Vasoactive Intestinal Polypeptide and Seasonal Flocking and Aggression in Four Emberizid Sparrows

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During the breeding season, many bird species display territorial and aggressive behaviors; however, while some birds continue to display these behaviors throughout the year, others form winter- flocks. Seasonal variation in group size has important fitness implications, but the neural and endocrine mechanisms that promote seasonal flocking are not well understood. Vasoactive intestinal peptide (VIP) is a polypeptide distributed throughout many brain areas involved in social behavior. Previous work indicates that VIP signaling is important in both grouping and aggression. Seasonally flocking sparrows show higher VIP fiber density during the winter than do non-flocking species in both the paraventricular nucleus (PVN), which regulates a variety of affiliation behaviors, and the medial bed nucleus of the stria terminalis (BSTm), which influences social behavior. In the lateral septum (LS), which influences anxiety and affiliation, infusions of VIP promote territorial aggression. To determine whether VIP receptor regulation is associated with seasonal flocking, we collected brains from male field sparrows (*Spizella pusilla*) and dark-eyed juncos (*Junco hyemalis*), which seasonally flock, and from male song sparrows (*Melospiza melodia*) and eastern towhees (*Pipilo erythropthalmus*), which do not seasonally flock. We collected brains in both spring and winter, and collections were preceded by field-based assessments of aggression. We quantified binding sites of [I125]-labeled VIP in numerous brain areas. All four species show a winter increase in VIP receptor density in all areas examined. In the LS there is also a main effect of species. In the BSTm there is a non-significant trend towards a greater winter increase in binding sites in the seasonally flocking species. VIP receptor density in the BSTm is positively correlated with individual aggression while receptor density in the PVN is negatively correlated with aggression. Receptor density in the LS shows both positive and negative correlations with aggression.

Expression of Arginine Vasotocin in Brain Regions that Influence Sexually Dimorphic Electrocommunication in *Apteronotus leptorhynchus*

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Gymnotiform weakly electric fish produce an electric organ discharge (EOD) which functions as a signal communicating intrasexual competition, courtship and mating rituals, and interspecies and intraspecies disctinctions. Transient changes in EOD frequency called chirps are also important communication signals. In brown ghost knife fish, two types of chirps are produced: 1.) low frequency chirps in which EOD increases 30 to 150 Hz above its baseline frequency used in competitive situations and 2.) high frequency chirps in which EOD increases 30 to 150 Hz above its baseline frequency used in courtship situations. EODs and chirps are regulated by the pacemaker nucleus (PMN) and pre-pacemaker nucleus (PPN), respectively. Arginine vasotocin alters chirping behavior in male brown ghost knifefish by increasing amount of high frequency chirps (courtship) and decreasing the number of low frequency chirps (competition) (Bastian *et al.* 2001). In the present study we will see if AVT is expressed in the brain regions that directly regulate chirping, such as PMN or PPn, or indirectly regulate the chirping through upstream brain regions. We looked for AVT expression in brain regions that may influence chirps directly or indirectly and sought to quantify potential sex differences using immunohistochemistry densitometry techniques. AVT was not expressed in the pacemaker nucleus or prepacemaker nucleus. The AVT antibody labeled cells that produce AVT in the preoptic area and periventricular nucleus. An upstream structure, the lateral hypothalamus, showed extensive expression of AVT as fine fibers and varicosities. Quantification of AVT staining in the hypothalamus to assess potential sex differences in expression is ongoing.

Larger Shoals Disrupt Rheotaxis Performance in Zebrafish (*Danio rerio*)

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Group characteristics, such as size, have noticeable affects on the performance of the aggregate. Collectives can enhance or depress the performance of individuals, either non-additively or linearly. Here we assess the effects of group size on task performance in zebrafish (*Danio rerio*) by evaluating behavioral sensitivity of fish to orient towards water currents, positive rheotaxis. We built a fluvial tank to present multiple constant velocities of water flow to groups of fish. After a five-minute acclimation period groups of four and eight zebrafish were presented a randomly ordered series of three different two-minute water velocities separated by a two-minute inter-trial interval. At all current velocities a greater percentage of individuals in a group of four fish showed positive rheotaxis in comparison to individuals in a group of eight fish. For both group sizes, the percentage of fish showing positive rheotaxis increased in parallel with current velocity. In conclusion, groups of four and eight fish differ in their responsiveness to water currents. These behavioral differences likely arise from an interference effect, where shoal mates negatively affect behavioral sensitivity of rheotaxis.

