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Symposium for Young Neuroscientists and Professors of the Southeast

Abstracts

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Nicotine Facilitates Learning In Zebrafish, *Danio rerio*

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Losses in memory have been a devastating reality for millions of Americans, especially with modern increases in age and the onset of neurodegenerative diseases such as Alzheimer's disease. Studies have shown that in many model systems, nicotine increases memory. A recent addition to the group of standard models is the zebrafish Danio rerio, a small fish used traditionally in the studies of developmental biology and genetics. In our study, the fish were tested using a rapid-conditioning test to observe learning of side-preference in a tank. Previous studies used the salt nicotine ditartrate in their tests; however, in order to standardize results, this study used pure nicotine. Results revealed a dose-dependent curve, with optimum concentrations providing higher learning than control fish showed. We also determined that long-term exposure to nicotine produced results no different from fish not exposed to nicotine, suggesting the development of tolerance to the chronic presence of nicotine.

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The effect of perinatal DHT and E2 on copulatory behavior in the female musk shrew

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Testosterone (T) has three metabolic pathways: it can bind via androgen receptors, be aromatized to estradiol (E2, which acts via estrogen receptors) or it can be irreversibly reduced to dihydrotestoterone (DHT). Data suggest that altricial rodents require aromatization of T to E2 to differentiate copulatory behavior, while in primates rely more on the DHT pathway (Wallen and Baum, 2002). We studied the female musk shrew, an altricial insectivore in which female copulatory behavior is regulated by T. On postnatal days 1-5, female pups were injected with DHT, E2 or sesame oil vehicle. At 2-4 months, all animals were ovariectomized and implanted with small T capsules to ensure male-typical activational hormone levels. During behavior testing, the experimental shrew was placed in a test box with a stimulus female and observed for two 60-minute copulatory behavior tests by an experimenter blind to hormone treatment. After performing a 3X2 ANOVA (hormone treatment by trial), we found that there was a significant difference between DHT vs. E2 and DHT vs. controls in time to initiate sexual behavior and time until first mount. However, no significant differences were found with hormone treatment in total number of mounts, and overall mounting rate was less than half that previously reported in shrews injected with prenatal T (Freeman et al., 1998). Thus, the data suggest that while DHT may be an effective masculinizer in the initiation of male copulatory behavior, both metabolites may be necessary in the musk shrew to fully differentiate sexual behavior.

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Tetrodotoxin Increases Retinal Ganglion Cell Neurite Branching in vitro

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Retinal ganglion cells (RGCs) are the only neurons that relay visual input from the eye to the brain. RGC axons form the highly organized optic nerve, which innervates the optic tectum of the midbrain in Xenopus laevis. Neuronal activity is involved in RGC axon refinement via NMDA-receptor-mediated mechanisms, implicating visual cues in the development of retinotopic connections. Moreover, RGCs are spontaneously active even before their axons synapse with tectal neurons. Thus we investigated the role of neuronal activity on initial RGC morphological differentiation by exposing RGCs to tetrodotoxin (TTX), which inhibits voltage-gated sodium channels, reducing neuronal activity. Xenopus retinal neurons

were dissociated and cultured for three hours. Cultures were then randomly assigned to one of three experimental conditions: (1) Development in the absence of TTX (control), (2) low TTX (1 uM), and (3) high TTX (10 uM). Cultures were fixed six hours later and immunostained to identify RGCs. Fluorescence microscopy was used to image and quantify neurite length and branching. Neurons reared in the presence of TTX exhibited a significant dose-dependent increase in branching without affecting total neurite length or number of primary neurites. This increase in neurite branching in TTX-treated neurons suggests that neuronal activity plays an early role in RGC morphological differentiation. Similar enhancements in axon branching have been observed in TTX-treated Xenopus RGC axons in vivo. Thus, neuronal activity plays a role in determining the shape of Xenopus RGCs.

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Sex Difference in Spatial Learning Found in Mice via Paddling Pool Maze

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The Paddling Pool Maze (PPM) has been proposed as an alternative to the Morris Water Maze (MWM) as a more suitable measure of spatial learning in mice (Deacon, 2004). The PPM is a circular, shallow water maze with up to 12 possible escape tubes on the perimeter (Fugger et al, 1998). Since males mice show better spatial learning than females in the MWM we hypothesized that they would show a similar sex difference in the PPM. During 8 training trials we gave the mice a 3-minute opportunity to find the only open escape route out of the 12 on the perimeter of the pool. We found no sex difference in escape time during training, $F(1,20) = 1.28$, $p = 0.27$. We moved the exit 120 degrees and gave the mice 3 probe trials. During the probe trials, females found the new exit significantly faster than the males, $F(1,20) = 17.179$, $p = 0.001$. Subsequent testing found that there was no sex difference in A) thigmotaxis in open field conditions, B) preference for a dry platform over water, and C) preference for entering a dark tube. Thus, we concluded that the sex difference could be attributed to spatial learning with males having stronger memory for either intra- or extra-maze cues, making it harder for them to learn the location of the new exit.

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Long Term Exposure to Voluntary Exercise Decreases Cocaine's Reinforcing Efficacy

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Exercise has been shown to improve general physical and mental health and has been implicated as a potentially effective alternative to drug use. A handful of previous studies have shown that exercise impacts the self-administration of drugs. The present study examined whether chronic, voluntary exercise influenced the self-administration of cocaine using a progressive ratio schedule of reinforcement. Female, Long-Evans rats were obtained at weaning and separated into an exercise condition in which the home cages were modified with an exercise wheel attached or a sedentary control condition without an exercise wheel. Following 6 weeks in these conditions, rats underwent surgeries to implant intravenous jugular catheters and were tested in the cocaine self-administration procedure. After a period of acquiring the lever-pressing response on a fixed-ratio 1 (FR1) schedule, breakpoints on a progressive ratio schedule of reinforcement were determined for multiple doses of cocaine (0.0, 0.3, and 1.0 mg/kg/infusion) and compared between the two groups. Whereas the two groups did not differ in the number of days necessary to acquire the task on the FR1 schedule, the exercise group responded significantly less than the sedentary group for cocaine on the progressive ratio schedule at the 0.3 and 1.0 mg/kg/infusion doses. These results indicate that exercise decreases the reinforcing efficacy of cocaine and may be useful in therapeutic settings to decrease the likelihood of addiction.

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Changes in Cutaneous Peptidergic and Nonpeptidergic Nociceptors Associated with Fetal Ethanol Exposure

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As fetal alcohol syndrome disorder (FASD) occurs in 1-10 in 1000 births, there is a need for the complications of this disorder to be better understood. In particular, an animal model from our research group has shown that the peripheral neuropathy resulting from fetal alcohol exposure is characterized by decreased sensitivity to non-noxious mechanical stimuli and increased sensitivity to noxious thermal stimuli. It is thought that perhaps fetal alcohol exposure interferes, delays, or even inhibits proper peripheral nerve development and mylineation; however, the mechanism for this difference and its sensory consequences is not understood. In this study, we set out to examine the difference in the peripheral nerve endings in the skin of rats on a cellular level using immunohistochemistry. Specifically, we used the following antibodies: the PGP 9.5 which is a pan marker for cutaneous nerve endings, Isolectin B4 which stains for non-peptide

containing, pain-sensing nerve endings, CGRP (Calcitonin Gene Related Peptide) which stains for peptide containing, pain-sensing nerve endings, and Neurofilament 200 which stains for A-Beta afferent, large diameter myelinated touch fibers. Preliminary results suggest a decrease in the PGP 9.5 immunoreactivity, and an increase in both CGRP immunoreactivity and IB4 binding in the animals exposed to alcohol versus the controls. This suggests a decrease in peripheral nerve endings in general, and an increase in both peptide and non-peptide containing pain-sensing endings in the skin. These changes are likely to have a profound impact on how children with fetal alcohol exposure experience sensations like touch and pain.

