The Role of Recency and Memory Load in Olfactory Working Memory

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Working memory is the transient holding and processing of new and stored information. Common examples of tasks used to model working memory in nonhuman animals include the Morris water maze, the radial arm maze, and delayed alteration. A novel task used to model working memory, the odor span task (OST), is an olfactory non-matching to sample task developed by Dudchenko, Wood, and Eichenbaum (2000). Working memory is typically considered to have a limited capacity; however, OST studies of working memory in nonhumans have largely found no debilitating decrease in accuracy as memory load increases (April, Bruce, & Galizio, 2013). Given the nature of non-matching to sample tasks as well as the rats' sensitivity to odors, the OST allows for the number of stimuli to be easily manipulated in search of an effect of memory load. However, memory load and recency are inherently confounded; as memory load increases, it becomes more likely for a randomized comparison odor to have been presented less recently. This study aimed to investigate a possible capacity to olfactory working memory by expanding the total number of stimuli to 101 odors while separating these confounding variables. As in the aforementioned study, rats chose between a novel odor and a single, comparison odor from a gradually increasing set of old odors. The recency of comparison odors were randomized, allowing us to independently measure the effect of recency and memory load on accuracy. While no significant main effect of memory load or recency was found, we did observe group difference of recency. Our findings support the notion that memory load may not influence olfactory working memory accuracy while suggesting the possibility that the decrease in accuracy found in some studies of olfactory working memory as memory load increased were due to an effect of recency.

Individual Variation in Thermal Physiology and Maternal Care

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Parent-offspring interactions are complex, dynamic and reciprocal. The actions of one influences the actions of the other and consequently their interaction. Successful parenting results from the parents' attraction to the infant, appropriate infant-directed care, and successful solicitation by the infant. Dependent offspring can influence the amount of care they receive with a suite of behaviors and physiological traits. Here, we used rodents as a model to understand how individual differences in infant thermal physiology influence maternal care. In two experiments with Postnatal Day 4 litters of C57Bl/6J mice we quantified the amount of maternal care received by the warmest and coldest pup in that litter. In the first experiment, using an infrared camera we selected the warmest and coldest pups from each litter. Subsequently, in the second experiment we selected pups at random and anesthetized them to manipulate their body temperatures and eliminate ultrasonic vocalizations. We scored maternal investigative behaviors and pup retrieval order. The mothers did not show pup dependent differences in investigative behaviors. Moreover, the mothers were able to discriminate between warm and cold pups; mothers retrieved the warmer pup first 67% of the time. This type of study may give us insight to possible reasons why mothers show more care towards certain offspring and may be the beginning to understanding how pups can influence maternal care.

How Changes in Flow Rates and Visual Stimuli Affect Shoaling Behavior in Zebrafish, *Danio rerio*

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A wide variety of animal species use multimodal systems for increasing accuracy in social communication, predator detection, searching of mates, and ultimately to adapt to their environment. Zebrafish are a good example of an animal species which uses such multimodal perception to adapt quickly to external changes. They have also become a popular model in use with biomedical research, their visual response systems resembling those of humans. Our experiment aims to expand the literature and knowledge of the underlying mechanisms of their sensory systems, in the hope of learning more about the evolutionary, environmental, and biomedical implications of shoaling in Zebrafish. In the first portion of the experiment, we tested 10 groups of two and four zebrafish of equal male to female ratios in a flow tank under different flow and spatial frequencies. Once all the groups of fish were subjected to the stimulus, the movements were analyzed using Ethovision® Software as well as manual scoring. The data will be interpreted using R statistical software. It is expected that, for higher rates of flow, there should be higher cohesion in both groups, and at a certain threshold of special frequencies, the fish should create a positive reaction to the visual stimulus. This information will give insight on the evolutionary adaptive mechanisms behind zebrafish behavior, and in the future, may allow us to predict what will happen to zebrafish populations in the wild as a result of climate change, as well as update the models used for biomedical research.

Electrophysiological Recording of Basolateral Amygdala in Animal Model of Negative Urgency

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Negative urgency, the tendency to act rashly when in a negative affective state, has gained attention as a predictor of substance abuse. Although negative urgency is associated with substance abuse, studies have yet to examine neural substrates underlying this trait. Recently, an animal model of negative urgency called the reward omission task (ROT) was developed in which a reward is unexpectedly omitted, creating an aversive state, and subsequent responding for the reward is recorded. To investigate the neural correlates of negative urgency, the current study implemented electrophysiological recording in basolateral amygdala during the ROT to examine differences in neural activity during omission versus non-omission trials. In Phase 1 (Pavlovian), rats were trained to associate a light and tone with the presentation of a sucrose pellet reward. In Phase 2 (operant), rats were trained to press a lever on a fixed-ratio 10 (FR-10) schedule of reinforcement for delivery of a sucrose pellet. In Phase 3 (combined Pavlovian/operant), we presented a sucrose pellet following the light and tone, and, directly thereafter, the lever was extended for a 45 s interval in which the rat could respond on a FR-10 schedule for sucrose pellets. Rats were then surgically implanted with microelectrodes in basolateral amygdala and will be tested using the same procedure detailed in Phase 3. We expect to find differential basolateral amygdala activity during the omission trials compared with the non-omission trials, as well as an increased rate of responding and total number of responses following omission trials.

