Does early postnatal stress exposure lead to sex-specific telomere dynamics in wild tree swallows (Tachycineta bicolor)?

Sol Beltran, Sarah Wolf, Dr. Kimberly A Rosvall

Early life stress can have long-term implications on an individual's behavior, development, and physiology; in particular, early life stressors may influence how animals cope with their environment by 'programming' telomere dynamics. Telomeres are the guanine-rich, protective ends of chromosomes that shorten with age and exposure to stressors (i.e. corticosterone), and can predict measures of lifetime fitness. While previous studies have separately shown stress-induced telomere loss and sex-specific growth rates, few have explored whether stress-induced, sex-specific telomere dynamics occur. In this study, we hypothesized that early postnatal stress leads to sex-specific telomere dynamics in wild tree swallows (*Tachycineta bicolor*) via differences in growth rates and/or stress reactivity. To test this hypothesis, we injected female tree swallows with either saline or lipopolysaccharide (LPS), which elicits an acute immune response, when chicks were five days old. We then sexed chicks, measured growth and telomere length throughout the treatment period, and quantified corticosterone (CORT) after handling stress. Our results indicated that following an LPS-induced reduction in parental care and growth rates, LPS chicks exhibited telomere elongation exclusively in males. While this sex-specific trend was not related to growth rate, LPS male chicks did exhibit lower elevated CORT levels that positively correlated with both 12 day-old telomere length and the change in telomere length over the treatment period. Additional research is necessary to determine the causation of these sex-specific patterns (i.e. parental care, developmental rate, protection mechanisms), which may provide insights into whether sex differences in telomere dynamics shape, and are shaped by, sex-specific life-history trade-offs.

Steroid sensitivity and conversion vary by brain region and breeding stage in a competitive female songbird

Allison Bishop, Elizabeth George, Dr. Kimberly A. Rosvall

Males and females of many species can increase their reproductive success by using aggressive behaviors to compete for limited resources, such as breeding territories. Several studies show that male vertebrates can mediate aggression by elevating circulating testosterone (T) levels (i.e., the 'challenge hypothesis'). Though female vertebrates are sensitive to and can produce gonadal T, they often face steep costs from maintaining high T levels, such as the disruption of parental care. Therefore, we hypothesize that females should regulate aggression throughout the breeding season by producing, modifying, and binding steroids (e.g. testosterone and its metabolites) *locally* in socially-relevant brain regions. To test this hypothesis, we studied female tree swallows (*Tachycineta bicolor*), cavity-nesting songbirds in which females aggressively compete for nesting sites. Though competition is most intense during territory-establishment, breeding females must respond to challenges from non-breeding females ('floaters') throughout the entire breeding season, including during incubation, when circulating T levels are low. Females were previously euthanized during two breeding stages, territory-establishment and incubation (n=5 each), and RNA was extracted from dissected brain regions thought to regulate aggression (hypothalamus and nucleus taeniae), as well as the hippocampus, hindbrain, and cerebellum. Here, we used qPCR techniques to measure the gene expression of steroid-modifying enzymes and receptors (androgen receptor, estrogen receptor α, aromatase, and 5α-reductase), as well as an immediate-early gene (c-Fos), whose expression is a proxy for neural activity. We found that the hypothalami of early-season females showed greater relative expression of steroid-related genes and neural activity than those of incubating females, while relative expression levels in the nucleus taeniae did not differ between breeding stages. These novel findings suggest that females can alternatively use mechanisms that modify and bind to steroids in their brains especially in the nucleus taeniae to mediate aggression while avoiding costs of high T levels, especially during incubation.