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Enhanced Capsaicin-Induced Thermal Hyperalgesia and Neuronal Activation in Fetal Alcohol Exposed Rats

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In 9-10 out of every 1,000 human live births, the infant is born with fetal alcohol spectrum disorder (FASD). Among the many effects of FASD is increased sensitivity to painful thermal stimuli. We hypothesize that fetal alcohol exposure causes this alteration in sensitivity via changes in the activity of small diameter, unmyelinated primary afferent neurons that respond to temperature (C fibers). To test our hypothesis, we used a rodent model of FASD and examined responses to thermal stimuli and neuronal activation following application of the C fiber agonist capsaicin. Capsaicin is responsible for the "hot" taste of chili peppers. On postnatal day 21, an injection of 20μl of capsaicin was administered subcutaneously in the left plantar hind paw, activating C fibers. Ethanol-exposed animals exhibited increased capsaicin-induced thermal hyperalgesia as compared to control animals. Two hours post-injection, animals were perfused and spinal cords were isolated. Spinal cords were sectioned at 30μm and processed for c-fos using immunohistochemistry. C-fos is a marker of neuronal activity; higher levels of c-fos in the dorsal horn corresponded to greater neurotransmission from peripheral C fibers. We found an increase in the number of c-fos positive neurons in the superficial dorsal horn of ethanol-exposed rats as compared to control rats. These findings suggest that fetal ethanol exposure increases pain sensations that result from the activation of C fibers expressing the capsaicin receptor. Understanding the physiological basis of the FASD-induced increase in sensitivity to painful thermal stimuli is a fundamental first step in developing treatments for this disorder.

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Using Herpes Simplex Virus-1 to Over-express the Mu-Opioid Receptor in Afferent Neurons

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Neuropathic pain is a chronic condition that millions suffer with on a daily basis. Opioids are often administered at high dosages to help with the symptoms associated with chronic neuropathic pain. In this project, Herpes Simplex Virus-1 (HSV-1) was used to over-express the mu-opioid receptor (mOR) in primary afferent neurons. Four groups were included: a control virus encoding the E. Coli LacZ gene (SGZ), a HSV-1 encoding cDNA for mOR (SGMOR), a HSV-1 encoding cDNA for preproenkephalin (KPE), and a combination of the SGMOR and KPE viruses. HSV-1 viral constructs were administered to the left hindpaw via topical inoculation. Spinal cords were collected at 4 weeks post-infection to examine expression of the mOR using immunohistochemistry. Preliminary results show that when compared to the SGZ infected control group, the SGMOR infected mice have double the density of mOR immunoreactivity in the dorsal horn of the spinal cord. This increase in mOR immunoreactivity was observed in lamina I-III as compared to a more limited expression in lamina I-II in SGZ control infected mice. Also, infection with SGMOR+KPE virus increases mOR immunoreactivity in lamina I-III of the spinal cord. These results suggest that HSV-1 mediated viral vectors can increase expression of mOR in primary afferent neurons and may be used to enhance opioid analgesia in the treatment of chronic neuropathic pain.

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The Effects of Acute verse Chronic Alcohol intake on Brain Glucose Utilization in Cannabinoid 1 Receptor Knockout Mice

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Alcohol has a number of physiological effects, none greater than its abuse potential. Studies have linked the behavioral effects of alcohol, including its abuse, with the brain's CB1 receptor - a part of the endocannabinoid system. The CB1 receptor is the most abundant neurotransmitter receptor in the brain and is localized in several areas, including the nucleus acumbens, amygdala, thalamus, superior colliculus, periaqueductal gray, and rostral ventromedial medulla. In this

study, access to water for both the CB1 knockout (KO) and Swiss Webster (SW) mice was gradually decreased to one hour per day to simulate binge drinking. Animals were assigned to one of three doses: control (0 g/kg EtOH), low (0.5 g/kg EtOH), or high (1.5 g/kg EtOH). In the acute study, mice had access to ethanol only once (test day), while for the chronic study, they drank the same dose everyday for one month. On test day, mice were given 30 μCi of tritiated-2-deoxyglucose ([3H]2-DG) via the lateral tail vein immediately prior to them given access to ethanol for one hour. After the hour, blood glucose and EtOH concentrations were taken; the brains were extracted and later imaged using a Beta Imager. The acute study revealed that CB1 KO mice had significantly lower [3H]2-DG binding than their SW counterparts in both the striatum and hippocampus, two areas associated with the abuse of alcohol. Results from the chronic study are currently being analyzed and will be compared to the acute study. These results will be extremely valuable in the mapping of ethanol abuse pathways.

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An Investigation of Cadmium Effects on Mitochondria Isolated from Mouse Brain

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Cadmium (Cd) is a relatively abundant environmental contaminant. Accumulation of Cd in nervous tissue causes neuropathy and while its cytotoxic effects are well-documented, it is unclear exactly how Cd kills cells. One potential mechanism involves inhibition of cellular energy production. In this study, we used fluorescence microscopy to monitor the effects of Cd on mitochondrial transmembrane potential (∆ψm) in individual mitochondria isolated from mouse brain. Mitochondria were adhered to microscopy glass and loaded with rhodamine 123, a fluorescence indicator that collects in energized and respiring mitochondria with a robust ∆ψm. We found that Cd at relatively low concentrations quickly and irreversibly dissipated ∆ψm. Cd mitotoxicity was relatively potent and efficacious when compared to two other well-characterized mitotoxic metals, Ca and Zn. These results demonstrate that Cd can substantially inhibit mitochondrial function, and provide important insight regarding the mechanism of Cd-mediated neurotoxicity.

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A dose-response analysis of methylphenidate locomotor sensitization in adolescent D2-primed rats

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Past studies from our laboratory have shown that neonatal quinpirole (dopamine D2/D3 agonist) treatment produces increases in dopamine D2 receptor sensitivity that persists throughout the animal's lifetime, a phenomenon known as D2 priming. A common drug typically used to medicate attention deficit-hyperacivity disorder (ADHD) is methylphenidate (Ritalin), a drug that has stimulant properties and abuse potential. In this study, we analyzed whether male and female adolescent rats neonatally treated with quinpirole will demonstrate locomotor sensitization to methylphenidate (MPH) when this drug is administered in adolescence. Rats were administered quinpirole from postnatal days (P)1-21. Beginning on P33, male and female rats were administered one of three doses of methlphenidate (MPH; 1, 3, or 5 mg/kg) or saline beginning on P33 every other day through P49. Results showed that females an approximate 100% increase in locomotor activity to the 5 mg/kg dose of MPH compared to males, and non D2-primed females demonstrated sensitization to the highest dose of MPH (5 mg/kg) and D2 priming blocked this sensitization. The lowest dose (1 mg/kg) produced locomotor suppression in females compared to controls. In males, both the 1 and 3 mg/kg of MPH produced locomotor suppression compared to controls, and males did not demonstrate sensitization to any of the three doses. Additionally, neonatal drug treatment did not affect the locomotor response to MPH in males. These results show that females are more sensitive the locomotor activating effects of MPH than males, whereas males are more sensitive the locomotor suppressing effects of MPH than females.

