

# POSTER PRESENTATION ABSTRACTS

## 2022 ANIMAL BEHAVIOR CONFERENCE

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Organized alphabetically by presenter's last name. Numbers indicate poster numbers.

### 1 RELIABILITY OF SPONTANEOUS PAIN BEHAVIOR ASSAYS IN AN ACUTE INFLAMMATORY PAIN MODEL

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Worldwide it is estimated that 1.71 billion individuals suffer from musculoskeletal conditions that are often associated with chronic pain. The ability to replicate spontaneous pain in a laboratory model of musculoskeletal pain is imperative to further understanding the pain pathology and to better assess drug candidate's efficacy. The purpose of this study was to examine knee joint pain using an intra-articular injection of Complete Freund's Adjuvant (CFA) and validate measures of weight bearing associated pain and spontaneous joint pain across two laboratories. Twelve-week-old male C57BL/6 mice were used for this study. Mice were injected in the right knee with CFA (5 $\mu$ l, 1 $\mu$ g/ $\mu$ l) or vehicle control. Knee width was measured using a micrometer before CFA injection, and 1, 2, 3 and 29 days after injection. Spontaneous joint pain was examined using conditioned place preference (CPP) to pain relief induced by peripheral nerve block by intra-articular injection of lidocaine (4% w/v, 10 ml) administered to mice either 24 hours or 4 weeks after CFA injection. Weight bearing was tested following CPP on day 3 and day 29 using a static incapacitance device. A sample size of 31 mice (n=16 CFA; n=15 saline) was used to assess the effectiveness of both behavior tests. CFA significantly increased knee swelling from baseline values on day one and day three post injection ( $p < 0.0001$ ,  $p = 0.003$ ). Saline injection had no effect on knee swelling. CFA mice spent more time in the lidocaine-paired chamber at their posttest ( $p = 0.05$ ) for both laboratories 24 hours after CFA induction but not at the four-week time point; saline mice had no effect at either time point. Similarly, CFA mice developed weight-bearing asymmetry compared to the saline group at the early time point but not at the late time point ( $p < 0.0001$ ). Two different laboratories independently demonstrated that weight-bearing asymmetry and CPP can effectively discriminate pain behavior in an acute inflammatory model of joint pain.

## 2 DOES MIXED-SPECIES FLOCK SIZE AND COMPOSITION INFLUENCE FORAGING AND CALLING UNDER RISKY CONTEXTS?

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Carolina chickadees (*Poecile carolinensis*) and tufted titmice (*Baeolophus bicolor*) regularly form flocks with multiple species, including white-breasted nuthatches (*Sitta carolinensis*), through the winter months. Vocalizations are important for flock connectivity and cohesiveness. Of these vocalizations, the *chick-a-dee* call of chickadees and titmice functions as a ubiquitous multi-purpose signal within the mixed-species flocks. Earlier studies in captive aviary settings found that calling behavior of both chickadees and titmice was sensitive to the relative number of titmice in mixed-species flocks. We tested for the effects of flock composition on seed-taking and communicative responses following an alarm call playback. At our study area in eastern TN, we have 36 feeder sites set up with at least 400 m of separation between them to ensure that each site is in a different flock's territory. After a feeder had been stocked with seed and a flock was exploiting that site, a microphone was set up 1 m from the feeder so that we could digitally record calls at and around the site. We verbally noted seed-taking and calling behavior after the alarm playback had started. For chickadees and titmice, we encoded the seed-taking latency and call latency following the playback. Using linear regression, we found that both Carolina chickadees and tufted titmice had shorter seed-taking latencies as conspecific numbers in flocks increased. Chickadees called sooner after the alarm call playback with increasing numbers of chickadees. Conversely, titmice called sooner with increasing numbers of heterospecifics following the alarm call playback. These results point to individuals' sensitivities to the social context in foraging and seed-taking behavior, and, for titmice, add to the growing literature on the importance of mixed-species flock composition on calling behavior.