Effects of Early Life Stress on Adult Neuronal Morphology

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The stress response is an evolutionary adaptation that allows humans and animals to prepare for a fight or flight response. Furthermore, unpleasant memories acquired during stress are remembered with higher recollection than neutral stimuli, which suggests an enhanced encoding of memories during stress. Therefore, chronic stress can have strong effects on subsequent anxiety-like behaviors. Early life stress in humans can be a key risk factor for the development of psychiatric disorders, which are more prevalent in women. The amygdala, a brain structure critical for emotional learning and processing, is believed to play a central role in stress mechanisms. In recent studies of adult male rats, dendritic spines on pyramidal neurons in the amygdala increased in density after acute exposure to stress. Here we examined the effects of early life stress (15 mild footshocks on post-natal day 17) on spine density in the basolateral amygdala of adult male and female rats. We predicted that stressed rats would show an increase in spine density in the basolateral amygdala, with female rats exhibiting a more robust increase in spine densities compared to male rats. Spines were counted on terminal branches and were classified as thin, stubby, mushroom, or branched, according to standard morphological criteria. Although stress did not alter spine density overall, early life stress increased branched spine densities in female, but not male rats. This may indicate that females have the ability to more easily learn stress-induced fear, and could help explain why women have higher rates of psychiatric disorders than men.

Does Social Experience Influence Serotonergic Fiber Density in the Auditory System?

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Past social experience plays an important role in shaping how receivers process social signals. In the auditory system, serotonin is a potential mechanism for linking social experience with auditory processing for three reasons: 1.) serotonin influences single neurons' response to auditory stimuli; 2.) changes in serotonin reflect contextual features of social encounters, and 3.) the serotonergic system is sensitive to social experience in other brain regions. In this study, we investigate how past social experience affects the serotonergic fiber density of the inferior colliculus (IC), an auditory midbrain region that demonstrates selectivity for species-specific vocalizations. Male CBA/J mice (*Mus musculus*) were given different social experiences by using a classic manipulation: housing for four weeks either individually or in a social group of three mice. Subsequently, we used immunohistochemistry with florescence to label the serotonin transporter (SERT) as a marker for serotonergic fibers. Tissue sections were imaged, and ImageJ was used to quantify the fiber density as the percent area of the IC covered by fibers. We expect there will be a negative correlation between serotonergic fiber density for individually verses socially housed mice. A difference in serotonergic fiber density would suggest that the mice differ in the ability to release serotonin, which could lead to differences in the ability of serotonin to reflect social information in this auditory center.

The Evolution of Sexual Dimorphism and Communication in Electric Knifefish

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Behavioral, molecular, and hormonal mechanisms work together to impact sensory processes, communication, and mate choice. This study investigates the evolution of communication and sexual dimorphism through an analysis of intrasexual variation in behavioral and molecular mechanisms in *Apteronotus leptorhynchus*, or the brown ghost knifefish. Although knifefish behavior is well described, gene expression studies in the brain are fairly novel, and few have looked at the correlation between the two within individuals. The brown ghost knifefish has unique, sexually dimorphic communicative behaviors that can be systematically quantified by measuring its electric organ discharge frequency (EODf) and chirp rates (rapid frequency modulations). Furthermore, quantifying gene expression of hormone receptors in the pacemaker, whole brain, and gonads can test molecular dimorphism. In this study, we investigated the hypothesis that intrasexual variation in behavioral and molecular traits will be higher in males than in females due to sexual selection pressures. The behavioral results support this hypothesis. We confirmed behavior is dimorphic in brown ghost knifefish, both in terms of baseline frequency and chirp number. Males have higher baseline frequencies and chirp rates than females. In addition, there is significantly more intrasexual variation in chirp rates within males. Molecularly, we found estrogen receptor ESR2A was expressed in gonads, but not the pacemaker regions of either sex, preliminarily suggesting perhaps receptor gene expression levels are not dimorphic. This work supports the hypothesis that behavioral variation is greater in male knifefish, but the presence or absence of molecular dimorphism in the brain could not be confirmed.