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Fluorescence Detection of MAO Activity in Mitochondria Isolated from Mouse Brain

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Monoamine oxidase (MAO) enzymes degrade dopamine, serotonin, and norepinephrine and other monoamine neurotransmitters, and are important targets in the management of depression and psychiatric disorders. The two major isoforms, MAO-A and MAO-B, are both associated with the outer mitochondrial membrane, but their distribution varies depending on tissue. Specifically, MAO-A is abundant in liver, while both are found in brain. In this study, we developed a plate reader-based, fluorescence assay to detect MAO activity in mitochondria isolated from mouse brain. We used the

indicator amplex red to monitor H2O2 production resulting from the oxidation of benzylamine and tyramine, and we tested MAO activity in the presence of various inhibitors. We have used these results to produce a relatively efficient and simple means of assaying MAO activity in a high-throughput fashion.

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A Comparison of Reactive Oxygen Species Production in Mitochondria Isolated from Mouse Brain and Liver

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Mitochondria are a major source of reactive oxygen species (ROS), which includes free radicals such as superoxide and non-radicals such as hydrogen peroxide. At controlled levels, ROS participate in cell signaling and are probably beneficial. However, excess ROS are cytotoxic, and are thought to contribute to numerous clinical pathologies such as Parkinson's disease and amyotrophic lateral sclerosis (ALS). In the present study, we investigated ROS production in mitochondria isolated from mouse brain and liver using the fluorescence ROS sensor Amplex Red in a spectrofluorophotometer-based assay. Pharmacological manipulations revealed that ROS production in brain occurs by substantially different mechanisms compared to liver. Specifically, brain mitochondria produce ROS at high levels when supported by substrates metabolized by complex II of the electron transport chain, but ROS production was low when complex I substrates were used. In contrast, liver mitochondria produced ROS at relatively high levels regardless of substrate conditions. Additionally, brain ROS production was partly reliant on an intact transmembrane potential, whereas liver mitochondria produced ROS regardless of the transmembrane potential. Our results demonstrate that ROS production is differentially regulated in mouse brain and liver and that mitochondrial ROS production varies between different tissues.

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Histological and Behavioral Analysis of rAAV9-induced Rodent Model of Taupathy in Alzheimer's Disease

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One of two hallmark features of Alzheimer's Disease is tau neurofibrillary tangles. The project seeks to develop an accurate rodent model of AD taupathy, which can be used in the future to develop drugs that prevent tau pathologies in the brains of human Alzheimer's patients. The hippocampus is implicated in memory formation and one of the first brain areas affected in Alzheimer's Disease. Therefore, brain surgery targeting the hippocampus was performed on 20 male Sprague-Dawley rats. Ten rodents received 4 hippocampal injections (3 µl per injection) of replication-deficient adeno-associated viral vector serotype 9 (rAAV9) containing the gene for human tau with mutation P301L, which predisposes tau protein to hyperphosphorylation, increasing its tendency to aggregate into tangles. Ten control rodents received 4 hippocampal injections (3 µl per injection) of rAAV9 containing the gene for green fluorescent protein (GFP), a protein from the jellyfish Aequorea Victoria that naturally fluoresces under blue light and that reports expression. rAAV9s were obtained from the lab of Dr. Ronald Klein, Louisiana State University. Multiple subjects were tested daily on a Y-maze alternation task for six months. Preliminary findings indicate that spatial memory deficits occur in the group with the mutated tau gene. Throughout data collection, the researchers were blind to the conditions. Histology testing to confirm GFP and tau presence is ongoing in collaboration with Dr. Michael King, University of Florida.

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Progesterone Pretreatment Selectively Attenuates Reinstatement of Cocaine-Seeking in Estrous Female Rats

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Clinical research indicates a role of gender differences in mediating certain aspects of cocaine addiction, specifically, the rate at which dependence develops from casual use and the length of time spent abstaining from drug use. Preclinical studies have examined cocaine addiction with rat models of cocaine self-administration. These studies support the findings of clinical research and show that female rats respond at higher rates than males during self-administration, early extinction, and cocaine-primed reinstatement. These differences in behavior of cocaine use have been correlated with the female estrous cycle and plasma progesterone levels. Higher levels of progesterone during the proestrus phase correspond with decreased cocaine seeking behavior, whereas lower levels of progesterone during the estrous phase yielded higher cocaine-seeking behavior. Therefore, based on this inverse relationship between progesterone levels and cocaine seeking, the current study hypothesized that pretreatment with progesterone should yield diminished cocaine seeking behavior. Female Sprague-Dawley rats received jugular catheter surgery and were subsequently trained to self-administer cocaine (0.5 mg/kg per infusion). Cocaine seeking behavior was measured as a function of the rate of

responding and the number of lever presses emitted by the rats in order to receive cocaine infusions during 2 hour daily sessions of self-administration. After the self-administration phase, the cocaine reinforcer was removed and rats no longer received infusions of the drug following lever pressing. This extinction phase continued for each rat until the rat extinguished the lever pressing behavior to a set criterion (i.e. 25 lever presses for 2 consecutive days). Following extinction, rats received an injection of cocaine (10 mg/kg, IP) before reinstatement testing began. To assess the effects of progesterone pretreatment on cocaine-primed reinstatement behavior, either progesterone (2 mg/kg, SC) or vehicle was administered to rats at 20 hours and 2 hours before reinstatement testing began. Responding on the active lever was measured throughout each of the three phases of the experiment. Vaginal smears and blood samples were collected during the extinction and reinstatement phases in order to determine estrous phase and measure plasma progesterone levels, respectively. This study found that females in the estrous phase of their cycle displayed increased responding on the previously cocaine-paired lever during early extinction and reinstatement. Furthermore, female rats in estrus, but not the other phases of the estrous cycle, who received progesterone pretreatment displayed attenuated lever responding during testing. The clinical applications of the results from this study may provide a better understanding of relapse in women in that it may depend on progesterone levels present during different phases of the menstrual cycle. Therefore, this research study could contribute to the development of a progesterone treatment strategy for women who are susceptible to cocaine relapse.