## 3 THE SCENT OF A FEMALE: FEMALE OLFACTORY CUES MODULATE ABR AMPLITUDES IN MALE MICE

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Both acoustic and non-acoustic stimuli are influential in the behavior of mice. Incoming olfactory cues can influence mice behavior, while incoming somatosensory and visual cues can influence their auditory processing and perception. In this study, we are looking at whether the olfactory cues of mice conspecifics and predators can also modify the auditory processing of mice. To measure this, we looked at the ABR's of anesthetized male mice while playing complex tone bursts with a frequency spacing reflecting female squeaks. During the presentation of these sounds, we also presented female urine odor and cat fur through an olfactometer. To look for changes in auditory processing, we compared the amplitudes and latencies of waves I-IV from each mouse's ABR. We looked at the differences right before (our control) and after odor presentations. We found that female urine increased the amplitude relative to the control period, but not the latency in waves I, III, and IV, with wave II showing no significance, since this is the smallest wave. This result

suggests that the presentation of female urine caused an increase in the neural response or neural synchrony. In contrast the cat fur changed the latency of wave II only relative to the control period but did not change amplitudes. This result suggests that the presentation of cat fur can cause a delay in the response to auditory stimuli. Overall, these results suggest that olfactory cues may have an effect on auditory processing and eventually behavior.

#### **4 LONELY AND LISTENING: HOW SEX AND SOCIAL ISOLATION INFLUENCE SOCIALITY DURING SQUEAK PLAYBACK**

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During social interactions, female house mice use vocal signals to communicate their receptivity to courtship. Broadband vocalizations (BBVs) are produced by females when they reject male sexual advances. When BBVs are played back, males decrease their courtship efforts, shown by decreasing production of prosocial ultrasonic vocalizations (USVs). In this study, we used a three-chamber social approach assay to evaluate the effects of BBVs on mouse sociability and motivation, comparing this behavior across groups of varying social experience and sex. We used a 10-minute playback consisting of 200 seconds of silence, followed by 200 seconds of BBV playback, followed by 200 seconds of silence. The focal mouse could move freely across three chambers, with one chamber containing a stimulus mouse in a small cage, one chamber with an empty cage, and an empty middle chamber. Trials were repeated with 10 minutes of complete silence. We hypothesized that mice would decrease their social approach and investigatory behavior during BBV playback, and that these behaviors would recover to baseline levels once playback ceased, similar to how male mice modulate USV production in response to BBVs. We also predicted that this trend would be most prominent among male mice compared to females, and among males that were socially isolated compared to those who lived in groups. We found that mice in the playback trials decreased their social investigatory behavior during BBV playback compared to baseline and increased it again after playback ended. This was primarily driven by male mice, as females showed less recovery after playback. Socially isolated males spent less time in the empty chamber and investigating the empty cage throughout the entire interaction than socially housed males. These patterns were not present in the silence-only trials, indicating that during exposure to BBVs, mice modulate their social approach behavior and this modulation varies with sex and social experience.

#### **5 SEX DIFFERENCES IN RESPONSE TO DRUGS OF ABUSE IN ADOLESCENT RATS**

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Compared to males, females may exhibit a greater initial response to the same dose of a drug and show more drug seeking. Moreover, compared to adults, adolescents are thought to be more sensitive to the rewarding effects of drugs. Such differences in drug response may facilitate progress to compulsive drug taking and addiction, serving as determinants of drug addiction and predicting dysregulation of behavior. In this study, we examined sex differences in behavioral response to cocaine and morphine in adolescent rats (N=36), postnatal days 44-51. Spontaneous activity and simple learning (responding on a fixed ratio

5 or FR5) were measured following brief exposure to cocaine and morphine. Rats were housed in groups of 3 on a 12/12 light/dark cycle. The acute drug effects on activity were measured each day for 4 consecutive days, immediately after drug injection. The effects of drug on FR5 were measured during the withdrawal period. In the absence of drug, female rats showed a higher level of spontaneous activity, compared to males. Following cocaine (100 mg/kg), males and females showed comparable spontaneous activity and stereotypy, with a slightly higher activity in males. Cocaine impaired performance on FR5, with a significantly slower response in females, compared to males. Morphine (10mg/kg) substantially decreased activity, with a greater suppression in females than males. Morphine impaired performance on FR5, with a significantly slower response in females, compared to males. Our data suggest that in adolescents, drugs of abuse may produce greater effects in females. Such differences in drug response may reflect a greater vulnerability and facilitate progress to compulsive drug taking.