Habitat Heterogeneity Influences Social Interactions in Zebrafish (*Danio rerio*)

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While grouping (shoaling) is an effective anti-predatory behavior; excessive, unnecessary grouping may lead to competition and aggression among conspecifics. Animals may utilize spatial variability and the accessibility of shelter as an indirect cue of predation risk. The purpose of our study was to test whether shoal cohesion and aggression depend on the immediate availability of refuge or on recent habitat experience. We used Zebrafish (*Danio rerio*), a small freshwater fish native to India, that form small shoals of 4-10 fish and display aggression via chases. We predicted that the fish would be more aggressive in habitats with an increased abundance of shelter, but shoal more in simple habitats where no refuge was provided and the threat of predation is potentially perceived as higher. We found changes in both aggression and shoaling tendency that suggest that zebrafish modify their behavior flexibly to accommodate current and recent surroundings.

Energetic trade-offs between the reproductive and immune systems in female Siberian Hamsters (*Phodopus sungorus*)

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Organisms that live in seasonally fluctuating environmental conditions must adapt to the available energy resources of the present season. The amount of accessible energy in the environment affects the amount of energy available to an organism's physiological systems. Since there is a limited amount of energy available to maintain all of an organism's physiological systems, energetic trade-offs between specific physiological systems occur. In our study, we experimentally limited energy availability to female Siberian Hamsters (*Phodopus sungorus*) with 2-Deoxy-D-glucose (2-DG), a non-metabolizable glucose analog that disrupts cellular utilization of glucose. We observed how treatment with a low (750 mg/kg) or high (1750 mg/kg) dose of 2-DG affected energy allocation to the reproductive and innate and acquired immune systems. Specifically, we predicted that limiting energy availability would lead to decreases in reproductive or immune functions. In addition, a subset of hamsters was treated with leptin, an adipose tissue hormone that provides a direct signal of available fat stores. We predicted that leptin treatment would provide a "false signal" of energy reserves and thus would reduce the energetic constraints imposed by 2-DG. We found that 2 -DG treatment reduced, but leptin did not restore, reproductive mass. Furthermore, neither 2-DG nor leptin treatment affected innate immunity, as measured by a bacterial killing assay. While 2-DG treatment alone did not decrease IgG production in response to a foreign antigen, the combination of the high dose of 2-DG and leptin resulted in decreased IgG production. Our results show that the 2-DG treatment alone decreased energy allocation to the reproductive system, but combined 2-DG and leptin treatment decreased energy allocation to both the reproductive and immune systems. Collectively, these findings suggest that an animal's current energy balance can affect both reproductive and immune responses and that leptin acts, at least in part, to regulate energy allocation among important physiological systems.

Why Do Some Animals Produce More Testosterone Than Others?

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Testosterone (T) mediates a wide range of different phenotypes, including aggression, parental care, mating behavior, and immune response. In other words, T relates to many traits that are thought to be important for survival or reproductive success. Dark-eyed juncos (*Junco hyemalis*) are songbirds that exhibit natural individual variation in the maximum amount of T produced by the gonads. Importantly, this measure of hormonal phenotype predicts survival and reproductive success in the wild, suggesting that variation in T production may be of critical importance to phenotypic evolution. However, a key unanswered question is why individuals vary in T production. We hypothesize that this individual variation in hormones relates to the abundance of certain key enzymes that produce T in the gonads.

