



REU ABSTRACTS

Center for the Integrative Study of Animal Behavior

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REU RESEARCH ABSTRACTS



Evolution – Convergence, Divergence, and Adaptation Lessons Developed to Teach Evolution and Ecology to Middle and High School Students.

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Understanding the processes and implications of evolution are crucial to understanding the concepts of biology at all levels from the single cell to the entire biosphere. Most states, including Indiana, require the teaching of evolution to middle and high school students in life science and biology courses. Some teachers are comfortable with this task, while others, due to their own lack of understanding and/or experience or due to pressures from the community, school, parents, and/or local school boards, find it difficult to teach. It was the goal of this project to develop educational materials and a field trip experience that would assist the accurate teaching of a few evolutionary topics in a meaningful and engaging manner. Five areas in Jordan Hall at Indiana University were identified as “exhibit” area where students could engage in a learning activity. The five areas were the vivarium, the Birds of Indiana collection, the specimen displays, the public greenhouse, and the prairie plantings. Pre-visit activities to teach the concepts of convergence, divergence, and adaptation were developed for teachers to use before the students came to view the exhibits. On-site activities were developed to engage the students in the learning process as they viewed the various exhibit areas in Jordan Hall. Post-visit activities were developed to help review the concepts and to extend application and understanding beyond the field trip experience. Maps, teacher background resources, and a website were developed as part of this project as well. The on-site educational materials were tested on students from the Jim Holland Summer Enrichment Program. Conversation with the local science museum Wonderlab also took place, and possible future partnerships between IU and Wonderlab were explored.

Role of DHEA in Mediating Seasonal Aggression in male Siberian Hamsters (*Phodopus sungorus*)

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In many species of animals, aggressive behavior persists or, in some cases, increases during the non-breeding season when testosterone (T) levels are low. Animals that display this disassociation of aggression from gonadal steroids, specifically (T) levels suggest the need for further investigation of alternative physiological mechanisms in mediating seasonal changes in aggression. Siberian hamsters (*Phodopus sungorus*) are an ideal study species for this type of research because they display increased territorial aggression during the non-breeding season, and this increase is inversely related to T levels. The goal of the present study was to examine the role of the adrenal steroid dehydroepiandrosterone (DHEA) as an alternative endocrine regulator of seasonal aggression in male Siberian hamsters. In this experiment animals were housed in either long-day (LD) “summer-like” photoperiod or short-day (SD) “winter-like” photoperiods in order to increase the aggressive behavior in those animals housed in SD. After 8 weeks in photoperiod we randomly select the hamsters that received the DHEA capsule. Controls animals received empty capsules. In this experiment all animals were tested using a resident–intruder model of aggression. With respect to the aggressive behavior (numbers of attack, chase, latency of initial attack) we found that there was a significant photoperiod effect but there was no significant treatment effect of DHEA with respect to the aggressive behavior nor a significant interaction between DHEA and photoperiod. Collectively, the results of the present study do not support the hypothesis that seasonal changes in territorial aggression in Siberian hamsters are mediated by circulating levels of dehydroepiandrosterone (DHEA).

Sexual asymmetry in *Sceloporus graciosus* visual performance

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Differences between the sexes may evolve for several reasons, including sexual selection pressures such as mate choice and competition. Animals may use complex visual displays to communicate territoriality and courtship to male and female receivers in different functional contexts. During courtship, male *Sceloporus graciosus* lizards utilize motion specific displays, whereas for territorial/aggressive communication, males utilize stilted postures which exhibit their blue ventral coloration. Using a psychophysical assay (optomotor response), we studied full-field motion perception in male and female *S. graciosus* to determine whether the differential display production was associated with differences in visual performance. Previous results from an acute/peripheral motion detection assay found that female *S. graciosus* were better able to detect motion. Our current results show that males likely have a higher threshold for full-field motion perception. Results from these studies provide evidence that sexual asymmetry may exist between the sexes at different levels of visual processing. The optomotor response is a useful behavioral assay because it produces an unambiguous, robust response. This assay could be utilized to test diverse taxa in comparative studies and field trials for non-invasive experiments on full-field vision.



Are Ape Limb Proportions Adaptations for Physical Aggression or Arboreality?

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Among the apes, long forelimbs compared to hindlimbs is a distinctive feature. That is, apes have a high intermembral index (IMI). In 2007, Carrier put forth the hypothesis that short legs and long arms among great apes were an adaptation for aggressive behavior. He hypothesized that having short legs both lowered their center of gravity and that long arms improved bipedal hitting during interspecies physical interactions (Carrier 2007). Prior to Carrier's aggression hypothesis, the high ape IMI had been attributed to various selective pressures related to arboreality. The climbing hypothesis suggested a high IMI was an adaptation for climbing trees; the balance hypothesis suggested short hindlimbs aide stability on branches; the reaching hypothesis saw long arms as increasing reach while foraging. Previous research has shown that lowland gorillas (*Gorilla gorilla gorilla*) are more arboreal and that mountain gorillas (*Gorilla gorilla beringei*) are more aggressive. It was expected that in lowland gorillas females would be more arboreal than males, but no difference in arboreality was expected in mountain gorillas due to the limited amount of time spent in trees. Aggression was expected to be greater in males of both subspecies, but greater aggression was expected in the male mountain gorillas. We tested the different hypotheses by comparing arm length, leg length, and intermembral indices for two different subspecies, and for males and females. Mountain gorillas had significantly longer arms and legs, compared to lowland gorillas. Intermembral index did not vary between subspecies, or between males and females. However, arm and leg length did vary. Male mountain gorillas had shorter arm and leg length than male lowland gorillas and female mountain gorillas. Female lowland gorillas had shorter arm and leg length than male lowland gorillas and female mountain gorillas. These results support Carrier's aggression hypothesis as explaining differences between the subspecies for male gorillas, but these results suggest the arboreal balance hypothesis explains female gorilla differences. Further research on physical aggression in gorillas is needed to better discriminate between the various hypotheses.