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An Evaluation of the Health Belief Model as Applied to Helmet Use in College Undergraduates

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abstract.: Helmet wearing practices and attitudes about helmet use were examined in 274 undergraduates who bicycle (Mean Age = 19.5 years, 78% females, 87% Caucasian). Participants completed a 57-item scale developed to assess components of the Health Belief Model (HBM; Rosenstock, 1974) and other items assessing demographics, bicycling habits and helmet use. We examined hypotheses that compared helmet wearers vs. non-wearers along dimensions of the HBM. According to the HBM, perceptions of vulnerability to injury and the severity of consequences associated with injury should relate positively to helmet use as do perceptions about the perceived benefits and exposure to cues to action. In contrast, perceived barriers are hypothesized to relate negatively to helmet use (Rosenstock, 1974). Consistent with previous research (e.g., Coron & McLaughlin, 1996), only 12.4% of the sample reported regular helmet use while bicycling. Overall, helmet users differed from non-users on HBM subscales [Omnibus F (10,197) = 20.63; p.< .001; Eta-squared = .512]. Consistent with model predictions, follow-up ANOVAs revealed group differences on all HBM subscales. These findings highlight the utility of the HBM in explaining helmet usage among college students and may help explain why traditional advertising campaigns or health promotion events are not more effective. Our findings highlight the need for interventions that inoculate individuals against negative peer pressure, encourage parents and significant others to insist on helmet wearing beyond childhood, and emphasize the emotional benefits of helmet use in addition to the risks associated with bicycling without helmets.

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UNDERGRADUATE NEUROSCIENCE EDUCATION: USING IMPULSE AS A TEACHING TOOL

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The journal IMPULSE is in its fifth year of publishing articles both written and peer reviewed by undergraduates. Started in 2003 (Soc. Neur. Abs. 29:25.3; first issue 2004, Soc. Neur. Abs. 30:28.6), the journal provides an opportunity for students to publish their original research and review articles. It also offers undergraduate neuroscience faculty a mechanism to mentor their students through an authentic publishing experience, where the students write their own manuscript and experience the subsequent submission, peer review, and revision process. Further, faculty wanting to train students in the review process can work with IMPULSE review-team students at their institution and use the manuscript reviewing as a mechanism to teach experimental design, scientific writing, ethics in communications, etc. (see course suggestions in JUNE Spring 2006 Vol. 4, Issue 2 http://www.funjournal.org/results.asp?juneid=159). The IMPULSE review team has membership from around the world and serves the additional purpose of increasing undergraduate understanding of the international character of science. The journal is hosted at the University of South Carolina (http://impulse.schc.sc.edu), and is listed through the Directory of Open Access Journals. Growth in submissions has lead to creating a second Reviewer Training

Site at Middlebury College, where Kim Cronise now serves as an additional Faculty Advisor. Supported by the SC Honors College.

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The effects of transcranial magnetic stimulation over the left motor cortex on pain perception in healthy adults

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A number of neurostimulation methods of pain relief have been developed that act at various levels of the nervous system in order to relieve symptoms of chronic neuropathic pain. Transcranial magnetic stimulation (TMS) is a relatively painless and noninvasive form of stimulation that does not require sedation. There is evidence that fast (> 5 Hz) repetitive TMS (rTMS) over the motor cortex produces analgesic effects in both chronic pain patients and healthy adults. The exact frequency and intensity of stimulation to employ in order to achieve maximal pain relief, however, is not known. In an effort to elucidate this variable, this study examines the effects of various stimulus frequencies (1 Hz, 10 Hz, and 50 Hz triplets) and intensities (80%, 90% and 100% of resting motor threshold (rMT)) of rTMS over the motor cortex on pain perception in healthy adults. Pain measurements investigated include thermal pain thresholds, intensity and unpleasantness of supra-threshold pain stimuli, intensity of thermal wind-up pain, and mechanical pain thresholds. Results indicate that 10Hz TMS at 80% rMT seem to inhibit hyperalgesic effects significantly during thermal pain testing. However, perhaps due to a limited data set, it appears that motor TMS with all other intensities and frequencies did not produce any reliable effects on pain perception in healthy adults.

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Nicotine-conditioned hyperactivity in adolescent male and female D2-primed rats.

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The aim of this study was to determine the ability of a nicotine-conditioned context to elicit locomotor hyperactivity in a neonatal quinpirole animal model of psychosis, and whether this conditioned hyperactivity could be blocked by the D2 antagonist eticlopride. Sprague-Dawley rats were treated with either saline or the dopamine D2 receptor agonist quinpirole from postnatal days (P) 1-21 to create priming of the dopamine D2receptor, a phenomenon that we have shown persists throughout the animal's lifetime. Beginning on P33, animals were injected i.p. with either nicotine (0.5 mg/kg), the D2 antagonist eticlopride followed by nicotine, or saline and placed into the arena 10 min after injection every other day through P49. A non-paired group was included. Results showed that, D2 priming blocked the typical initial hypoactivity produced by nicotine, and these animals also sensitized to nicotine more rapidly than controls. Eticlopride blocked sensitization to nicotine in both D2-primed and non D2-primed rats. On P50, a portion of these animals were administered a drug-free test in which rats were given saline before being placed into the locomotor arena. Interestingly, control animals administered nicotine demonstrated conditioned hyperactivity on the drug free test that was blocked by eticlopride, but eticlopride did not affect nicotine-conditioned hyperactivity in D2-primed rats. This result indicates priming of the D2 receptor was able to overcome D2 receptor blockade in adolescent rats. Further studies are analyzing the role of nicotinic receptors in this phenomenon, as collaborators have shown that D2 priming produces alpha7 nicotinic receptor upregulation.
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Nicotine sensitization in adolescent Beta Arrestin-2 knockout mice: Correlations with BDNF

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abstract.: Beta Arrestin-2 is a protein that regulates hydrolysis of the G-protein and is co-localized with the dopamine D2 receptor. In this study, 3-4 week old adolescent BA-2 KO and wild type C57/B6 mice were administered either nicotine tartarate (s.c, 0.5 mg/kg) or saline 10 min before being placed into the locomotor arena on each of seven (Experiment 1) or 14 (Experiment 2) consecutive days. A drug-free abstention period of seven days followed nicotine sensitization in each experiment, at the end of which animals received a nicotine challenge (0.5 mg/kg free base). Experiment 1 results showed that BA-2 KO mice were slightly hypoactive and did not demonstrate sensitization by day 7, whereas wild type controls did not demonstrate an increase in activity as compared to saline-

treated wild types by day 7, but did show an increase in activity over the 7 days of nicotine treatment. On the nicotine challenge, BA2 KO blocked expression of nicotine sensitization. In Experiment 2, BA-2 KO mice demonstrated sensitization although not the levels of the controls. On the challenge, beta arrestin-2 again blocked expression of sensitization. Brain tissue was taken in both experiments for analysis of Brain-derived neurotrophic factor (BDNF) in the nucleus accumbens, and showed that nicotine produced a significant increase in BDNF which was blocked by the knockout of beta arrestin-2. These results show that beta arrestin-2 plays a more important role in expression as compared to induction of nicotine sensitization, and blocks nicotine-induced increases in BDNF in the nucleus accumbens.

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Amphetamine sensitization in a rodent model of psychosis

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This study was designed to analyze the effects of amphetamine on locomotor sensitization in a rodent model of psychosis developed in our laboratory. Past studies have shown neonatal quinpirole (dopamine D2/D3 agonist) produces a significant increase in dopamine D2 receptor sensitivity that persists into adulthood, a phenomenon known as D2 receptor priming. An increase in D2 receptor sensitivity is consistent with several behavioral disorders, including schizophrenia. Rats were administered quinpirole (1 mg/kg) or saline from postnatal days 1-11 and raised to adulthood (postnatal day 60). Beginning in adulthood, rats were administered d-amphetamine sulfate (1 mg/kg) or saline every other day for 14 days, resulting in a total of seven exposures to the drug. Approximately 10 min after drug injection, rats were placed in a locomotor arena and overall activity was analyzed. Results showed that D2-primed rats receiving amphetamine demonstrated a significant increase in locomotor activity across all seven days of testing relative to all other groups. Controls receiving amphetamine also demonstrated a significant increase in activity over days, demonstrating sensitization. Interestingly, D2-primed rats given saline demonstrated a lack of ability to habituate to the environment, and actually increased activity over days. Seven to fourteen days after locomotor sensitization testing was complete, cerebrospinal fluid samples were taken via microdialysis from the nucleus accumbens core to be analyzed for dopamine levels, and results showed that D2-primed rats demonstrated a significant 5-fold increase in dopamine levels compared to controls administered d-amphetamine. Supported by NIH grant 1 R15 DA 020481-01 to RWB.

