**Ethanol Self-Administration and Conditioned-Place Preference are Altered in Desensitization-resistant Cannabinoid 1 Receptor ($CB_1$) Mutant Mice**

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2010 CISAB REU Program Abstract

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The endogenous cannabinoid signaling system is involved in modulating the motivation to consume a wide range of drugs of abuse including ethanol. Recent work has found that alcohol preference and self-administration is attenuated, while sensitivity to acute ethanol is enhanced, in mice lacking the neuronally expressed $CB_1$ receptor. In this study, desensitization-resistant $CB_1$ "knock-in" mice were used to determine if this "hyper-sensitive" form of $CB_1$ on ethanol sensitivity, self-administration, and conditioned-place preference. The $CB_1$ receptor in these mice contained two point mutations where serines at residues 426 and 430 have been substituted for non-phosphorylatable alanines (S426A/S430A). This mutation was designed to prevent receptor desensitization by blocking phosphorylation of the receptor by G-protein coupled receptor kinases. Ethanol self-administration was examined in wild-type and S426A/S430A mice using a two-bottle choice paradigm. Mice were given stepwise increases in ethanol concentration every 3 days (3%, 6%, and 7% ethanol). Consumption and preference for 7% ethanol was determined by weighing bottles containing water and ethanol every 7 days. Conditioned-place preference for 2g/kg ethanol was also measured. Wild-type mice demonstrated the expected post-conditioning preference for the ethanol-paired chamber. In contrast, S426A/S430A mice exhibited an unexpected conditioned-place aversion to ethanol. Locomotor activity (in the CPP apparatus) was measured to determine whether alterations in activity could account for the conditioned-place aversion in the S426A/S430A mutants. Although S426A/S430A mice exhibit hyperactive locomotor activity prior to ethanol conditioning, this hyperactivity is lost during conditioning trials. S426A/S430A mice were also found to be significantly more sensitive to the hypothermic effects of a 2 g/kg ethanol injection. This study further demonstrates the ability of the endocannabinoid system to modulate ethanol consumption and preference. However, in contrast to other studies using mice with elevated endocannabinoid levels, we find that mice with a "hyper-sensitive" $CB_1$ receptor are more sensitive to ethanol and exhibit conditioned-place aversion to ethanol.

**Dietary lipids increase activity levels and behavioral stress in zebrafish (*Danio rerio*) exposed to a social stimulus.**

2010 CISAB REU PROGRAM ABSTRACT

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Lipids decrease social stress and stabilize mood in humans. We tested for a similar effect in male zebrafish by measuring whether supplemental dietary lipids decreased stress in fish exposed to a social stressor. In contrast to our prediction, we found that supplemental lipids increased activity levels and the time it took subject fish to recover from a mild disturbance, regardless of social treatment. Repeated visual exposure to novel fish increased activity levels of male zebrafish but did not alter behavioral measures of stress. We are conducting further analyses to determine whether our social manipulation influenced levels of urinary cortisol. These results suggest that although male zebrafish behavior is influenced by dietary lipids, the specific direction and interaction with social context may differ from that in humans.

**Lipidomics on the Hypothalamus and Midbrain after Acute Peripheral Inflammation**

2010 REU Animal Behavior Abstract

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The endocannabinoid system, along with playing a regulatory role in sleep, immune function, appetite, and mood, has also been shown by several studies to play a central role in the inflammation and pain pathway. However, studies that have documented the analgesic effects of endocannabinoids and structurally similar lipids have focused on only a small number of endogenous lipids. For this reason, by comparing the relative concentrations of endocannabinoids and structurally similar lipids present in the hypothalamus and midbrain between rats that received injections of the inflammatory agent Carrageenan and rats that either received no injections or only injections of the vehicle, this study was undertaken in order to gain insight into the family of endogenous lipids that play a role in the pain response and to characterize endogenous lipids that have not yet been identified in the pain response. Through using lipid extraction and HPLC/MS/MS methods, this study found that significant changes in the levels of 2-arachidonyl glycerol (2-AG) in the hypothalamus and midbrain, arachidonyl ethanolamide (AEA) in the hypothalamus, palmitoyl ethanolamide (PEA) in the hypothalamus, and oleoyl ethanolamide (OEA) in the hypothalamus occur in response to chronic pain. Furthermore, elutions from these lipid extractions are currently in the process of being analyzed using HPLC/MS/MS methods for significant changes in the concentrations of over 100 endogenous lipids, many of which have not yet been analyzed in the context of the pain response.

