appetitive paired-associate learning task.

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EFFECT OF BINGE MORPHINE EXPOSURE ON PAIN THRESHOLDS AND OPIOID ANALGESIA IN NEONATAL AND ADOLESCENT RATS
It has been shown that a history of substance abuse can make pain management more difficult. Studies show that adult methadone maintenance populations have chronically altered pain thresholds, but no studies have been done on the effects on pain thresholds and analgesic efficacy in prenatal or adolescent opiate exposure. This study characterizes how binge% morphine exposure in neonatal and adolescent rats affects pain thresholds, exogenous morphine efficacy, and endogenous analgesia.
Starting on the day of birth (for neonates) or postnatal day 32 (P32, adolescents) morphine (3 mg/kg) was subcutaneously administered to the animals for 9 consecutive days. Saline vehicle and naive controls were used for both ages. For adolescent animals, paw withdrawal thresholds and latencies were measured every other day pre- and post-injection from P32-P40, and done a single time P42-44. For the neonates, paw withdrawal thresholds and latencies were measured during an abstinence period of P12-P49. Swim-induced analgesia tests were performed on P45 for adolescents and P50 for neonates. A morphine dose response curve was performed in the neonates on P50 as well. Morphine exposure in both neonatal and adolescent rats produced decreased thresholds and latencies. Both age groups exhibited decreased efficacy of exogenous morphine. Only the neonatal exposure decreased swim-induced analgesia. In conclusion, binge morphine exposure in both neonates and adolescents decreases pain thresholds and morphine efficacy, but only affects endogenous swim-induced analgesia as the result of neonatal exposure.

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EFFECTS OF CHRONIC MELATONIN INGESTION ON TUMOR PROGRESSION AND MOOD BEHAVIORS IN A RAT MODEL OF MAMMARY ADENOCARCINOMA

Melatonin, a hormone produced in the pineal gland primarily at night, has been shown to decrease the size, number, occurrence and growth rate of tumors as well as have anxiolytic and antidepressant properties. This study investigated if mammary adenocarcinoma can induce changes in anxiety-like and depression-like behaviors in a tumor-bearing rat model and to ascertain if melatonin can attenuate both the development and progression of mammary adenocarcinomas and the altered mood behaviors. Female Fisher 344 rats, age 8 weeks, were handled and monitored daily for food and water intake, and melatonin intake was initiated 2 days prior to tumor cell injection at a dose of 4mg/kg/day in the drinking water. Estrous cycles were also tracked daily by vaginal lavage. Animals were injected subcutaneously in a mammary pad with either serum free medium or 1.0x10^6 MTLn3 cells for tumor growth. Once significant tumor growth occurred, palpable tumor size was recorded daily. For the assessment of anxiety-like behaviors, animals were tested on the elevated plus maze 19-20 days following control or cell injections. Two days later, depression-like behaviors and the antidepressant effects of melatonin ingestion were evaluated using the Porsolt forced swim test. Results showed both circulating and brain melatonin levels were higher, even in daylight hours, in animals treated with melatonin compared with controls. Melatonin-treated animals also showed attenuated tumor progression compared with control rats. The presence of tumors induced modest increases in anxiety and depression-like behaviors in both testing paradigms, although these changes appeared to be attenuated with nocturnal melatonin supplementation consistent with the anxiolytic and antidepressant profile of this hormone.

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STARTLE RESPONSE FACILITATION BY SOCIAL ANTICIPATION

The acoustic startle eyelink response has been used in previous research as an index of fear and aversive affect. The present study investigated the effects of social encounter, evaluative feedback, and trait anxiety level on startleblink modulation in 67 healthy college participants. Startleblink responses were elicited by a 50 ms duration, 100 dB burst of noise. Participants were tested in four groups, with the control group receiving 5 blocks of 8 startle trials each. A second group experienced the same trials, but was told after the first block that they would be observed later in the experiment by a person who would be in the testing room with them (anticipation). This social evaluation encounter took place during the third block of trials. The remaining two groups experienced the same anticipation and encounter, but also received positive or negative feedback about their performance after the fourth block. Each participant’s state anxiety and emotional affect (PANAS) were measured after each block of startle trials. Results show that the anticipation of a social encounter contributed significantly to startle modulation by delaying startle habituation. However, trait anxiety, the social encounter, and the administration and type of feedback had no significant effect on startle modulation. These results demonstrate that anticipatory anxiety about a social encounter delays the habituation of the startle response, and that this startle potentiation is a state effect, not related to trait anxiety. The effect of anticipation of a social encounter is analogous to fear potentiation of startle responding.