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Differential Effects of α-MSH on Social Behavior in Mice Depending upon the MC1R Receptor

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Research supports that α-MSH (melanocyte-stimulating hormone) modulates pain sex-dependently. This sex-dependent mechanism involves the abundance of melanocortin-1 receptors (MC1R) in the periaqueductal gray (PAG). Because this region influences social behavior, the relationship between α-MSH and the MC1R, was studied with social interactions in mice; female estrous cycle state was also considered. Possible α-MSH effects in the absence of the MC1R propelled testing on MC1R mice. 160 adult, naïve Swiss Webster and MC1R knockout mice were randomly assigned a partner of the same strain and opposite sex to one of eight experimental conditions, which varied based on drug and estrous state. Based on vaginal appearance, females were verified as estrus or non-estrus. Following intracerbroventricular (ICV) injections of α-MSH or saline, the pair was placed in a social interaction box. The following behaviors were quantified: locomotor activity (line crosses), flees, tailfollows, anogenital investigation, frontal investigations, rears, self-groomings, mountings, initiate contacts, and squeaks. MC1R females displayed greater sensitivity to α-MSH than SW females. α-MSH showed little or no effect in SW or MC1R males. α-MSH affected social, rather than non-social, interactions.

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Treatment of injured peripheral nerves with chondroitinase ABC leads to improved functional recovery

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Degrading chondroitin sulfate proteoglycans (CSPGs) with the bacterial enzyme, chondroitinase ABC (ChABC), enhances axonal regeneration after nerve transaction and repair. However, whether enhanced regeneration leads to

improved functional recovery is not known. In this study, we investigate the extent of functional recovery in ChABC-treated rats. The sciatic nerve was transected unilaterally and the distal stump treated with ChABC prior to conventional end-to-end repair with fibrin glue. Beginning two weeks later, video records (120fps) of level rat treadmill locomotion at a speed of 11m/m were obtained once a week for the next ten weeks. Kinematic analysis of hindlimb movements during treadmill locomotion was used to evaluate functional recovery in ChABC-treated animals and compared to untreated controls. Following sciatic nerve transaction, muscular control of ankle joint movement is lost. No ankle flexion occurs during the swing phase (F epoch), there is a larger and more prolonged period of ankle flexion during the early stance phase (E2 epoch), and there is very little ankle extension to achieve limb push off during late stance (E3 epoch). Rats attempt to compensate for this lack of control using greater hip flexion during swing, less knee flexion during E2 and hip flexion instead of extension during E3. In ChABC-treated rats, ankle flexion during F epoch is restored, beginning as early as four weeks after nerve repair surgery, and compensatory changes at the hip and knee are reduced or eliminated. In untreated controls, none of these signs of functional recovery was noted, even ten weeks post-operatively. Chondroitinase ABC treatment of injured peripheral nerves leads to enhanced axon regeneration and also improved functional recovery.

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The Role of AMPA/Kainate and Dopamine Receptors on Reinstatement of Heroin Seeking

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The nucleus accumbens is a crucial structure in the neural circuitry of drug reward and addiction. It is known that both dopaminergic and glutamatergic innervations of this area play important and necessary roles in the modulation of several drug seeking behaviors. In this study we examined the effect of antagonism upon dopamine and AMPA/kainate receptors in the nucleus accumbens core (NAc) and how the blockade of these receptors relates to reinstatement of heroin seeking behavior in rats. We introduced the AMPA antagonist CNQX and the dopaminergic antagonists fluphenazine, sulpiride, or SCH-23390 and assessed the behavioral effects of this receptor blockade upon heroin primed reinstatement. We found that each of the antagonists significantly blocked heroin primed reinstatement compared to controls, thus indicating that both AMPA/kainate and dopamine receptors in the NAc are necessary for reinstatement of heroin seeking in rats. This shows that both glutamatergic and dopaminergic pathways within the nucleus accumbens play key roles affecting heroin reinstatement.

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Neuroprotective efficacy of estrogen and calpeptin is enhanced in motoneurons co-cultured with astrocytes exposed to glutamate-toxicity

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Estrogen mediated neuroprotection is well established in single cell cultures, but, no data has yet been presented examining the effects when motoneurons are co-cultured with astrocytes. Astrocytes are an integral part of the nervous system that serves as supporting cells to the motoneurons. In the case of injury or neurotrauma, they form glial scars, which can hinder cell growth and prevent axon penetration. This study focused on the neuroprotective role that astrocytes offer to the motoneurons in glutamate induced neurotoxicity. Co-culturing astrocytes with motoneurons enabled the motoneurons to withstand twice the concentration of glutamate compared to motoneurons cultured alone, as shown by quantitative cell viability assay using MTT and morphologically by In Situ Wright staining. Estrogen and calpeptin were also shown to prevent glutamate-induced cell death in co-cultures. Calpeptin rendered protection via inhibition of the neutral protease calpain, which was evident from attenuation of active m-calpain and downstream active caspase-3 expression in the co-cultures using Western blot. The mechanisms by which estrogen protected the motoneurons in co-cultures remain to be elucidated by further studies.

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Characterization of Drosophila Palmitoyl protein-thioesterase 1's role in Cellular Trafficking

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Infantile Neuronal Ceroid Lipofuscinosis (INCL) is a pediatric neurodegenerative disease caused by mutations in the human CLN1 gene. CLN1 encodes palmitoyl-protein thioesterase 1 (PPT1) suggesting an important role for the regulation of palmitoylation in normal neuronal function. To further elucidate Ppt1 function, we recently performed a

gain-of-function modifier screen in Drosophila using a collection of enhancer promoter transgenic lines to suppress or enhance the degeneration produced by over-expression of Ppt1 in the adult visual system. Modifier genes identified in our screen connect Ppt1 function to synaptic vesicle cycling, endo-lysosomal trafficking, synaptic development, and activity-dependent remodeling of the synapse. Our results compliment recent work on mouse Ppt1-/- cells that shows a reduction in synaptic vesicle pools in primary neuronal cultures and defects in endosomal trafficking in human fibroblasts. We have now followed up on our results by examining general endocytic mechanisms and synaptic function in Drosophila Ppt1 mutants. Like human fibroblasts, we have shown that larval garland cells have defects in general endocytosis. Furthermore, we have characterized gain- and loss-of-function genetic interactions between Ppt1 and the Drosophila dynamin gene, shibire. Finally, we will present developmental and functional analysis of Ppt1 mutant neuromuscular junctions. Taken together our work suggests that trafficking defects may be one of the underlying cellular causes of neuronal dysfunction in INCL patients.

