

Olfactory discrimination capabilities of the Dark-Eyed Junco (*Junco hyemalis*)

2009 REU Animal Behavior Abstract

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Recently olfactory cues have proven important for foraging (Nevitt *et al*, 1995), nest recognition (Minguez, 1997), and nest maintenance (Petit *et al*, 2002) in various avian species; however, the role of olfactory cues in avian social interactions is still unknown. Chemical analyses have revealed differences in volatile components in preen oil between different species, different sexes and between different populations of conspecifics (Soini, unpublished), but behavioral studies have yet to test a songbird's ability to discriminate among these groups using olfactory cues. We ran multiple habituation-discrimination trials using subjects and preen oil donors of the species *Junco hyemalis*, a small passerine species. Each trial was filmed for scoring and videos are currently being analyzed via ODlog software. We predict that *Junco hyemalis* can discriminate among the scents of conspecifics vs. heterospecifics (specifically, brown-headed cowbirds, a known brood parasite), own vs. different populations, and same vs. opposite sex. Preliminary data suggest that juncos are able to discriminate between the odor of conspecifics and the odor of cowbirds. Findings from this study may not only increase our understanding of avian sexual selection, but could lead to a better understanding of brood parasite recognition as well.

Female mate choice for condition-dependence and genetic compatibility

2009 REU Animal Behavior Abstract

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Although natural selection is one mechanism that can lead to evolution of a species, sexual selection in the form of mate choice can be a strong selective force affecting polymorphism of a population. Female mate choice is accomplished through condition-dependent mating when females look for indicator traits, assortative mating targets males who are genetically similar, or disassortative mating that selects males who are genetically dissimilar from the females. Using a prosobranch snail species, *Potamopyrgus antipodarum*, females were tested for condition-dependent and disassortative mating. Snails were grown from juvenile to maturity in designated environments for appropriate trials. After snails were identified and isolated, reciprocal mating pairs were created and then videotaped at identical times. Data was collected from the number of contacts, rejections, and matings and later used in a McNemar's test for data analysis. Condition-dependent assortative mating was not shown among females since there was no preference for males of better health by choosing males based on state of health. Disassortative mating was not shown among females since there was no preference for males of greater genetic dissimilarity.

**Relative dependence of rats on allocentric vs. egocentric cues in locating
a safety platform in a water maze or a food location in an arena**

2009 REU Animal Behavior Abstract

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Spatial navigation is a key to survival in many animal species, from humans to birds, rats and insects. Two common ways to orient one's self are through egocentric search, which is based on starting at a specific location and following a specific route defined as a sequence of oriented responses; and allocentric search, which is defined by approaching a specific location defined by a set of surrounding landmarks after starting at any location within sight of the landmarks. In Experiment 1, I studied the effect of egocentric vs. allocentric cues on rats' ability to find an escape platform in a water maze. The results showed that initial learning of an egocentric strategy did not interfere with subsequent learning of environmental landmarks. In Experiment 2 I examined whether the use of self-motion cues was influenced by an environmental light cue on rats trying to find a food location in a large dark room. It was found that the removal or displacement of the light cue had no effect on the rat's ability to find the food. In conclusion, after training, an egocentric strategy, based on a specific route or self-motion cues, appears to be independent of environmental cues.

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Does food supplementation enhance immune function in reproductive female Sagebrush Lizards, *Sceloporus graciosus*?

2009 REU Animal Behavior Abstract

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The immune system is vital in maintaining health and longevity for reproductive success, yet it is costly to maintain. Thus, trade-offs may exist during energetically taxing periods, such as during the reproductive season. However, trade-offs can be context-dependent. In this study, we consider the role of food availability on immune function in female sagebrush lizards. Specifically, we tested whether food supplementation could eliminate energetic trade-offs between immune function during the reproductive season. We experimentally manipulated food availability in a natural population of female sagebrush lizards. We measured immune function by calculating the bacterial killing capability of collected plasma exposed to *E. coli* *ex vivo* and compared bacteria-killing capabilities in the food-supplemented and control group. We found no significant difference in the number of bacteria killed between the test groups. Thus, we found no difference in immune function between food-supplemented and control female lizards. This finding suggests excess energy gained from food supplementation may have gone towards other physiological functions rather than immunity during the reproductive season.