## 6 A SYSTEMATIC REVIEW OF METHODOLOGIES TO STUDYING BEHAVIORAL IMPRINTING

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Since the classic studies on precocial waterfowl by Lorenz (1935), behavioral imprinting has been described in diverse animal lineages. Due to the varied types of specific processes and the diversity of taxa in which it occurs, researchers have used a variety of methodologies when documenting imprinting. We systematically reviewed the relevant literature to describe the range of behavioral methodologies used to study imprinting across these diverse taxa. After gathering 60 behavioral imprinting-focused papers using a key-word and internal citation driven approach, we categorized them by the different types of imprinting processes studied, the focal species used, the ontogenetic stage addressed, and the methods applied for both training and testing. The largest proportion of studies focused on filial imprinting in precocial birds. Non-filial processes, such as sexual imprinting or territorial imprinting, as well as altricial and non-avian study species were far less represented. Filial imprinting is thoroughly studied through different sensory modalities and applying artificial stimuli while non-filial imprinting studies have mainly used live animals as the stimuli without isolating the relevant sensory modalities. Most studies of filial imprinting employed either spatial proximity, a follow response, or the suppression of alarm calls as a measure for imprinting, whereas most studies of sexual imprinting employed the attempt frequency of sexual behaviors. We also found a one-sided potential publication bias in our literature search output as 94% of our papers reported significant patterns of the addressed imprinting process(es).

## 7 SEXUALLY TRANSMITTED PARASITES AND POSTCOPULATORY SEXUAL SELECTION IN THE *DROSOPHILA-GAMASODES* SYSTEM

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Sexually transmitted parasites (STPs), which encompass a broad spectrum of organisms ranging from mites and nematodes to protists and viruses, are expected to favor the evolution of female mate preference through direct fitness benefits females may receive by

avoiding infected males. Whereas there are several described cases of STPs in insects, little is known about their influence on host sexual selection. We tested whether the ectoparasitic, sexually transmitted mite, *Gamasodes pachysetis*, generates sexual selection in its natural drosophilid host, *Drosophila albomicans*. Mite transmission in the *Drosophila-Gamasodes* system occurs from male to female during copulation. In the laboratory, 419 mating trials were conducted where virgin females were individually paired with a male that was either parasitized or not. All matings that occurred were noted, and copulation latency and duration were measured. The behaviors of a subset of females during mating were recorded and analyzed. It was found that females mated with parasitized males with similar frequency relative to unparasitized males. These results suggest that females do not discern parasite status of their would-be mates prior to copulation. In contrast, significant female postcopulatory responses to the parasite status of males were discovered. During copulation females exhibited a significantly greater frequency of kicking against parasitized males relative to unparasitized males. Interestingly, copulation duration with parasitized males was significantly reduced (by 24%) relative to unparasitized males. The results suggest that females have evolved postcopulatory choice against parasitized males, either to avoid becoming infected themselves and/or to reduce the quantity of sperm received from parasitized males. Experiments are underway to identify the precise fitness advantages that may accrue to females from these pronounced behavioral responses to sexually transmitted *Gamasodes* mites.

## 8 EXPRESSION OF STEROID-RELATED GENES IN SENSORY BRAIN REGIONS OF TWO SPECIES OF APTERONOTIDS THAT DIFFER IN SEXUAL DIMORPHISM

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The production of reproductive communication signals can be modulated by hormones acting on the brain regions that regulate these signals. However, less is known about whether perception is modulated by hormones. The electrocommunication signals of weakly electric fish are sexually dimorphic, sensitive to hormones, and vary across species, making them an excellent model for studying how steroids modulate communication. The goals of this study were to examine (1) whether hormone receptor genes are expressed in sensory brain regions that process electrocommunication signals and (2) whether this expression differs between sexes and species that have different patterns of sexual dimorphism in their signals. *Apteronotus leptorhynchus* (brown ghost knifefish) and *Apteronotus albifrons* (black ghost knifefish) produce an electric organ discharge (EOD) that is used for communication and electrolocation. These species differ in their sexual dimorphism as brown ghost males have a higher EOD frequency (EODf) than females, but in black ghosts, males have the lower EODf. Two brain regions, the electrosensory lateral line lobe (ELL) and the torus semicircularis (Torus), process EOD signals and chirps, a type of communication signal in which fish temporarily increase EODf in aggressive and courtship interactions. Here, we investigate the mRNA expression of genes for androgen receptors (ARa, ARb), estrogen receptors (ESRa), and aromatase in the ELL and Torus in brown ghosts and black ghosts. We extracted RNA from tissue punches from the ELL and Torus and performed RT-PCR and qPCR to confirm expression. Punch locations were verified by

staining the brain slices. Preliminary results confirm that these steroid-related genes are expressed in sensory brain regions and vary between individuals. ARb gene expression was also higher than ARa across all individuals. This suggests that hormones may play a role in modulating the perception of EOD signals.