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THE EFFECTS OF TAURINE ON HUMAN MOOD AND COGNITIVE PERFORMANCE

Taurine is an amino acid that is found naturally in the brain and sold as a dietary supplement in the United States. Although it is an active ingredient in many energy drinks (e.g., Red Bull), few studies have examined its behavioral and psychological effects. The purpose of the present study was to examine the effects of taurine on human mood and cognitive performance at a dose approximating that found in dietary supplements and energy drinks. Using a double-blind, cross-over, within-subjects design, 16 participants ingested either 1000 mg taurine or placebo, and measures of mood and cognitive performance were taken 0, 40 and 80 min after administration. Relative to placebo, taurine...
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**PRENATAL TETRAHYDROCANNABINOL (THC) EXPOSURE DISRUPTS SOCIAL AND OPEN FIELD BEHAVIOR IN MALE LONG EVANS RATS**

Marijuana is the most frequently used illegal drug among women of reproductive age, but little is known about the consequences of using marijuana during pregnancy. THC (delta-nine-tetrahydrocannabinol), one of the active chemicals in marijuana, has been shown to cross the placental barrier as well as to be present in breast milk. In this study, pregnant Long Evans rats were assigned to one of three treatment groups (THC-exposed, vehicle control, and non-treated control) on day 1 of gestation. Drug exposure consisted of 2 mg/kg of natural THC, administered twice daily by subcutaneous injection, from gestational day 1 through the entire pregnancy. Pups continued to receive drug exposure through postnatal day 10. Male rats from each group were tested starting on postnatal day 90 in a battery of tests, including open field activity, active social interaction, operant alternation with delay, and forced-swim test. Results: There were no significant differences in weight gained by dams or weight of offspring when compared to controls. THC-exposed rats showed decreased distance traveled in the inner part of the open field and an increase in investigation time in the test of social interaction compared to both control groups. Conclusions: Prenatal delta-9-THC exposure can result in increased susceptibility to anxious behavior and may impair social functioning in offspring. (Supported by NIAAA RO1 11566 to SJK and the University of South Carolina Honor’s College.)

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**THE DISCRIMINATIVE STIMULUS PROPERTIES OF THE ATYPICAL ANTI-PSYCHOTIC CLOZAPINE IN C57BL/6 MICE**

The atypical antipsychotic drug (APD) clozapine (CLZ) has increased therapeutic efficacy and reduced side effect liability compared to typical APDs such as haloperidol for the treatment of schizophrenia. Its discriminative stimulus properties have been studied in rats, monkeys and pigeons using a two-choice drug discrimination task demonstrating important cross-species differences. Consequently, Philibin et al. (2005) established a CLZ drug discrimination procedure in C57BL/6 mice using a standard two-lever operant task. Using this procedure, the atypical APDs olanzapine, ziprasidone and risperidone fully substituted the CLZ cue, while the typical APD haloperidol did not. These data suggest CLZ drug discrimination in C57BL/6 mice may differentiate between atypical and typical APDs. The goal of the present study was to further characterize this procedure in C57BL/6 mice with additional atypical and typical APDs. C57BL/6 male mice were trained to differentiate between 2.5mg/kg CLZ and vehicle in a two-lever drug discrimination task. The atypical APDs zotepine, iloperidone and melperone and the typical APDs chlorpromazine and fluphenazine were tested for the ability to substitute for the CLZ cue. CLZ substituted fully at both the 2.5 and 5.0 mg/kg doses. Zotepine, iloperidone and melperone fully substituted. The typical APD chlorpromazine fully substituted the CLZ cue. This may be due to the fact that chlorpromazine exhibits a much stronger binding affinity to 5-HT2A receptors than other typical APDs such as fluphenazine and haloperidol (Schotte et al. 1996). Fluphenazine failed to substitute. These data extend previous results suggesting the importance of 5-HT receptor mediated actions in the discriminative stimulus properties of CLZ in C57BL/6.