Vegetation and water condition effects on zebrafish (*Danio rerio*) behavior

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Zebrafish are model organisms that are used in a variety of science experimentation, yet their natural behavior is not greatly documented. Inspection of common behaviors such as aggression, stress recovery, shoaling, and boldness in different environmental conditions may assist in connecting natural zebrafish behavior to already identified genetic components. Because zebrafish come from habitats that vary in vegetation complexity and water turbulence, an experiment combining these factors would greatly influence zebrafish studies. Our study includes testing both vegetation and water condition variation effects on the behavior of three different strains of zebrafish. The three strains were all derived from different backgrounds including two naturally captured types and one laboratory bred. Several groups of six fish all from one strain were tested in four different environments: non-vegetated with flowing water, vegetated with flowing water, non-vegetated with stagnant water, and vegetated with stagnant water. Incidence of foraging efficiency, aggression, shoaling, and boldness were recorded. Our findings exhibit that the lab bred scientific hatchery strain ate much faster than the other two naturally derived strains in the foraging efficiency test. This is probably due to being accustomed to a predator-free environment their entire lives. Fish were more aggressive in environments that were inconsistent with their rudimentary habitats. Shoal cohesiveness was highest in naturally derived fish that were from large, vegetated lakes. This behavior was most likely a result of the high predation risk in their natural habitat. Lastly, boldness increased as time increased in observation. Fish must have recognized the predator model as harmless as they became more accustomed to it over time.

The Effects of Cocaine-Related Cues on Triggering Relapse and Activating Dorsal Prefrontal Cortex

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The prefrontal cortex (PFC) has been implicated in processing cocaine-related information as well as mediating drug-seeking behavior to cocaine- and cue-induced reinstatement after repeated exposure to cocaine. The dorsal prefrontal cortex (dPFC) in particular has been identified as forming one part of a series circuit regulating drug-seeking behavior. In addition, the PFC in behaviorally sensitized rats has been shown to undergo neuroadaptations that are persistent even after lengthy periods of withdrawal. Using a self-administration paradigm, the purpose of this study is to investigate the role of the dPFC in signaling drug availability when rats previously exposed to cocaine are presented with cocaine-related cues after a period of extinction. Once rats have successfully undergone operant training to press a lever for sucrose solution, microwire electrodes will be chronically implanted into the dPFC. Rats will then press for cocaine in which a compound conditioned stimulus (CS) will also be simultaneously presented. After a period of extinction, dPFC activity will be measured when the compound CS is presented in the absence of cocaine. Based on current evidence, we predict that neurons in the dPFC will be activated during reinstatement when only the cue is presented, confirming findings from previous studies that activation of the dPFC is responsible for signaling the availability of cocaine in addition to validating the claim that drug-associated cues can trigger relapse even after long periods of abstinence.



Voltammetric study of the effects of autoreceptor activation on serotonin in the inferior colliculus of mice

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Serotonin (5-HT) plays an important role in a wide range of behaviors including sleep, mood and feeding. Another important role for serotonin is the modulation of auditory processing in the inferior colliculus (IC). Serotonin in the IC is released by neurons that project from the raphe nuclei. The activity of these neurons changes with behavioral state and is regulated, in part, by 5-HT_{1A} autoreceptors on the raphe neurons. By blocking or activating these autoreceptors and increasing or decreasing the firing of the raphe neurons, we predict that we can alter the amount of 5HT present in the IC. In vivo cyclic voltammetry was used to investigate the effects of a known 5-HT_{1A} agonist (8-OH-DPAT) and antagonist (WAY100135) on extracellular 5-HT in the IC of mice. Voltammetry was performed during anesthesia-induced sleep and awake behavior. As predicted, the levels of 5-HT decreased after treatment with 8-OH-DPAT and increased after treatment with WAY100135. Also, pre-treatment with WAY100135 prevented the effect of 8-OH-DPAT. This effect is seen in anesthetized and awake mice. The results show that altering the firing patterns of 5-HT releasing cells with 8-OH-DPAT and WAY100135 changes the amount of 5-HT in the IC in both awake and anesthetized animals. Since the activity of 5-HT releasing cells is known to change with behavioral state, this supports the hypothesis that neuro-modulation by 5HT changes with behavioral state and may serve as a mechanism for state dependent auditory processing.