## 9 CLOSE ENCOUNTERS OF A CRUSTACEAN KIND: PREDATOR-PREY RELATIONSHIP BETWEEN STREAM CRAYFISH AND LARVAL SALAMANDERS

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The predator-prey relationship between the stream crayfish (*Cambarus tenebrosus*) and larval Long-tailed salamanders (*Eurycea cirrigera*) was investigated. We established 6 pool microcosms and placed 15 larval salamanders in each pool. Two pools were designated as controls and did not receive any crayfish, whereas 4 crayfish were placed in each of the remaining 4 pools. Over the subsequent 3-weeks, the position of the crayfish and salamander larvae was recorded. At the conclusion of the trial period, the pools were broken down and the number of surviving crayfish and larvae was recorded. Larvae in the control pools had a 100% survival rate. Despite abundant alternative food, 25% of larvae in the crayfish pools were consumed over the duration of the trials. Additionally, larvae in the crayfish pools shifted microhabitat use to the top of shelter objects relative to their control counterparts. These results suggest that crayfish may be an important stream predator for *Eurycea* larvae and that behavioral modifications, including preferred microhabitat use may be an important predator avoidance strategy by these prey animals.

## 10 NEUROGENOMIC PROFILES OF REPRODUCTIVE BEHAVIOR IN NORTHERN JACANAS

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Differential selection on the sexes may shape behaviors involved in competition and parental care, along with the neuroendocrine phenotypes that facilitate those behaviors. We examined the extent to which aggression and parenting may be mediated by different neurogenomic mechanisms, by comparing females versus males in the Northern Jacana *Jacana spinosa*, a sex-role reversed shorebird. Sex-role reversal stems from a unique case of sexual selection in which females compete for mates and males conduct the majority of parental duties. We found that levels of testosterone in circulation were similar between females and parenting males, and higher in courting males, similar to patterns from species with conventional sex roles. However, testosterone levels did not correlate with aggression for either sex or breeding stage. We hypothesized that sex differences in neural transcriptomic profiles explain sexual dimorphism in behavior. We conducted RNA-Seq in two brain regions within the social behavior network: the nucleus taeniae and preoptic area of the hypothalamus. If female jacanas regulate aggression using conserved mechanisms shared among species with conventional sex roles, we expect to find aggression-related gene regulatory networks enriched for neural sensitivity to sex steroids, along with neurotransmitters like catecholamines and monoamines. Likewise, we expect to find sex and stage-specific differences in the expression of genes that regulate parental care,

including galanin, prolactin, and oxytocin. Alternatively, jacanas may regulate aggression and parental care by way of novel mechanisms, and as such, we may identify new candidate genes. Determining the molecular basis for variation in the sex-specific phenotypes is critical to understanding the extent to which selective forces operate on females and males in parallel.

**11 SHIFTING SANDS: AN ASSESSMENT OF LANDMARK LEARNING POTENTIAL IN AQUATIC RUSTY CRAYFISH**

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In all ecological communities, organisms exhibit different types of spatial learning in order to increase survival rate. Landmark learning, a type of spatial learning, may be critical in aquatic habitats which may be exposed to frequent structural changes such as tides, currents, or flooding. Learning trials were conducted to determine whether rusty crayfish (*Orconectes rusticus*) could instinctually learn to associate a physical landmark (rock) with the presence of food. The results from this study indicate that burrowing crayfish may be able to associate a landmark with food and are likely capable of landmark learning. In addition, varying the total duration of training produced a correlated response, with longer training producing a more intense response and shorter training intervals producing weak reactions. Learning is an important component of survival for many species, and these results suggest that species who occupy unstable environments, such as aquatic habitats, may be capable of rapidly making associations between landmarks and environmental stimuli.

**12 AMBASSADOR SLOTH: IS "GOOD FOR THE SPECIES" ALSO GOOD FOR THE INDIVIDUAL?**

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Over 75% of zoos globally offer visitors opportunities to interact one-on-one with animals. Contact with animals, commonly referred to as Animal Ambassadors, is presumed to increase feelings of connectedness with the species and, perhaps, motivate participants to protect wildlife and the environment more generally. However, such human-animal interactions (HAI) have the potential to cause stress for the animal, which could compromise physical and/or psychological wellbeing. Alternatively, some HAIs may be enriching for Animal Ambassador, thereby increasing quality of life. Guidelines published by The World Association of Zoos and Aquariums recommends that Animal Ambassadors are regularly evaluated for negative and positive effects of HAIs but does not provide a standardized assessment framework. In the present study, we take a multifaceted approach to evaluate the effects of HAIs on a Linnaeus's two-toed sloth (*Choloepus didactylus*) that is featured in the "Sloth Experience" at the Louisville Zoo. During a Sloth Experience, zoo visitors spend approximately 30-45 min inside the sloth habitat and are permitted to approach and pet the back of the sloth as it is hand-fed by a familiar trainer. Cameras mounted inside the sloth habitat recorded randomly selected "Sloth Experiences" and control feeding sessions over a 6-month period to determine whether the presence of visitors affected the latency and/or duration of the feeding sessions. We also recorded the