Interspecific differences among three local entomopathogenic nematodes

2009 REU Animal Behavior Abstract

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Spatial distribution of a species is determined by its abiotic and biotic environment, plus its behavioral responses to this environment. We examined three species of insect-parasitic nematodes (in the genus *Steinernema*) found in sympatry on a hill at Indiana University's Research and Teaching Preserve, Moore's Creek. In 2007, the three species were found segregated by altitude along the hillside. This summer, we returned to the hill at Moore's Creek to examine the microhabitat variation of each species. We sampled the soil to determine temperature and moisture differences across six sites. The two sites furthest downhill had significantly more moist soil and higher maximum temperatures. Thus, we hypothesize that these abiotic factors may be limiting the nematode expansion. We also examined the soil samples for potential arthropod hosts to see if the biotic environment among the sites was variable. We found the following potential host species: six diptera, four coleoptera, and one diplopoda. There was no difference in arthropod species or density across the sites, so further arthropod field surveys are needed to make inferences about the potential natural hosts of each species. Two behavioral assays were conducted to study foraging strategy differences among the three species. In the first behavioral assay we examined the ability of the nematodes to travel horizontally through 1, 5, or 10 cm of soil to a host. One species was shown to be significantly more infective over a longer distance. Secondly we inspected the jumping behavior of each of the nematodes, and found only one nematode of the three species was able to jump. Our results show that abiotic environmental differences plus the behavioral responses of the nematodes to the environment could be related to the spatial distribution on the hillside.

Habitat and sex differences in task performance in zebrafish

2009 REU Animal Behavior Abstract

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The zebrafish, or *Danio rerio*, is a small, striped freshwater fish found in India in lakes (rice paddies with murky and slow-moving water) and rocky hill streams with clear water. Males and females are very similar in morphology and behavior but have small size and color differences. The objective of this project was to test males and females from both lake and stream populations in the ability to accomplish two tasks associated with foraging for food and predator avoidance. Our hypothesis was that stream fish would be more skilled than lake fish at finding food and avoid predators, and we expected few differences between males and females. We placed one small male and one large female in each of 50 testing arenas, and used either a red or blue card on the back of the tank to mark the side. We then presented each pair of fish with either food (foraging) or a moving stick (avoidance) on the marked side of the arena, and recorded the position of each fish for 20 min. We repeated the procedure three times/day for 2 days, and then shifted to the alternate task so that each pair of fish was challenged with both task types. We found that females performed better than males at both tasks, that some zebrafish may have learned to associate the color card with the better side of the tank, and that there was no significant difference between lake and stream zebrafish in performance of either task. Future studies are needed to determine whether the body size also played a role in our results.

The effects of cat odor on unlearned and learned defensive behavior in responding and non-responding Sprague Dawley rats

2009 REU Program Abstract

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Cat odor is the most common predator odor used to study unlearned defensive behavior in laboratory rats. Our data continues previous work showing that rats encountering cat odor introduced on a cloth or collar near food in one arm of a familiar Y maze produces prompt withdrawal from that arm. Withdrawal to the cat odor is more pronounced and persistent than to the cloth. Most importantly, the cat odor conditions defensive behavior to the exposure arm. On subsequent exposures to the apparatus the rats show continued avoidance of that arm, and continued wariness in the form of “head flagging” from a distance. We have also discovered that other biological odors, such as mint, produce initial withdrawal by the rats, but show little evidence of subsequent (conditioned) avoidance of the location of the mint. Finally, it appears that the Sprague-Dawley strain of rats is divisible into responders (rats exhibiting the full set of avoidance reactions to cat odor and avoidance to mint), and non-responders (rats who continue to eat food in the presence of the mint or cat odors on subsequent trials). This suggests the possibility of a more general deficit with respect to the perception and/or effect of odors in this subset of rats.

The functional effects of endocannabinoid signaling on Siberian Hamsters across photoperiod

2009 REU Program Abstract

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Siberian hamsters are a seasonally breeding species that undergo seasonal changes in energy balance, losing ~30-40% of their body mass in short “winter-like” days. These changes are driven primarily by changes in food intake, but the underlying neuroendocrine mechanisms are still unknown. Hypothalamic peptides traditionally attributed to regulation of energy balance give little explanation for this seasonal change. Endocannabinoids (EC), specifically those acting at CB₁ receptors, have been linked to the regulation of ingestive behavior in other rodent species but the functional effects of endocannabinoid signaling on Siberian hamsters has yet to be tested. To determine the functional effects of EC signaling we injected animals in long days and short days with either a CB₁ receptor agonist (ACEA) or antagonist (SR141716) for five days and measured food intake and hoarding. We found that blocking CB₁ receptors decreased food intake in long day animals only. Also, we found that long day animals have a greater orexigenic drive than short-day animals, i.e. they eat and hoard more than animals in short day. These findings suggest that endocannabinoid signaling may affect Siberian hamsters differently across photoperiod.