In this study we focused on three enzymes: steroidogenic acute regulatory protein (StAR) which transports cholesterol into the mitochondria, cytochrome p450 side chain cleavage (p450scc) which converts cholesterol to pregnenolone, and 3-beta-hydroxysteroid dehydrogenase (3-βHSD) which is a catalyst for the synthesis of pregnenolone to progesterone and dehydroepiandrosterone to androstenedione. Previous studies have demonstrated that these three enzymes are among the rate limiting steps in the production of T. Determining whether these enzymes are linked with T production is a crucial piece of knowledge in understanding how hormones and hormone-mediated traits evolve. Specifically, we asked whether the variation in the amount of T that can be produced by a given individual is correlated with the amount of gene expression for StAR, p450scc, or 3BHSD. T production ability was tested in two populations of juncos using a GnRH challenge, which measures the maximum amount of T that can be produced by a given individual. We used extracted RNA from gonadal tissue to make cDNA. The cDNA was amplified using real time qPCR to measure the amount of starting mRNA that was in the tissue sample. This amount was quantified as a relative amount of each target gene, compared to a standard housekeeping gene using the $2^{-\Delta\Delta Ct}$ method.

Our results showed that StAR transcript was the most abundant of the three enzymes, followed by 3-βHSD, and lastly p450scc. For StAR and 3-βHSD, we found a significant difference in the amount of transcript in the sexes and population, with males having a greater abundance than females and the Virginia (Va) males having a greater abundance than South Dakota (SoDa) males. We also found that the amount of StAR and 3-βHSD positively co-varied with the amount of T produced in the Va population, but not in SoDa. P450scc mRNA abundance showed no significant sex difference, but there was a difference among the populations, with SoDa having the greater amount. However, p450scc showed no correlation with the levels of T in either sex or population. We found a positive

correlation between the amount of StAR and 3-βHSD transcript, but these showed no correlation with p450scc. Our results suggest that StAR and 3-βHSD may be important predictors of T in VA, though there must be some other mechanism regulating the amount of T produced in SoDa, e.g. differences elsewhere along the T synthesis pathway or HPG axis. The results, at least for the Virginia population, suggest that small alterations in gene expression within the gonad could potentially alter hormones or hormone mediated traits. This suggests that there are multitudes of ways in which selection can change hormone levels, and that these mechanisms may even not be conserved across closely related subspecies.

Analysis of Morphine Reward and Dependence in Desensitization-Resistant Cannabinoid 1 Receptor (CB_1) Mutant Mice

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The endocannabinoid system is involved in many physiological processes, including regulation of food consumption and energy expenditure, learning and memory, motor control, pain, and drug addiction. It is also involved in modulating the dependence and the motivation to consume a wide range of drugs of abuse, including opiates. In this study, desensitization-resistant CB_1 "knock-in" mice were used to determine whether a "hyper-sensitive" form of CB_1 potentiated conditioned place preference and dependence on morphine. These mice contain 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 preventing phosphorylation of the receptor by G-protein coupled receptor kinase. In these experiments we measured conditioned place preference (CPP) for 10 mg/kg morphine administered via sub-cutaneous injection. We find that mice with "hyper-sensitive" cannabinoid signaling exhibit a non-significant trend towards increased CPP for morphine relative to wild-type littermates. Morphine dependence was measured by scoring the severity of precipitated withdrawal from morphine in S426A/S430A mutant and wild-type mice implanted with 75 mg morphine pellets. We find that desensitization-resistant S426A/S430A mutant mice showed a non-significant trend towards an increase in the somatic effects associated with morphine withdrawal demonstrating that they have greater physical dependence on morphine than wild-type mice. These results suggest that exaggerated CB_1 signaling increases physical dependence to opiates, but may not affect opiate reward.