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Role of β-endorphin in behavioral despair, stress, and anxiety

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β-endorphin , a natural opioid, is released in response to psychological stress as well as physiological stressors, such as alcohol (EtOH) and is believed to play a role in the regulation of the effects of stress and EtOH. To investigate the role of β-endorphin in stress and its relation to alcohol consumption, a line of male and female transgenic β-endorphin mice (Rubinstein et al., 1996) having different basal levels of the opioid in their brains were subjected to two tests—modified versions of Porsolt's (1977) Forced Swim Test (FST) and Steru's (1985) Tail Suspension Test (TST)–of behavioral despair (akin to depression in humans) after being given an intraperitoneal injection of EtOH or saline. These tests measure behavioral despair and anxiety through the amount of mobility or immobility mice show when placed into aversive environmental situations. Adrenal glands were also removed from naïve male and female KO, HT, and C57/WT β-endorphin mice and weighed to determine any fundamental differences in adrenal gland size (and, thus, physiological responses stress) among the three genotypes. Behavioral test results have been inconclusive so far. FST and TST tests should produce similar results since they supposedly measure the same concept (behavioral despair). This has not been the case in our initial set of experiments. Our preliminary behavioral findings do, however, suggest an interaction among EtOH, β-endorphin levels, and stress. This relationship seems to vary between different sexes and strains. The results from the adrenalectomies suggest that adrenal gland weights differ significantly among the three strains of mice with the KO mice having the largest adrenal glands, probably due to the fact that they do not produce β-endorphin , resulting in a chronically stressed state. Additional research will need to be conducted to allow us to gain additional insight into the relationship between β-endorphin , EtOH, and stress.

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Investigating the Role of β-endorphin in Mediating Alcohol Reward Using in vivo Microdialysis

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Rationale: Alcohol reward has been implicated in dopamine and, to a lesser degree, glutamate release in the nucleus accumbens.
Objectives: This study hoped to elucidate the relationship between the endogenous opioid β-endorphin, which has been implicated in stress reduction and feelings of euphoria, and the release of dopamine and glutamate in the nucleus accumbens of transgenic mice possessing different "levels" of β-endorphin production capacity in response to alcohol administration.
Methods: In vivo microdialysis and HPLC was used to assess the relative concentrations of extracellular glutamate and dopamine in the nucleus accumbens of β-endorphin knock-out (KO), heterozygous (HT), and wild-type control (C57) mice at normal, baseline conditions and after acute ethanol administration.

Results: Results are currently preliminary and tentative as the glutamate HPLC assay has currently been performed on only one of each strain of mouse. Glutamate levels appear to be fairly stable in the C57 mouse and most variable in the HT mouse. The KO mouse showed a marked decrease in glutamate levels after ethanol administration while the HT mouse's glutamate levels spiked, then declined after receiving the drug.
Conclusions: Clear conclusions cannot be drawn based on our current results. The addition of more glutamate data, which is forthcoming, and the beginning of dopamine HPLC analysis on all microdialysis samples collected will assist in elucidating a possible relationship between α-Endorphin, glutamate, dopamine, and the induction of "reward" or "pleasure" in the nucleus accumbens as a result of alcohol administration. Determining the relationship between these varied neurological components will assist in creating a possible explanation for alcohol addiction and may offer insight into future areas of research related to the treatment of alcoholism.

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Differences in Analgesic Effects of Lidocaine HCl and Lidocaine Docusate Topical Solutions in Mice

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Topical pain medications are a commonality today - therefore, finding one that is more effective at blocking pain is important. This study tests two formulations of Lidocaine - a hydrochloride solution and a docusate solution - both diluted to proper concentration in DMSO. Lidocaine HCl is a commonly used form of lidocaine and acts as the control test compound. We predict that the docusate solution will have a different effect from the hydrochloride solution. We tested three concentrations of each solution. Subjects were male and female Swiss-Webster mice (n=45). Subjects were tested for analgesic effects by the tail flick method. Talk flicks were measured 10 and 20 minutes prior to a minor thermal burn injury to get a baseline reading. They were measured again twice post-injury, then treated with one type and dose of medication. Then tail flicks were measured for four hours at even intervals. Data was analyzed for between groups differences, area under the curve, and dose-response relationships. The docusate solution had a significantly greater effect than the hydrochloride solution. It acted faster and blocked pain longer. Tail flick latencies were as much as two fold higher in mice treated with the docusate solution. This shows good promise in potentially increasing the analgesic effects of topical pain ointments by using a different formulation.

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Effect of Social Environment on Estrogen Receptor Alpha in Prairie Voles

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Social environment, particularly isolation, has a significant impact on neuroendocrine responses and social behaviors. Prairie voles are socially monogamous rodents (displaying life long pair bonds and biparental care) and may be particularly sensitive to the effects of isolation relative to other rodent species. Isolated prairie voles (Microtus ochrogaster), demonstrate deficits in individual recognition and discrimination and are more reactive to social stressors. Isolation also affects central vasopressinergic responses in this species, particularly in females. Species comparisons among rodents (including voles) demonstrate a relationship between estrogen receptor alpha (ERα) and the degree of prosocial behavior. This association is particularly notable within discrete limbic system nuclei. In the present experiment, juvenile prairie voles were housed with a sibling, stranger or in isolation for three weeks following weaning (at 21 days of age). ERα expression was then quantified in the central nervous system using immunofluorescence. The number of neurons containing ERα were counted in the medial preoptic area (MPOA), medial amygdala (MeA), bed nucleus of the stria terminalis (BST) and ventromedial hypothalamus (VMH). Isolate females displayed significantly fewer ERα labeled cells in MPOA and MeA areas compared to the stranger housed females. ERα expression in the MPOA and MeA of males was not significantly different among housing conditions. This suggests that females may be more reactive to the stress of isolation in this species

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Gender Differences In Stimulant-Mediated Smoking Behavior

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Previous studies suggest that a large majority of stimulant abusing and dependent individuals smoke cigarettes. Conversely, cigarette smokers are more likely to use illicit drugs like stimulants. However, cigarette and stimulant co-use remains poorly researched. While the results of previous studies indicate that sex may differentially influence physiological and behavioral responses to cigarette smoking, the literature is mixed regarding whether males and females are differentially sensitive to the acute effects of stimulants. It is thus unclear whether males and females are differentially sensitive to stimulants, and what effect this could have on smoking behavior. In this experiment, the influence of sex on the acute effects of several doses of methylphenidate (0, 10, 20, and 40 mg) and ad libitum smoking was examined using a retrospective analysis of data from two previously published studies using identical procedures and measures. One hour after ingesting drug, participants were allowed to smoke and consume food and non-caffeinated beverages ad libitum for four hours. Primary dependent measures were number of cigarettes smoked, number of puffs taken, expired CO level, and number of calories consumed. Significant dose by gender interactions were observed for peak carbon monoxide levels (men > women). Significant main effects of gender were observed for subject ratings of Willing to Pay For Drug (women > men). As expected, methylphenidate dose-dependently decreased measures of food consumption, and dose-dependently increased heart rate, blood pressure, and several measures of cigarette smoking rate. This analysis indicates that men and women differed only in the biological measure of cigarette intake as a function of methylphenidate dose but not on other measures of cigarette smoking behavior.