Chronic stress induces sex-specific dendritic reorganization in medial prefrontal cortex of adult rats that experienced social instability in adolescence

Michaela R. Breach, Kelly M. Moench, Dr. Cara L. Wellman

Women are more susceptible to stress-linked psychological disorders in which dysfunction of prefrontal cortex is implicated, including depression and posttraumatic stress disorder. Chronic stress induces sex-specific changes in rodent medial prefrontal cortex. For example, apical dendrites of adult male rats retract after 10 days of chronic stress, which is followed by outgrowth after 7 days of rest. Conversely, apical dendrites of stressed female rats exhibit minimal changes throughout the post-stress period. However, little is known about how stress in adolescence affects these sex-dependent stress-induced changes in adulthood. As adolescence is a critical developmental period for HPA axis development and synapse maturation, stress during this time could alter stress-induced changes in the adult brain. We examined dendritic remodeling in the prelimbic region of medial prefrontal cortex of rats who had experienced social instability stress in adolescence, followed by chronic restraint stress with or without a rest period in adulthood. Brains were collected either on the day after chronic restraint or after 7 days of rest, and were stained using a Golgi-Cox method. Dendritic length and spine density analyses revealed a delayed retraction of apical dendrites and an increase in mushroom spine density in chronically stressed male rats. Apical dendritic length was not altered in chronically stressed females, though there was a decrease in mushroom spine density after the rest period. Thin spine density was increased in chronically stressed males and females following a rest period. These results corroborate previous evidence of sex-dependent stress-induced changes in mPFC and suggest that adolescent stress may modulate stress-induced dendritic changes in adulthood.

Gene expression of hormone-related genes influence signal perception

Molishka A. Flores, Melissa R. Proffit, Dr. G. Troy Smith

In many animal taxa, hormones can modulate signal perception in order to detect socially relevant stimuli. Southamerican electric knifefish signal production is modulated by steroid hormones, but little is known on how signal perception is modulated. In electric fish, signal production and signal reception are uniquely linked. Ghost Knifefish are able to sense their environment through perturbations of their own electric signal. Based on previous literature, we know hormones (specifically androgens and estrogens) are shifting the sensitivity of the electroreceptors to match the fish's own signal, therefore we predict that there must be receptors for steroid hormones expressed in the skin. The species used in the experiment (*Apteronotus leptorhynchus*) presents a sexual dimorphism in their electric organ discharge (EODf), therefore we expected to observe a sex difference in gene expression for steroid hormones and a relationship between EODf with the genes expressed. We measured quantification of gene expression in the skin to observe if there are steroid-related genes expressed in skin. RNA was extracted from the skin, converted into cDNA, PCR was performed to confirm steroid-related genes were present and gene expression was quantified using qPCR. We found no sex differences in gene expression for 5-alpha reductase, Androgen receptor β and Estrogen receptor β ($p > 0.05$). There was no correlation between EODf and gene expression in any of our three genes of interest ($p > 0.05$). However, there was a correlation between Gonadal Somatic Index and estrogen receptor β expression in female skin ($p = 0.01$). The results of this study suggest that electroreceptor tuning is likely more related to hormone concentration than to receptor expression.

Oxytocin receptor densities in the nucleus accumbens and medial preoptic area and PPD-associated behavior in *Microtus ochrogaster*

Jennifer Gonzalez, Dr. Allison M. Perkeybile, Dr. C. Sue Carter

Postpartum depression (PPD) is a form of human maternal depression that is diagnosed in approximately 19% of pregnant women and new mothers, affecting not only the mother and her infant, but also members of their social network. PPD may be a result of biological and psychological factors, such as dysregulation of the oxytocin system and lack of social support, respectively. Effects of the oxytocin system on PPD may be analyzed in *Microtus ochrogaster* females, which exhibit many human-like parental behaviors such as a natural reliance on additional caregivers to raise young. This study first observed vole maternal and PPD-like behavior in maternal care observations, sucrose anhedonia, pup retrieval, and open field arena tests, and then investigated connections to quantified oxytocin receptor densities in the nucleus accumbens and medial preoptic area of the same female specimen. These brain regions have relatively high oxytocin receptor densities and contribute towards social and maternal behavior, respectively. It was hypothesized that females with no male assistance would display decreased levels of maternal care and increased PPD-associated behaviors, and would contain lower oxytocin receptor densities. Results indicate that females from both conditions displayed similar levels of maternal behavior in maternal care observations and pup retrieval tests, and also had similar oxytocin receptor densities in the nucleus accumbens and medial preoptic area. However, females with no male assistance displayed decreased sucrose preferences and exhibited greater overall movement in the open field tests, correlating to anxiety-like behavior. Future studies include analyzing oxytocin receptor densities in other brain regions associated with social behavior, as well as investigating regulation of the oxytocin gene. Results from this study may provide insight into the oxytocin system and possible treatments for PPD in humans.