**Lessons Developed to Teach Third Grade Students Scientific Investigation Design Related to Animal Behavior**

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2010 CISAB REU Animal Behavior Abstract

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Indiana's academic standards for science presently contain three standards with emphasis on scientific investigation design, scientific thinking, and the living environment. Through students' curiosity about living things, and how living things relate to each other, what they are like, and how they behave, I plan to teach my students how to become a scientist and researcher as we observe animal behavior. Animal behavior is the scientific study of everything animals do, and it is an expression of an effort to adapt or adjust to different conditions. Through the unit of lessons created, my goal for this project is to allow my students to take the role of a scientist and researcher to make connections as a result of their observations of Zebrafish behavior. Tanks with small groups of Zebrafish will be set up in the classroom to support this project. Lessons and activities will focus on immersing the students in practicing making observations, designing investigations, and forming theories based on evidence gathered. At the conclusion of our lessons and activities, we will take a trip to *WonderLab: The Museum of Science, Health, and Technology*, where we will follow up our work with scientific design and animal behavior. The students will participate in an animal behavior lab, and complete a teacher adapted *Wonder Challenge*, with emphasis on animal behavior. The students will be given opportunities to use scientific design throughout the school year as a routine way to answer questions based on our observations.

**Seasonal sociality and territoriality in sparrows correlate with neuropeptide and aromatse anatomy**

2010 CISAB REU Program Abstract

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Song sparrows (*Melospiza melodia*) and field sparrows (*Spizella pusilla*) exhibit different nonbreeding season (winter and autumn) social behavior. Field sparrows are territorial during the breeding season but flock during the nonbreeding season, whereas song sparrows are territorial year-round. The mechanisms responsible for maintaining territoriality in the nonbreeding season are poorly understood. The purpose of our study was to examine seasonal and species differences in socially-relevant neuropeptides and enzymes in the medial bed nucleus of the stria terminalis (BSTm), a brain region that plays an important role in the neuroendocrine regulation of both territoriality and flocking. Brain tissue was immunocytochemically labeled for multiple socially relevant neuropeptides, including arginine vasotocin (VT; homologue of vasopressin), mesotocin (MT; homologue of oxytocin), corticotropin-releasing factor (CRF) and vasoactive intestinal polypeptide (VIP); in addition to tyrosine hydroxylase (TH; the rate-limiting enzyme for the synthesis of catecholamine neurotransmitters) and the steroidogenic enzyme aromatase (ARO). ARO is essential for the production of estradiol from androgens and is essential for winter aggression in song sparrows. In our study, free-living male field and song sparrows were exposed to simulated territorial intrusions (STIs) during both breeding season and nonbreeding season, and latency to respond measures were recorded for each individual. Relative to spring birds, winter birds showed lower optical densities of ARO, VT, VIP, and CRF immunoreactivy (-ir) in the BSTm, with similar trends for MT. Field sparrows exhibited higher winter densities of VIP than did song sparrows, suggesting a potential relevance to flocking. Furthermore, we found negative correlations between optical density and response latency for VIP-ir and VT-ir in both species and ARO-ir in song sparrows. These data suggest that VIP, VT, CRF, MT, and ARO may have roles in mediating seasonal aggression and flocking in sparrows.

**Electric organ discharge modulations in Brown ghost knifefish (*Apteronotus leptorhynchus)* and the effect of pheromones**

2010 CISAB REU Program Abstract

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Brown ghost knifefish produce electric organ discharges (EODs) that allow them to locate objects in their environment and communicate with each other. By modulating the frequency of the EOD, the fish can produce 'chirps' to communicate with nearby fish. Male brown ghosts use chirps in male-male competition and female displays.

Electric signals are one sensory modality well-studied in electric fish, but not much is known about other ways of communication. Other species of fish use pheromones to communicate. These progestins 17,20βP; 17,20βPS; and prostaglandin PGF2α are three hormone derived pheromones that are used to signal the reproductive condition of a female fish. The aims of this experiment were to observe male brown ghost knifefishes' chirps in response to different EOD signals while in the presence of these three different pheromones and a cocktail of all three.

In experiment design one, same day testing was performed on each male in the presence of nothing, the vehicle used to dissolve the pheromone, and the pheromone. Repeated stimulus on the same day created habituation that confounded the ability to detect potential effects of the pheromones. The experiment was revised to consecutive day testing, which eliminated the habituation.

With the revision, there was no significant effect of pheromone treatment on the amount of chirping. Our findings suggest that these pheromones do not affect the amount of chirping in a brown ghost knifefish.