Within-Host Competition between Two Sympatric Insect-Parasitic Nematode Species

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Competition between two parasite species can be affected by antagonistic interactions within host individuals, which may be affected by the timing of exposure. We tested the hypothesis that parasites exposed to a host earlier would have competitive advantage over a later infecting parasite using two sympatric species of *Steinernema* nematodes. These insect-parasitic nematodes are in a mutualistic, symbiotic relationship with bacteria in the genus *Xenorhabdus*. Different isolates of each nematode species carry different bacterial genotypes, which vary in their ability to produce antagonistic compounds (bacteriocins) effective against the bacteria of the other nematode species. We examined the outcome of within-host competition between three isolates of each nematode species, S. sp. Clade 4—*X. koppenhoeferi* (genotype 1, 2, or 3) vs. X. sp. Clade 1—*X. bovieni* (type 1, 2, or 3), hereafter KOP vs. BOV. The caterpillar host, *Galleria mellonella*, was infected by placing one isolate of KOP on a host and 22 hours later placing one isolate of BOV, and vice versa such that 18 co-infection treatments were performed (3 KOP isolates x 3 BOV isolates x 2 exposure orders). Additionally, 6 single species infections were performed. The outcome of competition was determined by identifying the nematodes emerging from each infected caterpillar. Nematode species identification was based on size and verified based on DNA extraction. When BOV was exposed to hosts first, BOV emerged from 42% of caterpillars and KOP emerged from 58%. In contrast, when KOP was exposed to hosts first, KOP emerged from 90% of caterpillars. This asymmetry matched the success of the single species infection, as BOV was less successful in emerging from caterpillars. Furthermore, we found that one KOP isolate was less successful than the other KOP isolates, but we found no consistent signature of the bacteriocins. From these results we conclude that KOP was the dominant species; however the timing of infection may lessen its dominance.

Acoustic Trauma and Sertonergic Plasticity: Dealing with Damage in a Noisy World

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Neuromodulation within auditory cortices via 5-Hydroxytryptamine (5-HT) has been proposed as a mechanism by which sound perception is modulated during social interactions. Among the many 5-HT receptors expressed in the inferior colliculus (IC), an integration center of auditory projections, the 5-HT_{1A} and 5-HT_{1B} receptors have been shown to modulate frequency tuning by respectively sharpening and broadening the neuron's frequency response curve. Damaging sensory systems, through auditory trauma can serve as a method to understand 5-HT's importance in sensory regulations. Studies of severe acoustic trauma (binaural, 116dB, 10k Hz tone, 3 hours) have shown that expression of the 5-HT_{1A} gene is upregulated relative to other 5-HT receptors after 4 weeks. We suspect that the expression of 5-HT_{1B} and other proteins decreases after permanent acoustic trauma, although this has yet to be demonstrated conclusively. However, we know little about how a less severe trauma (monaural 116 dB, 10 kHz, 90 minutes) affects the localization of the 5-HT_{1B} in the IC. We used Auditory Brainstem Responses (ABR) to measure relative hearing loss and stained for the $5HT_{1B}$ antibody after short trauma. Qualitative analyses have demonstrated a decrease in expression of the 5-HT_{1B} receptor in the inferior colliculus. This data gives further evidence to support the importance of 5-HT modulation in the brain as a response to sensory damage, and provides tools to advance our understanding of the connection between 5-HT, the inferior colliculus, and social interactions.

The Effects of Sexual Experience on Male Mouse Ultrasonic Vocalizations

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Mice produce ultrasonic vocalizations (USVs) in social contexts that are highly structured and complex. These USVs vary across individual and have recently been shown to correlate with behaviors important to mating; males produce the most harmonic calls when mounting. It has also been suggested that these vocalizations have been indicative of courtship calls due to previous work testing female preference, and USVs eliciting female approach. However, it has been recently found that males with sexual experience produce more vocalizations than naïve males to female urine. These findings lead to the idea that these USVs are learned responses to sexual experience. We tested the differences that sexual experience has on male ultrasonic vocalizations using the same group of males. We explored differences in syllable types, number of syllables, and behaviors usually involved in courtship of naïve versus experienced males. We found that naïve males produce more syllables, and mount more frequently than experienced males. We also found that naïve males produce significantly more harmonic syllables than experienced males. Lastly, there was a significant positive correlation between the percent of harmonic calls in five minutes and the percent time a male spent mounting in the naïve male group. These results were not consistent with past research and suggest that the experienced mice became sated to female presence, after the possibility of receiving too much experience.