Replay of Items in Context Using Episodic Memory in Rats

Oluwagbemisola Ibikunle, Danielle Panoz-Brown, Dr. Jonathon Crystal

Episodic memory in humans has been defined as the ability to remember when, the context, and the order in which an event occurs. Animals have complex cognition systems akin to humans and rodents have been found to have episodic memory as well as the ability to replay, or remember the sequential order of their episodic memories and the objective content therein, via their behavior. Currently, it is unknown if rats can replay episodic memories of events that occurred in different contexts. In this study, we sought to develop a model of context-dependent episodic memory replay in rats, by testing whether rats can replay items in context using their episodic memory to identify the fourth-to-last odor in a list. We presented the rats with two different variable length lists in distinctive contexts, each list consisted of between 5-12 items. Context-dependent memory assessments immediately followed presentation of both lists. Assessments involved the rats choosing between two odors from the lists. Each assessment took place in a different distinctive context (one for each list). One item will be the fourth-to-last item presented in the list and the other was a randomly selected foil odor that appeared in a different ordinal position in the list. When presented with an on-demand test to find events that occurred in different contexts, rats were above chance for selection of the fourth-to-last odor in both memory assessments. These preliminary results suggest that rats can replay their episodic memories in context; however, further studies must be done to rule out non-episodic memory alternatives.

The effects of prior experience and the role of serotonin in male response to female rejection in mice

Eric Navarro, Kayleigh Hood, Dr. Laura M. Hurley

House mice (Mus musculus) emit two classes of vocalizations, ultrasonic vocalizations (USVs), which have been widely studied, and audible broadband vocalizations (BBVs). During sexual encounters, BBVs are produced by females while physically rejecting males. Female BBV production is predictive of male sexual behavior and is correlated with increased serotonin in the male auditory midbrain. In order to investigate the relationship between prior social experience and BBV perception, 10 males were presented with BBV playbacks after a week of group (n=6) or isolated (n=4) housing. These males were placed on the opposite side of a barrier from a novel female and had USVs recorded for 15 minutes. During that 15 minutes, 5 minutes of BBVs were played back in between two 5 minute periods of silence. Both isolated and group housed males significantly decreased USV production in response to BBV playback ($p<.05$). Social group influenced vocal behavior and response to BBV playback. To investigate the role of serotonin signaling in BBV perception, six additional males were implanted with cannulae to infuse fenfluramine to promote serotonin release, or saline directly into the inferior colliculus (IC), a nucleus in the auditory midbrain. Directly following infusion, males were exposed to a 5 minute BBV playback. Pilot results suggest that serotonin in the IC could be useful to study mechanisms of animal signal perception. Next steps include looking at how prior experience and serotonin work together to change signal perception.

The effects of gut dysbiosis on reproductive physiology and aggression in Siberian hamsters following fecal transplantation

Desiree Nieves, Kathleen Munley, Elizabeth Morrison, Dr. Gregory Demas

A myriad of physiological systems facilitate and modulate social behavior. While it is well-documented that the immune and neuroendocrine systems can directly influence an organism's behavior, recent work suggests that the gut microbiome may communicate with these two systems and the brain via the gut-brain axis. Previous research in our lab has shown that disrupting the gut microbiome via antibiotic administration has sex-specific effects on aggressive behavior in Siberian hamsters (*Phodopus sungorus*). However, the neuroendocrine mechanisms underlying this change in aggressive behavior following gut dysbiosis have yet to be explored. Furthermore, it is unknown whether this behavioral phenotype can be transferred to novel individuals via fecal transplantation. In the current study, we transplanted adult male and female hamsters with fecal matter from donor animals that were administered either sterilized water (control) or an antibiotic (treatment) intermittently for 4 weeks. Following 7 days of treatment, we used a resident-intruder paradigm to quantify changes in aggressive behavior, and we weighed reproductive tissues and used these data as a proxy for reproductive physiology. We found no significant differences in any measure of aggressive behavior across treatments or sexes, including number of attacks, attack duration, attack latency, number of chases, and chase duration. Females didn't exhibit significant difference in ovaries and uterine horns masses between treatment groups. Males mirrored these results when comparing paired testes mass between treatment groups. For both males and females, there were no significant differences in reproductive fat mass between treatment groups. However, females had a significantly lower reproductive fat mass than males, regardless of treatment. Collectively, these results suggest that fecal transplantation alone is not sufficient to alter reproductive physiology or aggressive behavior in novel individuals. More broadly, this study provides insight into the neuroendocrine mechanisms that modulate communication between the gut microbiome and the brain.