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**A ROLE FOR FIBROBLAST GROWTH FACTOR-2 IN XENOPUS LAEVIS RETINAL GANGLION CELL DENDRITIC ARBORIZATION**

In the developing Xenopus visual system, fibroblast growth factor (FGF-2) has been implicated in retinal cell fate determination as well as retinal ganglion cell (RGC) axon pathfinding and target recognition, but FGF’s role in dendritic arborization is unknown. RGCs express FGF-2 receptors (FGFRs) and RGC axonal pathfinding is known to be sensitive to FGF levels in the brain. Recent in vivo and in vitro experiments have suggested that exogenous application of FGF-2 may affect the determination of RGC dendritic morphology by enhancing branching. To elucidate the role of FGF-2 in RGC dendritic arborization further, we impaired the function of the FGF-2 receptors (FGFRs) by applying the FGFR inhibitor DMBI into the retinæ of Xenopus laevis embryos at stage 38, when RGC dendritic arborization is beginning. At stage 45, when dendritic arborization is well underway, we visualized RGC dendritic arbors by retrograde transport of fluorescent rhodamine dextran injected into the target. RGC dendritic morphologies were then imaged with confocal microscopy. Preliminary results suggest that inhibiting the FGFRs significantly alters dendritic morphology, strengthening the hypothesis that FGF-2 may play a role in dendritic arborization.

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**EFFECT OF A NEONATAL VENTRAL HIPPOCAMPAL LESION ON WORKING MEMORY IN RATS**

Patients with schizophrenia have deficits in spatial working memory, a function dependent on the hippocampus and its target structures. Post-mortem analyses of schizophrenic brains have shown pyramidal cell disarray in the hippocampus. We used the neonatal ventral hippocampal lesion (NVHL) rat model of schizophrenia to determine if spatial working memory is disrupted in two radial arm maze tasks. In the non-delayed random foraging (NDRF) task, rats were required to forage for sucrose pellets in 4 arms of the 8-arm maze without re-entering any arms. In the spatial delayed win-shift (SDWSh) task, rats were first given a training trial in which 4 arms were baited and 4 were blocked. In the test trial, rats were required to enter only the 4 previously blocked arms, after either a 5 or 30 min delay. Rats were also tested for prepulse inhibition (PPI) of the acoustic startle response. Lesioned and sham-treated rats acquired the NDRF task in the same number of days, and did not differ on any behavioral measures on this task. However, lesioned rats took longer than sham rats to acquire the SDWSh task, as measured by days to criterion performance, at both the 5 min and the 30 min delay. Lesioned rats also exhibited a deficit in PPI, indicating impaired sensorimotor gating. These results suggest that the NVHL model reproduces some of the working memory and sensorimotor gating abnormalities observed in patients with schizophrenia. However, spatial navigation impairments are limited to task conditions which place higher demands on working memory.

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**FEMALE AVOIDANCE OF MALE SCENT IN THE MUSK SHREW (SUNCUS MURINUS)**

The Musk shrew, Suncus murinus, is a small carnivorous animal, which feeds primarily on insects. The shrew is unique in that the
under these conditions, the effects of various doses of cocaine were examined in the conditioned place preference procedure and in an open-field test of locomotor activity. In the conditioned place preference procedure, both low (5 mg/kg) and high (10 mg/kg) doses of cocaine produced a place preference in both groups; however, enriched rats were significantly more sensitive to these effects than isolated rats. Similarly, cumulative (3, 10 and 30 mg/kg) doses of cocaine increased open-field locomotor activity in both groups of rats, but enriched rats were significantly more sensitive to these effects. To our knowledge, this is the first demonstration that environmental enrichment enhances sensitivity to the locomotor and rewarding effects of cocaine in female rats. These data suggest that social and environmental manipulations may alter dopamine transporter function in females, which may in turn have functional consequences for sensitivity to drugs of abuse. Support Contributed By: Davidson College and US Public Service Grant DA14255