**The ontogeny of huddling in C57BL/6 mouse pups**

2010 REU Animal Behavior Abstract

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Huddling behavior is seen in many diverse species. By huddling together, animals can conserve body heat and reduce the amount of energy used to achieve normal body temperature. Rodent pups, typically born within large litters, are not merely one among many, but survive and behave as a single entity (Alberts, 2007). Recent findings from my laboratory (Harshaw & Alberts, personal communication) demonstrate group regulatory behavior in 8-day-old rat litters, whereby the pups interact so that they adaptively regulate the surface area of the group in relation to the ambient temperature. We studied the effects of temperature change on huddling in postnatal day (PND) 2, 4, and 8 C57BL/6 mouse pups. The goal of this research was to determine whether group regulatory behavior is present in PND 2 and PND 4 C57 mice, as it is in PND 8 litters. Data collected in this research showed PND 8 mouse litters demonstrate group regulatory behavior and alter the surface area of the litter in accord with ambient temperature. We observed a clear developmental trend, in which C57 mouse pups showed improved correlation of their huddling parameters (surface area and perimeter measurements) with increased age, between PND2 and PND8. 4-day-old pups showed significantly better correlation of their huddle perimeters (but not areas) with the change in ambient air temperature than 2-day-old pups.

In Experiment 2 of my summer research we continued the study of coordination of behavioral and physiological thermoregulation (brown adipose fat activation) during early development in C57 mice. During cold exposure, sympathetic activation of BAT warms blood that is then directed toward the heart, lungs, and cervical spinal cord, thus providing much-needed heat to critical organs during even modest thermal challenge (Smith 1964; Hull and Siegel 1965). In newborn rats, BAT's primary importance for autonomic thermoregulation is highlighted by the fact that these animals are hairless, show little vasomotor control, and cannot shiver until after they are 10 days old (Taylor 1960; Spiers and Adair 1986). Thus in this research we are using infrared thermography to measure the intra-scapular and rump temperatures of pups during cold challenge and quantifying BAT activation as $T_{IS}$ - $T_{rump}$. Future research will examine the coordination of physiological and behavioral thermoregulation during early development in C57 mouse pups.

**Lipids Affect the Behavior of Some Strains of Zebrafish: Geographic Origin Plays a Role**

Mia V. Pertilla, Mayté Ruiz, Emília P. Martins

2010 CISAB REU Program Abstract

Department of Biology & Center for the Integrative Study of Animal Behavior; Indiana University

Dietary lipids play a vital role in animal physiology and behavior, often enhancing courtship and reproduction. Zebrafish are a biomedically-important model organism, originating from India, Bangladesh, and Pakistan. They reside both in heavily- vegetated, still water near the edges of shallow lakes and in the clear running water of rocky hillside streams, two habitats likely to differ in the availability of dietary lipids. Here, we manipulated dietary lipids of zebrafish from four recently established strains: two from clear-water stream populations and two from murky lake habitats. After two weeks of manipulation, we found that higher lipid intake increased activity level and courtship behavior of fish from murky, but not clear-water strains, suggesting that lipid metabolism and behavioral response to lipid availability may be adapted to the habitat in which the animals evolved.

**Neuromodulators and Behavior: Social Regulation of Serotonin in the Auditory Midbrain**

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2010 CISAB REU Program Abstract

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Serotonin (5-HT) has been shown to fluctuate with social interaction between male mice. The current study examines the social interactions between pairs of female mice and the relationship the exhibited behaviors have with serotonin in the inferior colliculus as well as other factors such as age and mass. Findings include variation among serotoninergic response of the resident mouse to a conspecific but no change in non-social behavior. Extracellular levels of serotonin correlated with digging and proximity. Age and mass each had correlations with specific behaviors. Overall, these data support a hypothesis of a more complex role of 5-HT in social interaction among female mice as a model organism. This theory would go beyond a simple motor-activation response and could possibly be understood better as a correlate of a social activation index.

# The relationship between estradiol and stress, positive & negative affect, sex role, and self-esteem in men and women

2010 CISAB-REU Program Abstract

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The purpose of this study was to investigate the presence of possible associations between levels of the hormone estradiol and behaviors such as self-esteem, positive and negative affect, sex role, and perceived stress in men and women. From an evolutionary perspective, it would be advantageous for behaviors that may help attract mates to correspond with ovulation. Estradiol peaks at ovulation, therefore a shift toward desirable values could be associated with a higher probability of securing a potential mate. We hypothesized that high estradiol levels would correlate with high positive and low negative affect, low stress, high self-esteem, and androgynous sex role. Saliva samples were collected from male and female participants for three days. Participants completed a questionnaire that included the Positive and Negative Affect Scale, Perceived Stress Questionnaire, Rosenberg Self-Esteem Scale, and Bem Sex Role Inventory. Saliva samples were combined and estradiol levels were measured using enzyme immunoassay. None of the four hypotheses were supported by this experiment. This contradicts recent literature that indicates statistically significant associations between some behaviors and estradiol levels, but may indicate that our specific behaviors are not influenced by estradiol. For future studies, an in depth analysis of female hormone levels may yield more information about the relationship between these behaviors and estradiol.