The role of arginine vasotocin on aggression and electrocommunication signals in black ghost knifefish (*Apteronotus albifrons*)

Brandi Pessman, Megan Freiler, Dr. Troy Smith

Animal communication is context-dependent due to differential activation of distinctive brain regions that form highly conserved neural circuits in vertebrates. Arginine vasotocin (AVT), the non-mammalian homologue of the nonapeptide vasopressin, is one modulator of this circuit. AVT regulates aggression and communication signals in a species and sex specific manner, but little is known about its effect on the immense diversity of social behaviors across vertebrate taxa. Teleost fishes, the most diverse and ancient vertebrates, make an excellent model for studying the evolution of social behavior mechanisms. Weakly electric fish emit signals from their electric organ to mediate communication. Chirps are brief increases in the electric organ discharge frequency (EODf) that serve as social signals. Although AVT is known to modulate these communication signals in *Apteronotus leptorhynchus*, its effect on the more territorial, closely-related, *Apteronotus albifrons* is not known. To investigate this effect, male-female dyadic interactions of *A. albifrons* were analyzed for EODf modulations and aggressive behaviors after injections of saline, AVT, or Manning Compound. While treatment had no effect on aggression, I saw a strong effect of social experience and status on levels of communication and aggression. Contrary to previous studies that utilized artificial playbacks to elicit chirps, I found males chirped more than females. This highlights the importance of using a more naturalistic approach to study social communication. These results enhance what is known about the variation in context-dependent electrocommunication signals across weakly electric fish species.

Sensitivity to Testosterone in the Avian Eye and its Relationship with Sex, Species, and Trait Differences

Yvette D. Rodríguez Jiménez, Dr. Alexandra Bentz, Dr. Kimberly Rosvall

Certain animal behaviors, like aggression, may rely on sensory perception but the mechanisms mediating this are unknown. For example, if the left eye is covered animals will not respond aggressively, but if the right eye is covered they still will, suggesting the left eye is critical for the expression of aggression. Testosterone (T) mediates different functions of an organism, including aggressive behaviors via tissue level "sensitivity" to T through androgen receptors (AR). Consequently, there could be a link between vision, AR expression, and aggression. However, sex steroid receptors have only been found in the eye of mammals in biomedical studies. Therefore, we examined if AR abundance in the eye is sensitive to circulating T levels and is related to T-mediated traits. We used three bird species (American Robin, *Turdus migratorius*; Eastern Bluebird, *Sialia sialis*; and Tree Swallow, *Tachycineta bicolor*) that differ in phylogenetic relatedness and T-mediated life-history traits, like nest competition and feather coloration. Each species has different feather coloration and studies suggest that both pigment-based and structural colors are influenced by T. We measured AR abundance relative to the housekeeping gene RPL4 in the left lens of all species using qPCR and compared sex, species, and seasonal AR expression. In addition, rump feathers were measured with a spectrometer to explore relationships between hue and AR expression. We found that males presented significantly more AR and there was a trend for AR to change seasonally along with changing T levels in female Tree Swallows, suggesting that AR in the eye could be sensitive to circulating T levels. We also found species differences, Bluebirds and Tree Swallows have more similar expression of AR compared with American Robins, suggesting shared life-history traits rather than genetic relatedness predicts AR patterns. Finally, in male Bluebirds, there was a positive trend between hue and AR, suggesting a link between AR expression and feather color, a proxy of aggressive behaviors. This is one of the first studies to search for and find a relationship between eyes and T, which is a first step in demonstrating the mechanistic link between vision and aggressive behaviors.