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EFFECTS OF VALERIANA OFFICINALIS ROOT EXTRACTS ON [3H]FLUORORWILLARDINE BINDING IN RAT SYNAPTIC MEMBRANES

Valeriana officinalis root extracts are used primarily as mild sedatives in the treatment of insomnia and anxiety. The goal of the present in vitro study was to determine the pharmacodynamic mechanism(s) by which valerian whole-root extracts induce sedation in vivo. We examined the interaction of ethanolic and aqueous valerian root extracts with alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in the presence of 15mM of the AMPA agonist [3H]fluorowillardiine (FW). The interaction between valerian root extracts and AMPA receptors appeared to depend on the solvent, concentration, and age of the extracts. Both fresh aqueous and fresh ethanolic extracts competitively bound to AMPA receptors, although more binding was detected in the aqueous extract conditions, and this effect was positively correlated with the concentration of the extract. When not in use, the extracts were stored at 0-4°C. In 2-day old extracts, an interaction with AMPA receptors was detected only in the undiluted aqueous valerian extract. It is possible that protein degradation or time-dependent changes in the extract compositions confounded the results of the study. Therefore, more research is necessary before details of the interaction of valerian root extracts with AMPA receptors can be determined.

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PATTERNS OF TAIL WITHDRAWAL RESPONSES TO HEAT STIMULI IN CRESTED GECKO LIZARDS

Previous results in rats (Cleland and Bauer J. Neurosci.- Rapid Comm. 22:5265-5270, 2002) revealed that pinpoint heat stimuli applied to the tail evoked withdrawal responses whose direction depended on the location of the stimulus. Although responses were generally directed away from the stimulus, there was uniformly a deviation in the dorsal direction. This pattern of response, in which responses are largely dorsal and rarely ventral, makes functional sense for rats because they are terrestrial. These results raise two questions: 1) whether other terrestrial animals, such as lizards, exhibit similar patterns, and 2) whether arboreal lizards exhibit patterns of response different from terrestrial lizards. Terrestrial (crested gecko) and arboreal (long-tail grass, schneider skink) lizards were restrained vertically. Heat stimuli were applied with a laser to 8 locations circumferentially around the tail. Vision and three-dimensional high-speed video (250 frames/s) were used to determine the direction of tail response. Results revealed that both arboreal and terrestrial lizards responded primarily with lateral tail movement.
regardless of stimulus location. The lack of dorsal and ventral movements did not arise from an inability of the lizard to move its tail in those directions, which was especially evident in crested geckos which use ventral curling of their tail to move among branches. In conclusion, although lizards differ from rats in their preponderance of lateral movements, both rats and lizards share a bias against ventral movements.

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PHYSIOLOGICAL EFFECTS OF LESION-INDUCED HIPPOCAMPAL SPROUTING: THE EMERGENCE OF THE CAPACITY FOR LONG-TERM POTENTIATION IN THE CROSSED TEMPORODENTATE PATHWAY

Research has shown that recovery of function after brain trauma results from reorganization in the central nervous system. There is strong evidence to suggest that axonal sprouting is the mechanism by which denervated brain regions become re-innervated after injury. It remains unclear, however, how these lesion-induced modifications of hippocampal circuitry alter the physiological functions of the pathway in which they are found. The purpose of this study was to investigate the emergence of the capacity for long-term potentiation (LTP) in the crossed temporodentate (CTD) pathway, which sprouts in response to unilateral entorhinal cortex (EC) lesion. High-frequency stimulation (eight trains of eight pulses at 400 Hz) was used to elicit LTP in the CTD twelve days after male, Sprague Dawley rats received a one-stage, progressive, or priming lesion of the EC. Results indicate that the capacity for LTP, defined as a 15% or more increase in the amplitude or left rising slope of evoked field potentials, is not statistically significant in any group twelve days post-lesion. These results suggest that LTP is not responsible for the recovery of working memory because it does not coincide temporally with behavioral recovery after unilateral EC lesions.