proportion of time the sloth spent on the branch associated with the HAI. Preliminary analysis indicates the sloth does not avoid the designated branch but also does not spend much time there outside of feeding sessions. When combined with physiological measures of stress (salivary cortisol), these data should provide some indication to how this sloth is affected by interactions with unfamiliar humans.

**13 EFFECTS OF PREDATORY BURROWING CRAYFISH (*CAMBARUS DIOGENES*) ON EMBRYONIC DEVELOPMENT AND LEARNING IN CHORUS FROGS (*PSEUDACRIS TRISERIATA*)**

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Community interactions in an ecosystem can induce morphological and behavioral shifts among co-existing species. In spring vernal pools, burrowing crayfish (*Cambarus diogenes*) and chorus frogs (*Pseudacris triseriata*) interact seasonally through overlapping mating periods and mutual habitat use. We conducted a series of studies on the predator-prey interactions between these species. Recently fertilized chorus frog eggs were exposed to mechanical and/or chemical cues from predatory crayfish and morphological plasticity was assessed. After hatching, the antipredator behavior of the larvae in response to these stimuli was again evaluated to determine if embryonic learning had occurred. Our results indicate that burrowing crayfish are predators on all life-history stages of chorus frogs. In addition, chorus frog embryos utilize both morphological and behavioral plasticity in response to crayfish, likely to reduce exposure to crayfish and minimize predation risk. Despite a relatively brief period of potential interaction, these results suggest a complex predator-prey interaction between these species.

**14 EFFECTS OF MATERNAL MICROBIOME MANIPULATION AND STRESS ON PREFRONTAL CORTEX IN ADULT OFFSPRING IN SIBERIAN HAMSTERS (*PHODOPUS SUNGORUS*)**

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Maternal stress and disruptions of the maternal microbiome during development often have organizational effects on the brain that can alter the development and behavior of offspring. The prelimbic cortex is an adaptive region of the fetal brain that exhibits neural plasticity in response to maternal treatment. While maternal stress and microbiome manipulations can independently alter physiology and behavior in offspring, the potential interactive effects of these maternal factors on offspring development remain unknown. We investigated how maternal stress and disruption of the microbiome during pregnancy can affect offspring neural development in the prelimbic cortex in Siberian Hamsters, *Phodopus sungorus*. Pregnant hamsters were exposed to either a broad-spectrum antibiotic, social stress, a combination of social stress and antibiotics, or no manipulation (i.e., control). Adult offspring (n=58, PND 107-115) were euthanized, brains were stained using Golgi histology, and apical and basilar dendritic lengths of pyramidal cells in the prelimbic cortex were quantified. Our preliminary data suggests that in control hamsters, females have shorter apical dendritic length compared to males, and the combined effect



of maternal microbiome manipulation and stress on apical length in offspring may be sex-dependent.

**15 INCREASING SEROTONIN SYSTEMICALLY AND IN THE AUDITORY MIDBRAIN OF MALE MICE IMPACTS BASELINE VOCAL BEHAVIORS BUT NOT RESPONSE TO REJECTION VOCALIZATION PLAYBACKS**