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Fluorescence Methods for Detecting Heavy Metals in Solution

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Fluorescent probes have broadened our understanding of the role of metals such as Ca^{2+} and Zn^{2+} in cell biology. However, one critical weakness of ion-sensitive fluorophores is their tendency to respond to a variety of species. Thus metal contamination often leads to spurious results. In particular, Zn^{2+} and Cd^{2+} are similar in chemical properties, and consequently are difficult to distinguish biological samples. In the present study we tested a variety of fluorescent probes in order to establish a spectrofluorophotometer-based assay that can selectively detect Zn^{2+} and Cd^{2+} in KCl-based buffered solutions. We examined the spectral responses that Zn^{2+} and Cd^{2+} elicit from the ratiometric probes fura-2, fura-2FF, mag-fura-2, and the intensiometric probes Calcium Green-5N, FluoZin-3, and Newport Green. We found that the mag-fura-2 responses to Zn^{2+} and Cd^{2+} differed substantially in both quantitative and qualitative aspects. Specifically, Zn^{2+} resulted in an isosbestic point around 346nm, while the isosbestic point for Cd^{2+} was 353nm. Calcium Green-5N responded strongly to Cd^{2+} but only weakly to Zn^{2+}, with no spectral shift in either case. The spectral compatibility between mag-fura-2 and Calcium Green-5N allowed us to combine both dyes for simultaneous measurements, which increased resolution. We then added physiological amounts of Ca2+ and Mg^{2+} to the KCl solution in order to test the dual-dye method in a more biologically relevant situation. In this final medium, nanomolar Cd^{2+} produced a mag-fura-2 isosbestic point of 356nm and a strong response to Calcium Green-5N, while nanomolar Zn^{2+} resulted in a mag-fura-2 isosbestic point of 345nm and a weak Calcium Green-5N response. In summary, the dual-dye approach allows sensitive and discerning detection of Zn^{2+} and Cd^{2+} in biologically relevant solutions. Future experiments will determine the viability of this method for detecting metals in whole, living cells.

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Computational Model of CA1 Pyramidal Neurons in the Hippocampus of Mice

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In order to study the mechanism underlying the electrophysiological behavior of CA1 pyramidal neurons in the hippocampus of wild-type mice, a conductance-based single-compartment computational model is utilized. Fundamental Hodgkin-Huxley based currents are incorporated into the model as well as a glutamate ionotrophic receptor, NMDA (N-methyl-D-aspartic acid) because they are important for the normal electrophysiological behavior of these neurons. The model reproduces essential features of the hippocampal biological neuron and offers the basis for a large-scale study of networks involving CA1 hippocampal pyramidal cells.

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PLCa4 Gene Expression Profile in Mouse Brain and Liver Tissue

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Animals exhibit daily rhythms that are regulated by an internal timekeeper. This timekeeper maintains and synchronizes a number of body processes including sleep/wakefulness, rest/activity, body temperature, and behavior. Individual tissues show endogenous cycles of genes and proteins but these independent cycles are only synchronized if in communication with the suprachiasmatic nucleus (SCN) of the hypothalamus. We have recently shown that a protein which is linked to circadian function in the SCN, phospholipase C a4 (PLCa4), undergoes a circadian oscillation in both the SCN and liver tissue. Moreover, in the liver, PLCa4 translocates from the cytoplasm to the nucleus over the course of the day. To determine if this oscillatory profile is due to rhythmic transcriptional regulation, we are currently analyzing the temporal profile of plca4 gene expression in liver obtained from animals housed in constant darkness using RT-PCR. In the brain, we are employing in situ hybridization techniques to look at light and temporal regulation of gene expression. These data are designed to gain insight into the mechanism that regulates the transcription of the plca4 gene in the SCN and liver of the mouse.

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Catalytic Interlesion Interval Facilitates the Emergence of Long-Term Potentiation

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In rat brains, damage to one entorhinal cortex (EC) destroys the ipsilateral perforant pathway and thereby denervates the ipsilateral dentate gyrus. However, the crossed temporodentate pathway (CTD), which links the intact EC of the contralateral hemisphere to the denervated dentate, sprouts and has been shown to restore lost memory function. Two stage lesions of the entorhinal cortex accelerate CTD sprouting. In other hippocampal projections, interlesion intervals of 4 to 13 days have been found to induce the greatest amount of sprouting. We explored the ability of a 12 day interlesion interval to catalyze and accelerate the CTD's ability to support long term potentiation. Electrophysiological data from rats with 6 or 12 day interlesion intervals were examined to determine whether the sprouted CTD could support long term potentiation. Our results indicated that the 12 day interval produced a sprouted pathway that was able to support criterion-based long term potentiation while the 6 day interval did not. This finding suggests that a 12 day interlesion interval more effectively prepares the CTD to support long term potentiation.

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The effects of (+)- and (-)-cyclazocine on cocaine-induced locomotor activity

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Cocaine is a highly addictive substance whose behavioral effects are linked with increased dopamine concentrations in the mesolimbic dopaminergic pathway. Activity at opioid receptors and sigma receptors can modulate cocaine's effects. Benzomorphans are a class of morphine derivatives whose (+) and (-) isomers interact with opioid and sigma receptors, respectively, and therefore may influence cocaine's effects. The current study tests the combined effects of cocaine and two benzomorphans, (+)- and (-)-cyclazocine, on locomotor activity in male Long Evans rats. Consistent with our hypotheses, (+)-cyclazocine (a sigma receptor agonist) increased the effects of cocaine in an additive manner, whereas (-)-cyclazocine (an opioid receptor agonist) increased the effects of cocaine in a synergistic manner. These data indicate that benzomorphans can serve as useful tools to explore cocaine's interaction with the opioid and sigma systems.

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Chronic Excercise Enhances Cocaine Conditioned Place Preference

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The purpose of this study was to determine whether chronic exercise alters sensitivity to the conditioned rewarding effects of cocaine. Female rats were obtained at weaning and randomly assigned to either sedentary or exercise conditions. After 6 weeks under these conditions, the effects of cocaine were examined in the conditioned place

preference procedure. Cocaine produced a dose-dependent conditioned place preference in both groups of rats. Exercising rats were more sensitive than sedentary rats to cocaine in this procedure, and this effect was most pronounced at the highest dose of cocaine. These data suggest that chronic exercise increases sensitivity to the conditioned rewarding effects of cocaine.

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The Effects of Cue Induced Reinstatement of Heroin Seeking on Glutamate Levels in the Nucleus Accumbens Core

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Relapse is a prevalent and severe problem among opiate addicts in treatment. Exposure to drug associated environmental cues often trigger reinstatement of drug seeking. The transmission of glutamate to the Nucleus Accumbens core aids in the reinstatement of cocaine seeking. These findings suggest an association between cue induced reinstatement of heroin seeking and glutamate levels in the Nucleus Accumbens core. Heroin self administration sessions involving light/tone cues paired with heroin infusions for two weeks were performed with one group of subjects, along with a yoked/control group receiving noncontingent saline, followed by two weeks of extinction training. Upon completion of self administration, microdialysis was performed in the Nuclues Accumbens core during cue induced reinstatement of heroin seeking for both the self administration and yoked groups. An increase in glutamate was found during reinstatement of the self adminstration group but not in the yoke group.