Why are Big Chicks Better than Small Chicks?
A Look at Immune Function in Tree Swallow (*Tachycineta bicolor*) Nestlings

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Behavioral ecologists oftentimes use proxies for fitness because fitness is difficult to measure directly in the wild. For example, offspring mass in songbirds often predicts survival into subsequent years, so mass can be a good proxy of quality/fitness. However, the mechanisms underlying this phenomenon are unclear. We hypothesized that immune function may influence chick quality and explain why smaller chicks are less likely to survive. To test this hypothesis, we measured mass and feather length in 148 chicks from 33 tree swallow nests at sites around Bloomington, Indiana. We took blood samples from a subset (n=65) to look at gene expression of interleukin-6 (IL-6), a cytokine involved in inflammatory responses. We predicted that larger chicks would have higher IL-6 expression, suggesting that larger chicks are better at fighting off disease. One alternative, that larger chicks have lower IL-6 expression, would instead suggest trade-offs between growth and immune function. We found that larger chicks tended to have longer feathers and came from smaller broods. IL-6 was not related to mass, feather length, date, or brood size. However, chicks with parasites had higher IL-6, and higher chances of mortality than unparasitized chicks. Additionally, chicks tended to be smaller in mass in nests that had parasites. Parasitized, larger chicks also had higher IL-6 than parasitized, smaller chicks, suggesting that when the immune system is activated (i.e., in the presence of parasites), larger chicks perform better. Our findings suggest that measuring immune function could be a way of predicting chick quality/survival in tree swallows.

Basal Strain Differences in $5HT_{2A}$ receptor expression of the Orbitofrontal Cortex and Differential Response to Stress in $MAOA^{Neo}$ and WT Mice

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Monoamine oxidase A (MAOA) is responsible for the oxidation of the monoamine neurotransmitters, serotonin (5-HT) and norepinephrine (NE). Low levels of MAOA result in high levels of 5-HT and NE in the brain and may lead individuals to experience heightened impulsive aggressiveness. Individuals with low MAOA who have experienced early childhood trauma have a higher predisposition to commit violence. In order to model individuals with low MAOA, a mouse model was created ($MAOA^{Neo}$ mice), in which low levels of MAOA combined with maternal separation produces aggressive behavior in adulthood. The mechanisms underlying this gene x environment interaction are not well understood. Densitometry was used to examine $5HT_{2A}$ receptor expression in orbitofrontal cortex in WT and $MAOA^{Neo}$ mice that underwent maternal separation on postnatal days 1-7. Overall, we found a decreased relative optical density in wild type mice that underwent maternal separation, while there was no effect of maternal separation in $MAOA^{Neo}$ mice. Further, we also found a higher optical density in the deep layers of the left hemisphere of unstressed $MAOA^{Neo}$ mice compared to unstressed wild type mice. Finally, maternal separation resulted in an increase in optical density in $MAOA^{Neo}$ compared to unstressed wild type mice. The result suggests basal strain differences in $5HT_{2A}$ receptor expression and differential response to stress in $MAOA^{Neo}$ and WT mice. These differences could contribute to the aggressive phenotype observed in maternally separated adult $MAOA^{Neo}$ mice.

Social Activation of Dorsal Raphe Neurons

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Serotonin (5-HT) neurons regulate the neural circuitry that controls a variety of behavioral processes that include mood, and social behavior. During social interaction between mice, the dorsal raphe nuclei (DRN) release 5-HT into the auditory system. This corresponds with changes of the internal state of the mice. However, there are unresolved questions regarding which part of the DRN is responsible for the secretion of 5-HT during social interaction. We investigated whether changes in the activity of 5-HT neurons in different parts of the DRN fluctuate with the changes of social behavior in mice. We placed male and female CBA/J mice (*Mus musculus*) into a chamber and recorded video of their social interactions. As a control, we placed isolated male mice into a chamber with a novel object and observed their behavior. Social (n = 8) and control (n = 8) males were sacrificed and their brains were prepared for multi-fluorescence immunocytochemistry (ICC). We performed multi-fluorescence ICC to visualize antibodies for the immediate early gene product FOS, a marker for neural activation, in neurons containing tryptophan hydroxylase (TPH) marker for 5-HT neurons. Photomicrographs were taken throughout the extent of the DRN. Our findings show a significant amount of C-fos in the dorsal raphe ventral area of the social animals, when compared to the control group. In social animals, mounting attempts correlated positively with C-fos/TPH colocalization in caudal DRN. Our data support our hypothesis that different parts of the DRN are responsible for the neural response to a changing social environment.