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THE EFFECTS OF METHYLENE BLUE ON RAT PERFORMANCE IN THE 8-ARM RADIAL MAZE

Methylene Blue, commonly used to identify nerves because of its oxidative reductive properties, has also been used as a clinical treatment for methemoglobinemia. Recent studies have also shown that methylene blue can facilitate memory through an increase in brain oxygen utilization. However the specific type of memory-enhancement has not been identified. The current study investigates the type of memory that is affected by this agent. The performance of Sprague-Dawley rats that were injected with methylene blue following training on the 8-arm radial maze was compared to the performance of saline-treated controls. We hypothesized that rats treated with methylene blue would perform better than rats that were treated with saline.

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DRUG DISCRIMINATION WITH THE ATYPICAL ANTIPSYCHOTIC CloZAPINE IN C57BL/6 MICE

The atypical antipsychotic drug (APD) clozapine (CLZ) has increased therapeutic efficacy and reduced side effect compared to typical APDs such as haloperidol for the treatment of schizophrenia. The discriminative stimulus properties of CLZ has been studied in rats, monkeys and pigeons that demonstrate important cross-species differences. Consequently, Philibin et al. (2005) established a CLZ drug discrimination procedure in C57BL/6 mice using a standard two-lever operant task. Using this procedure, the atypical APDs olanzapine, ziprasidone and risperidone fully substituted for the CLZ cue, while the typical APD haloperidol did not. These data suggest CLZ drug discrimination in C57BL/6 mice may differentiate between atypical and typical APDs. The goal of the present study was to further characterize this procedure in C57BL/6 mice with additional atypical and typical APDs. C57BL/6 male mice were trained to differentiate between 2.5mg/kg CLZ and vehicle in a two-lever drug discrimination operant task. The atypical APDs zotepine, iloperidone and melperone and the typical APD chlorpromazine and fluphenazine were tested for the ability to substitute for the CLZ cue. CLZ produced full generalization at both the 2.5 and 5.0 mg/kg doses. The atypical antipsychotics zotepine, iloperidone and melperone fully substituted for CLZ. However, while the typical antipsychotic fluphenazine failed to fully substitute for CLZ, the typical APD chlorpromazine produced full substitution for the CLZ cue. This may be because chlorpromazine exhibits a much stronger binding affinity to serotonin (5-HT)2A receptors than other typical APDs such as fluphenazine and haloperidol (Schotte et al. 1996). These data extend previous results suggesting the importance of 5-HT receptor mediated actions in the discriminative stimulus properties of CLZ in C57BL/6 mice.

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IMPULSE: AN ONLINE, INTERNATIONAL JOURNAL FOR NEUROSCIENCE REPORTS WRITTEN, EDITED, AND PUBLISHED BY UNDERGRADUATES

IMPULSE is an online journal created by undergraduate students interested in neuroscience and/or publishing. It is hosted at the University of South Carolina, but remote reviewers are from universities around the world. The first issue was posted in 2004 (see Soc. Neur. Abs. (2003) 29:25.3 and Soc. Neur. Abs.(2004) 30:28.6). The journal shifted to rolling submission format in 05 (see Soc. Neur. Abs. (2005) 31:20.19). Currently, 2006 is open with one article posted and four others under review. After a slow start, interest in using this mechanism for publishing undergraduate research and reviews is gaining momentum, with submissions increasing each year. The review team has turned over, with the founding editorial team graduating and being replaced by a new editorial team and review team. The reviewers all take a Scientific Publishing course, which seems to help build confidence and a sense of community among team members. While the journal is accomplishing its purpose locally of fueling interest in neuroscience and teaching students about publishing in the sciences, there are several continuing challenges. A larger, regular influx of submissions is needed, with more reviewers from other institutions, both in North America and abroad, to fulfill the goals of serving that community as an outlet for publishing and a mechanism for encouraging young, international scholars to build the future global community of neuroscientists. Supported by the South Carolina Honors College, USC.