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Investigation of the Effects of LPA on Chicken Retinal Growth Cone Collapse

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Visual system development is dependent on axon guidance in retinal ganglion cells (RGCs). In chicken embryonic retina, axons of RGCs extend and travel through various tissue to reach their target in the brain, the tectum. These axons are guided by growth cones which respond to extracellular cues, mediating their directional pathway. Lysophosphatidic acid (LPA), a lipid signaling molecule, is known to play a role in a variety of biological pathways, including neurons. We are investigating LPA as an inhibitory guidance cue on RGC growth cones in the retinal system. Six day old embryonic chicken retinas were cut into explants and neurite outgrowth was achieved using laminin as a substrate. LPA was then administered to growth cones at various concentrations ranging from 1 nM to 1 μM. Observance of growth cone morphology reveals that LPA induces an in vitro inhibitory response of retraction or collapse. Preliminary results suggest that this occurs in a dose-dependent manner. LPA is known to signal through five G-protein coupled receptors (GPCRs) to induce an intracellular G-protein signaling cascade. Thus, we will continue our investigation with the identification of the G-protein coupled cellular pathways activated by LPA receptors. In addition, we are studying the expression of the LPA receptors at different stages of chicken retinal development to examine their role in RGC axon guidance.

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Wake up and smell the caffeine: Zebrafish locomotor activity following caffeine exposure

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Originally used in many cultures for ceremonial purposes or as a daily stimulant, caffeine has become one of the most ubiquitous drugs used in the western world. In particular, caffeine has become a regularly overused stimulant in the United States. Zebrafish have served as a model organism for the study of development for more than 30 years. These animals have also been useful for the study of effects of cocaine, nicotine, ethanol and melatonin on behavior and development. In this study, we examined the acute effects of caffeine on zebrafish locomotor activity. Animals were exposed to either 0.01 or 1.0 mg/L of caffeine or a control condition for 5 minutes. Fish were subsequently transferred to an observational arena and locomotor activity was assessed by manually counting the number of line crossings on a grid below the arena. Additionally, we used the Noldus® Ethovision system to digitally image the activity of fish in the arena. We hypothesized that caffeine exposure would stimulate activity in these fish. However, caffeine exposure produced a dose dependent decrease in locomotor activity. At the 0.01mg/L dose (N=15), we saw a decrease in mean activity counts of 18% compared to the control condition. A dependent t-test indicated that this reduction in activity was significant (t (15) = 3.18, p < 0.003). In a second group

of animals exposed to 1.0 mg/L (N=16), we saw a 34% decrease in mean activity counts. A dependent t-test indicated that this reduction in activity counts was significant (t (15) = 2.38, $p < 0.015$). An analysis of these data using the Noldus system is currently in progress. Future experiments will examine conditioned place preference following caffeine exposure.

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The absence of thrombomodulin expression in developing avian spinal cords on E10

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Thrombin is a serine protease involved in coagulation via activation of the PAR-1 receptor. It has also been detected within the developing murine and avian CNS and is believed to play a role during development. Here it has primarily been found to have destructive effects, including increased apoptosis in avian motoneurons in the lumbar spinal region. Thrombomodulin, a transmembrane protein known to regulate the coagulant properties of thrombin, has also been detected in the CNS of murine embryos. Therefore, this study investigated the presence of thrombomodulin in the developing avian spinal cord. Experimental embryos were given 200 ƒÝL of 100 ƒÝM SFLLRNP (an artificial PAR-1 activator) daily from E5 to E9 while control embryos were given equal amounts of 1X PBS; on E10 the embryos were sacrificed. The spinal cords were prepared for thrombomodulin immunohistochemistry. Thrombomodulin was not detected. This finding suggests a different mechanism for regulating thrombin in the avian developmental system, such protease nexin-1, which is a potent protease inhibitor. Perhaps thrombin regulation is less complex in avian developmental systems. It is possible that mammals require multiple regulatory compounds to monitor thrombin activity, whereas birds utilize a more simple mechanism involving protease nexin-1.

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Changes in the Hippocampal Expression and Distribution of the KA2 Subunit of the Kainate Receptor in Epileptic Rats

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Temporal lobe epilepsy (TLE) is a brain disorder characterized by recurring spontaneous seizures which can result from a brain injury. The seizures usually originate in the hippocampi. Glutamate is an excitatory neurotransmitter. Glutamate receptors can be divided into several subtypes, including kainate receptors (KARs). KARs are composed of a variety of subunits which can include KA2. KARs play a role in the initiation and spread of seizures, and the presence of KARs containing KA2 can increase the risk of seizures. However, seizures can increase the expression of KA2. We hypothesize that the increased presence of KA2 during epilepsy predisposes the brain to seizures. We examined changes in the hippocampal expression of KA2 in epileptic Sprague-Dawley rats. Rats were made epileptic using the pilocarpine model of TLE. This model induces status epilepticus (a prolonged seizure) via intraperitoneal injection of the convulsant pilocarpine. The status causes brain trauma, which leads to the development of epilepsy. We performed immunohistochemistry to assess changes in the expression and distribution of KA2 in the hippocampi of rats which had been epileptic for 12 weeks. A better understanding of the changes in KA2 expression and distribution during epilepsy could lead to a novel therapy for TLE.

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A role for neural activity in Xenopus laevis retinal ganglion cell dendrite morphogenesis in vivo.

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The morphology of a neuron's axonal and dendritic arbors plays a crucial role in how the neurons signal to each other and integrate information from various synaptic inputs. The development of retinal ganglion cells (RGCs) from their somas in the retina to their synaptic targets in the optic tectum is influenced by many molecular factors, and is used as a model for understanding the development of a neuron's axons and dendrites. While many factors influence the development of RGC axons, from axon initiation and pathfinding to their arborization in the optic tectum, have been identified, factors involved in the development of RGC dendrites are less well known. This study examined the effect of neural activity on Xenopus laevis RGC dendrite development in vivo. When TTX, which blocks voltage-gated Na+ channels, was used to silence retinal activity, Xenopus RGC axons developed more complex arbors (Cohen-Cory, 1999). It was therefore hypothesized that RGC dendrites would also increase their

complexity in response to the inhibition of retinal neural activity. Although early light exposure does not influence Xenopus RGC dendrite development (Rigel and Lom, 2004), it is important to examine the possible contribution of spontaneous activity toward shaping the developing dendritic arbor. Intraocular injections of 100 μM TTX were used to silence action potentials in the retinae of stage 38 Xenopus tadpoles, the stage at which RGC dendrites have just begun to extend from the cell soma. At stage 43-44, when the RGC axons have reached their target in the optic tectum and dendritic arborization is well underway, rhodamine dextran was microinjected into the optic tectum to retrogradely label RGC dendritic arbors in the retina. Tadpoles were fixed at stage 45 and confocal microscopy was used to visualize RGC dendritic arbors. TTX treatment resulted in a significant increase in the average number of primary dendrites extending from each RGC, without affecting the average number of branch tips per RGC, indicating that there was an increase in the overall number of branch tips per primary dendrite. Inhibition of neural activity with TTX treatment did not affect the total length of RGC dendritic arbors. Although activity plays a role in shaping RGC dendrites, other factors may have a greater influence on their development.

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