Medial prefrontal cortex activation in response to acute stress in male and female rats

Briam Rosado, Dr. Cara Wellman

Males and females respond differently to stress but little is known about how the sexually dimorphic effects of stress lead to psychological disorders. The medial prefrontal cortex (mPFC) is an important region of the brain that is involved in the stress response and executive functions (Radley et al., 2006). The current study will focus on analyzing cellular activity in the mPFC of male and female rats during exposure to acute stress. Activity will be measured using c-fos staining. C-fos is an immediate early proto-oncogene that is expressed when a neuron is active. We hypothesize that females would produce more c-fos than the males and thus have more activity in this brain region. In addition, the stage of the estrous cycle may have a significant influence on the production of c-fos in mPFC. Males (N=12) and females (N=15) were tested. Approximately half of each group were exposed to 30 min restraint stress under bright light and then left alone in their cage for 1hr before being perfused. The rats were euthanized and processed for immunohistochemistry. C-fos-positive cells in the prelimbic region (PL) of mPFC were then counted stereologically. We found that exposure to acute stress significantly increased c-fos expression in the PL in both, male and female rats. Also, results suggest c-fos expression may be decreased in PL during estrous phases in which estrogen levels are high. However, numbers were too low to confirm this statistically. Future projects will be focused on the expression of c-fos in stressed and unstressed females and comparing then throughout the different stages of the estrous cycle.

Modulation of the HPA Axis and Anxiety-Like Behavior Following Fecal Transplantation in Siberian Hamsters

Ayley Shortridge, Kathleen Munley, Elizabeth Morrison, Dr. Gregory Demas

The mammalian gut microbiome can communicate with the brain along multiple neuroendocrine pathways and may play a role in regulating social and affective behaviors. Gut dysbiosis, an imbalance of the microbiome, has been implicated in psychiatric disorders including anxiety. Sex-specific differences in the prevalence of anxiety are well documented, with women at higher risk than men. Previous research from our lab suggests that antibiotic administration disrupts the gut microbiome in Siberian hamsters (*Phodopus sungorus*) and yields sex-specific decreases in aggression. The current study builds on previous work by using fecal transplantation to induce gut dysbiosis. Our objective was to assess the sex-specific effects of a modified microbiome on serum cortisol levels and anxiety-like behavior. Adult male and female Siberian hamsters received fecal transplants from donor animals, which were administered either enrofloxacin or water over two one-week treatment periods. Our results suggest that this manipulation did not affect submissive behavior in recipient hamsters. There was also no significant difference in adrenal mass, body weight or fecal boli count between treatment groups. However, post-treatment serum cortisol levels were significantly lower in treatment animals compared to controls across both sexes. We also found that females had higher serum cortisol levels and greater adrenal mass than males. This study provides insight into anxiety-related sex differences and demonstrates that experimentally manipulating the gut microbiome can affect HPA axis function in Siberian hamsters.

Investigating the relationship between a social trait, bacteriocin production, and virulence in pathogenic bacteria

Valeria C. Toro Díaz, Amritta Bhattacharya, Dr. Farrah Bashey-Visser

Social behaviors of microorganisms can affect host-parasite interactions. These interactions have been studied using virulence, which is the damage caused to a host by a parasite. Theoretical modeling has shown that incorporating key social traits of bacterial parasites such as, bacteriocin production, can affect virulence. Bacteriocins are proteinaceous toxins that can kill closely related competitors and are ubiquitously produced by almost all known bacteria. Model results predict that higher virulence correlates with low bacteriocin production. In this project, we harnessed the power of experimental evolution to directly test this prediction. We used *X. nematophila* populations that were experimentally evolved and showed higher virulence to compare bacteriocin production between an ancestral and four evolved lineages. Four replicate populations within each lineage were examined. Bacteriocins were chemically induced and extracted from the lineages across all replicates. Then, bacteriocin production was estimated using a growth inhibition assay and the extracts were tested against two distinct sensitive strains in the inhibition assays. Our results across both sensitive strains are consistent with the prediction that evolution for increased virulence correlates with decreased bacteriocin production. We are conducting more replicates of the experiment to ensure that these results are reproducible. However, detailed analyses are underway. These results provide, to the best of our knowledge, the first direct test of how bacteriocin production and virulence coevolve in natural pathogenic populations. They also provide an insight into how host health can be affected by social behaviors of pathogenic bacteria.