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EFFECTS OF SHORT TERM STRESS, CHRONIC STRESS, AND RECOVERY TIME ON COMMUNICATION BETWEEN THE IMMUNE SYSTEM AND THE NERVOUS SYSTEM

Numerous studies have highlighted the close relationship between stress and immunosuppression. Chronic stress in particular is known to cause severe suppression of basic immune functions. Bidirectional pathways of communication between the immune system and the nervous system are responsible for immunosuppression following stress. These facts become especially important in today’s fast paced society, where people are taking on more and larger responsibilities than ever before. The present study uses a rodent model to examine the effects of short term stress and chronic stress on immune function, and explores the concept of recovery time and its ability to aid in
recuperation of proper immune function. Four cohorts, each containing four Swiss Webster male mice, were assigned randomly as follows: short term stress (ST), short term stress plus one week recovery time (ST+R), chronic stress (CS), and chronic stress plus one week recovery time (CS+R). Restraint and visual stress were used to create both short term and long term stress conditions. Blood was collected from each mouse via submandibular vein puncture. Blood was used to analyze peripheral lymphocytes. It will also be used in a corticotrophic assay in the future. Corticotrophin hormone is the primary communicator in the hypothalamic pituitary adrenal (HPA) axis which links the immune and nervous systems. The spleen was also harvested from each animal and used to analyze splenic lymphocytes and macrophages. At this time, data has only been collected from the ST group. However, statistical analysis will be performed to compare all four cohorts and to determine if recovery time has an effect on immune function. It is expected that chronic stress will cause greater immunosuppression than short term stress, and that recovery time will help reverse the effects of immunosuppression in the ST+R group. However, the effects of recovery time on the CS+R group are more difficult to predict. This study hopes to highlight the severe immunologic effects of chronic stress in relation to short term stress, and the importance of recovery time for restabilization of the HPA axis as well as recovery of proper immune function.

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MANIPULATION OF OPERANT DELAYED ALTERNATION PERFORMANCE BY MUSCARINIC AND GLUTAMATERGIC ANTAGONISM

The current study employed an operant delayed alternation task to assess working memory in rats. Once the subjects’ baseline performance was stable, the effects of an NMDA and a muscarinic antagonist were examined. Specifically, the effects of the non-competitive NMDA antagonist ketamine (3.0 mg/kg and 10.0 mg/kg ip) and the muscarinic cholinergic antagonist scopolamine (0.3 mg/kg and 0.5 mg/kg ip) on working memory were examined. Ketamine at 10.0 mg/kg caused a decrease in accuracy in the no-delay condition, but not under the delay condition, while 3.0 mg/kg ketamine had no discernable effects at either delay interval. However, this difference was accompanied by increases in the average trial latency, suggesting that nonspecific effects of the drug may have been involved. Scopolamine produced deficits at both doses and both delay conditions in percent accuracy in this task, with no accompanying increase in latency. Thus, these preliminary data suggest that the muscarinic cholinergic system is important for working memory as assessed by this task. However, the role of the glutamate system is unclear, based on these results.

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EFFECTS OF THE ACTIN INHIBITOR CYTOCHALASIN ON XENOPUS RETINAL GROWTH CONE EXTENSION RATES IN VITRO

Growth cones are the highly motile structures at the end of extending neurons that sense environmental cues and determine how and where an extending axon navigates to its synaptic target. Growth cones have two essential parts: the thicker lamellapodia, which is composed primarily of microtubules, and the filopodia, highly motile extensions emanating from the lamelapodia and contain actin filaments. Previous research has shown that inhibiting actin polymerization in vivo impairs retinal ganglion cell (RGC) growth cone navigation in the developing Xenopus visual system. We investigated how the actin inhibitor cytochalasin B affected Xenopus growth cone extension rates in vitro. We prepared explant retinal cultures from stage 24-28 tadpoles, and active growth cones were visible 16-24 hours later. We used time-lapse imaging to observe retinal growth cone dynamics for 30 minutes to establish baseline growth cone extension rates. After 30 minutes of observation, we then introduced either the actin inhibitor cytochalasin B, or DMSO, the solvent in which cytochalasin was dissolved, and continued imaging growth cone dynamics for another hour. Preliminary results indicate that cytochalasin may slow growth cone extension rates in vitro and that further investigations are warranted.