Maternal Contributions to Sensory Experience in the Fetal Mouse (*Mus musculus*)

2009 REU Animal Behavior Abstract

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A late-term fetus' intrauterine world is replete with sensory experiences, much of them derived from maternal behavior and physiology. To identify specific tactile and vestibular stimuli to which the mouse fetus is exposed, I observed maternal contributions to the prenatal sensory environment. Using videographic analyses, five categories of maternal behavior that provide to fetal tactile and vestibular stimulation were quantified. Pregnant dams were observed during the last week of a 19-day gestation period, during which time the emergence of tactile, vestibular and thermal function develops and there is a marked decline in amniotic fluid volume. My findings indicate that the general activity level of pregnant mouse dams is similar to that of non-pregnant females; nevertheless, the distribution of behavior differed during pregnancy. Grooming and nest building behaviors were more frequently displayed by pregnant mice, whereas rates of stirring and rearing behaviors overlapped. In conclusion, the uterine environment is highly stimulating to fetuses late in gestation. Thus, the fetus experiences accelerations during general activity, angular accelerations and pressure during rearing and grooming, as well as bursts of compressions and vibrations associated with stirring and scratching

Effects of food and social manipulation on zebrafish (*Danio rerio*) behavior

2009 REU Animal Behavior Abstract

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Many factors influence how animals respond to novel stimuli such as conspecific intruders or potential predators. For example, male vertebrates that have higher testosterone levels tend to be more aggressive and less wary of predators. Animals may be less aggressive in the presence of abundant food, possibly because they are satiated and less willing to fight over resources. Animals with higher food intake may also become more aggressive, because they have additional energy that can be used for aggression. In this experiment, we manipulated food availability and social context for two weeks to test the relative importance of these factors on subsequent aggression and wariness. We found an interaction effect between these two factors. Zebrafish that had been fed less than normal responded most dramatically to differences in social context. Specifically, those that had been exposed daily to potential conspecific intruders (in the form of a mirror presentation) were less aggressive and took longer to recover from a disturbance than did zebrafish that had not received the daily mirror presentations. In contrast, there was little, if any, effect of the mirror presentations on zebrafish that received abundant food. It appears that food stress accentuates the impact of social stress. Our next step will be to determine whether food and social manipulation also had an impact on steroid hormones, specifically cortisol and testosterone.

Social interactions alter serotonin levels in the inferior colliculus

2009 REU Animal Behavior Abstract

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In the inferior colliculus (IC), a site of convergence within the auditory system, serotonin changes the way that auditory neurons respond to sound, including communication vocalizations. Can social conditions, in turn, influence levels of serotonin in the IC and, if so, what aspect of a social interaction influences this change? To address these questions, we used *in vivo* voltammetry to measure levels of serotonin and behavioral analyses to examine social interactions between pairs of male CBA/J mice. Levels of IC serotonin were measured in a mouse while it: a) freely interacted with another male mouse, or b) interacted with another male mouse in a limited way, through a clear Plexiglas barrier. When mice were allowed to freely interact with one another, serotonin levels in the IC increased. Consistent changes in serotonin were not seen, however, during limited social interactions. Further, though a mouse's behavior changed during limited social interactions, levels of serotonin in the IC were not correlated with digging, rearing or motor performance. These results suggest that the qualities of a social interaction that influence levels of serotonin in the IC are not transmitted during limited social interactions and that physical social cues or reciprocated social behaviors are correlated with the neuromodulation of auditory processing.

Prenatal social stress affects Siberian Hamster (*Phodopus sungorus*) offspring social behavior and physiology

2009 REU Animal Behavior Abstract

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Prenatal stress has recently been shown to be a risk factor for adult diseases later in life, suggesting that the gestation period is a vulnerable time for mammals. The *in utero* environment is changed by the physiological stress response of the mother. These changes may affect the development of the offspring, resulting in modified physiology and behavior in adult life. Using a Siberian hamster model this study focuses on the effect of social stress on maternal behavior and physiology of an expectant mother and how this will affect adult offspring social behavior, hormonal response and immunity. We monitored mother's agonistic behavior such as submission, grooming and attacks, maternal behaviors, and pup growth. Treatment did not affect the mother's agonistic and maternal behavior or pup growth. We also tested agonistic behavior in adult offspring, serum glucocorticoid levels (i.e., cortisol) and innate immunity using a bacteria killing assay. We have found that prolonged prenatal social stress on Siberian hamster (*Phodopus sungorus*) offspring affected submissive behavior toward a same sex conspecific in female adult offspring while male offspring were not affected in this measure. Grooming, number of attacks and serum cortisol levels by control and prenatally stressed adult offspring were not significantly different. This study provides essential information for our understanding of nervous and immune system interaction in mammals including humans.