Injury Response of the pacemaker nucleus after spinal cord transection in *Apteronotus leptorhynchus*

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Voltage-gated potassium (Kv) currents play an important role in setting membrane potentials, regulating firing frequencies, and setting action potential duration. Previous studies have shown that Kv1.2 is primarily present in fine fibers throughout the pacemaker nucleus. In the current study, we used double-labeling immunohistochemistry with Kv1.2 and GFAP (glial fibrillary protein acidic protein) antibodies to examine whether the fine fibers found with labeled Kv1.2 were astrocytic. We also examined whether Kv1.2 expression in the pacemaker nucleus changed after acute spinal cord injury. Quantitative immunofluorescence microscopy showed that Kv1.2 and GFAP were co-localized within the fine fibers of the pacemaker nucleus. Using a complete transection model, we found that injury to the spinal cord decreased Kv1.2-like immunoreactivity within the pacemaker. However, by 7 days post-transection, Kv 1.2 expression was up-regulated to levels similar to those seen in control animals. This data suggests that the down-regulation of the Kv1.2 channels may be an injury response of the pacemaker, allowing it to maintain firing frequency after spinal cord injury.



Voltage Gated Potassium Channel Expression in Response to Spinal Cord Injury in the Electromotor System of *Apteronotus leptorhynchus*

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The weakly-electric teleost fish *Apteronotus leptorhynchus* uses a high frequency electric organ discharge (EOD) generated by a neurogenic electric organ (EO) for navigation and communication. It has been demonstrated that they regenerate nervous tissue and regain full electromotor function following spinal cord transection. However, not much is known pertaining to the physiological changes that occur in the electromotor system throughout the regeneration process. Voltage-gated potassium (Kv) channels of the Kv1 family have been suggested to play a role in regulating the excitability of the electromotor system of *A. leptorhynchus*. The distribution of these Kv1 channels in the intact electromotor system has been well characterized in previous studies. However, the change in distribution of these channels in response to spinal cord injury has not been previously examined. The present study used fluorescent immunolabeling with antibodies raised against particular Kv1 channels to visualize and characterize the effects on distribution of these channels in the electromotor system after spinal cord transection. Increase in EMN size, long term survival of the EMNs, and degeneration of the EO were observed that were in accordance with previous studies. Quantitative immunofluorescence microscopy showed that Kv1.3-like expression was not significantly changed after transection. However, Kv1.4-like expression decreased over long term survival periods. Kv1.6-like expression remained constant in the spinal cord with the exception of expression in the relay axons, which disappeared as the axons degenerated. This data suggest that these channels may not contribute to the spontaneous oscillatory discharge behavior of the electromotor neurons following transection. However, the presence of Kv1.4 long after transection in the EO, may indicate the presence of Schwann cells that may be involved in axonal guidance during EO regeneration.

How Many Injections of Nicotine can a Rat Anticipate in One Day?

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This study delved into the concept of drug anticipatory behavior in animals. Anticipatory behavior involving circadian rhythms has already been examined in animals using nicotine, cocaine, ethanol, fentanyl, and methamphetamines. However, all of these studies used a single injection model. This study took a closer look into the use of nicotine by establishing a multiple injection model. This model was used in order to develop a study that was in closer relation to human addictions. The main purpose was to determine how many injections a rat can anticipate in a 24 hour period. The rats went through four different injection phases consecutively rising from one to four injections per day. Thus far results have shown that the rats can anticipate at least two injections in a day. This is useful evidence towards the theory that in the absence of meal times, drug use can manipulate the part of the brain that is responsible for food anticipatory behavior. While this can not be confirmed with this study, it does provide insight for future studies testing this theory.



The effects of a context shift on chronically restrained animals tested in an instrumental appetitive-to-aversive transfer task

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Chronic stress has been shown to have many damaging effects on the body, including impairment of hippocampal-dependent memory. Long-term exposure to corticosteroid hormones following chronic stress can lead to a retraction of dendritic processes in the hippocampus, decrease in hippocampal volume, and suppression of neurogenesis in the dentate gyrus. The hippocampus is thought to be able to transmit information specifically relating to spatial and contextual information. Our study examined the effects of chronic stress on performance on an appetitive-to-aversive transfer learning paradigm, and subsequently the effect of adding a context shift at the point of transfer. Chronic stress was induced through restraint for six hours a day over the course of six weeks. The animals were tested in an operant conditioning chamber. Once the animals scored 90% or higher correct responses for two consecutive days in the appetitive task, they were transferred to 10 days of an aversive task. The context-shift rats received multiple changes in their environment at this time. We found that chronically stressed rats performed worse on both the appetitive-to-aversive and aversive-only learning. Although the impairments seem to be aversive-specific, they are similar to the effects of a hippocampal lesion, which suggests that chronic stress may impair hippocampal functioning in combination with affecting other brain structures. We also found that this deficit in the chronic restraint stress animals was moderated when the animals received a context shift at the point of transfer, which suggests that chronic stress has an impact on contextual encoding and retrieval in the hippocampus.