**The Brain-body connection: Does mRNA expression in the brain predict phenotype?**

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2010 CISAB REU Program Abstract

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A fundamental question in biology is whether behavior, morphology, and physiology evolve via correlated evolution, or whether each aspect of phenotype can evolve independently. Hormones, such as testosterone (T), likely connect many aspects of phenotype, since hormones have both organizational and activational effects on a variety of traits. It is not known, however, whether an individual's ability to respond to T at a cellular level is also correlated with other aspects of T-mediated phenotype. To better understand the evolutionary mechanisms shaping hormone-mediated traits, we asked whether tail white, a variable which correlates with circulating levels of T, co-varies with an individual's ability to respond to T in neural target tissues. As a measure of an individual's ability to respond to T, we measured levels of neural aromatase mRNA (AROM) relative to GAPDH, an established house-keeping gene. We expected either a tightly integrated system arising from correlated evolution or a system where T-mediated traits evolved independent of one another. Likewise, we expected either total or non-existent co-variation between neural gene expression and plumage to depend on whether or not natural variation in these traits existed. We found pronounced sex differences in tail white and also in AROM expression in nucleus taeniae (nT) confirming gene expression and plumage variation between sexes. Males however, did not co-vary tail white with AROM expression in the hypothalamus, while female tail white was significantly correlated with AROM expression in the hypothalamus. These preliminary results confirm known sex differences in morphology and provide new evidence for difference in neural coding. Furthermore, the results hint at possible sex differences underlying the brain's effect on phenotype, and that the degree of integration or independence of T-mediated traits in the brain and in phenotype may be sex dependent.

**Effects of Estraiol on Electrocommunication Behavior in *Apteronotus albifrons* on Populations of Peru and Columbia**

2010 CISAB REU Program Abstract

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*Apteronotus albifrons,* a weakly electric fish, produces electric organ discharges (EODs) used for communication and electrolocation. EODs are characterized by species-specific frequencies (EODf). Modulations in EOD frequency and/or amplitude are called chirps and are used in social interactions. Chirps fall into two categories, based on chirp frequency modulation (FM), called big and small chirps. EODf and chirp duration (length of each chirp) are sexually dimorphic in *A. albifrons* and sexually monomorphic in chirp rate and chirp FM. Our goal is to test the effect that estradiol has on these sexually dimorphic behaviors. The predictions were that EODf would increase and chirp duration would shorten (feminize) in response to estradiol due to these behaviors being sexually dimorphic. Fish were fed estradiol injected earthworms for three weeks. EOD responses were recorded by carbon electrodes that were placed at the head and tail, and at the sides to deliver a playback stimulus mimicking the presence of a conspecific fish. We found that elevations in blood levels of estradiol did not cause a feminization in EODf. Estradiol treatment increased chirp duration and chirp FM in small chirps, contrary to our predictions. No significant change was found in chirp duration, rate or FM in big chirps. These findings suggest that EODf and chirp behavior may be regulated by a hormone other than estradiol in *A. albifrons*.

**Leptin increases endocannabinoid receptor expression in Siberian hamsters (*Phodopus sungorus*)**

2010 REU Animal Behavior Abstract

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The regulation of energy balance is vital to the survival of all animals. Sex differences in the regulation of energy balance in mammals may be expected due to the high energetic demands of pregnancy and lactation experienced only by females, and should therefore be taken into consideration when studying mechanisms of energy balance. Two important orexigenic and anorexigenic factors are endocannabinoids and leptin, respectively, and both demonstrate sex differences in Siberian hamsters. Specifically, male hamsters generally have higher brain $CB_1$ receptor expression than females, and females have higher levels of circulating leptin. Additionally, deficient leptin signaling in other rodents is associated with upregulation of $CB_1$ receptor mRNA and protein, and increased endocannabioid levels in the brain. To determine whether differential leptin signaling accounts for sex differences in $CB_1$ expression in metabolically-relevant brain regions, we tested the effects of elevated leptin on $CB_1$ receptor expression in male Siberian hamsters. We administered injections of either murine leptin (1mg/kg), or saline (0.9%) to the animals for five days. Brains were extracted and analyzed using immunocytochemistry specific for the $CB_1$ receptor. We found that hamsters that received exogenous leptin showed higher levels of $CB_1$ receptor expression in the paraventricular nucleus, caudal area postrema, and nucleus of the solitary tract. The results suggest that leptin does affect $CB_1$ receptor expression but not in the directed that we hypothesized.