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Neuromodulatory systems like the serotonergic system can represent salient features of behavioral context within sensory systems, tuning sensory systems to relevant behavioral events. During sexual interactions, serotonin in the auditory midbrain of the male house mouse is inversely correlated to female rejection behavior. One of those female rejection behaviors is the production of broadband vocalizations (BBVs or 'squeaks'). When males are played back BBVs from females that rejected males, they decrease the number of courtship ultrasonic vocalizations (USVs) produced. To identify if this relationship between auditory midbrain serotonin is localized to the auditory system or the result of activation of the serotonergic system generally, we measured male behavior in response to BBV playbacks after being given either a systemic injection of a serotonergic precursor or saline or given a direct infusion into the auditory midbrain of a serotonin releasing agent or artificial cerebrospinal fluid. Males given systemic injections had significantly lower baseline USV production compared to infusion males ( $t=3.5863$ ,  $df=49$ ,  $p=0.0007$ ). Additionally, across both drug delivery methods, controls and males treated to increase serotonin did not differ in their baseline USV production ( $t=1.025$ ,  $df=49$ ,  $p=0.3104$ ), but treatment males had a significantly greater decrease in USV production in response to BBV playback ( $t=3.6375$ ,  $df=49$ ,  $p=0.00066$ ). These results indicate that while systemic drug delivery may reduce overall calling, it does not change the pattern of response male mice display when presented with BBV playback. Regardless of drug delivery method, males with either systemically increased serotonin or serotonin increased in the auditory midbrain showed a greater change in vocal behavior in response to squeak playback, indicating that the serotonergic system in the auditory midbrain may play a critical role in the perception of rejection vocalizations by male mice.

**16 FUNCTION OF CHIRPING DURING SOCIAL INTERACTIONS IN *STERNARCHORHYNCHUS* SPP.**

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An individual maintains or gains access to resources depending on how well they use communication to mediate agonistic interactions. In the unique example of weakly electric fish, individuals use electrocommunication signals to resolve conflicts and establish hierarchies. This is done by chirping, an electric signal that is seen in aggressive displays and courtship. Chirping involves increasing the fish's electric organ discharge (EOD) frequency by several hundred hertz over a short period of time. Weakly electric fish are ideal subjects to use to study social communication and neuroendocrinology because electric signals are

simple to measure. We are studying *Sternarchorhynchus*, a genus of ghost knifefishes. Previous studies on weakly electric fish did not examine natural interactions between dyads of fish and instead used playbacks. We aim to understand chirping in relation to aggressive behaviors by analyzing live interactions. We hypothesized that chirping is used in aggressive contexts as a way of indicating social status. Chirp function may also depend on the social context. Video and audio recordings of seven *Sternarchorhynchus* individuals, each paired two or three times, were captured for a total of twenty trials. The fish that chirped more attacked less, suggesting that chirping serves as a submissive signal. Conversely, the subordinate individual chirped as a stressor signal. Dominants and subordinates both chirped around the time of a dominant's attacks, suggesting chirping is temporally related to aggression. Chirp rate does not vary by social context but in social interactions, females attacked more and therefore appeared more aggressive than males.

**17 THE EFFECTS OF UNFAMILIAR MALE ODOR DURING SQUEAK PLAYBACK ON MALE MOUSE VOCALIZATIONS BASED ON DOMINANT AND SUBORDINATE STATUS**

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Male mouse mice who live within social structures have demonstrated significant alteration of behavior in response to social pressures. During courtship, male mice who differ in social rank also differ in reproductive output and effort which is essential for propagating offspring. Therefore, mice who demonstrate more dominance in their reproductive effort regardless of social context, may then be more successful than their subordinate counterparts. In previous studies, female rejection in courtship has shown significant change in male behavior following the playback of BBVs or broadband vocalizations. We also saw that vocal output by male mice would change as a result of the presence of the scent of another male. In this study, by using USVs or ultrasonic vocalizations by male mice as a measure of reproductive effort, we want to know if the presence of hierarchical partner will have effects on the reproductive effort displayed by dominant and subordinate mice. To observe this, we used male house mice and placed them within a model that allows us to observe behavioral changes during female rejection in the presence of different odor cues. To understand how context can manipulate behavior, we used the soiled bedding as the condition that we'd be altering in this experiment. We used the urinated bedding from a female mouse and that of a male mouse that the subject was previously housed with. We had 2 conditions: the female control which was just the bedding from the unfamiliar female and the other consisted of a mixture between female and familiar male bedding. We hypothesize that social pressures like female rejection and familiar male odor will significantly affect the ultra-sonic vocalization output of subordinate male mice during BBV playback more than it will affect dominant mice. Subordinate male mice will take longer to restart USV production after BBV playback than dominant mice. All males, regardless of social status, vocalized less during BBV playback in the presence of a familiar male odor. We also found that 2 out of 3 subordinate males displayed a disruption in calling, in which they vocalized more in the presence of a familiar male odor. Dominant males also showed a sharper decrease in calling rate in response to BBV playback when in

comparison with subordinates in the presence of a familiar male odor. This indicates that odor cues convey information that disrupts the behavior of male mice and alters their calling rate. This also shows that courtship interactions are highly-context dependent.

## 18 SOCIALLY LEARNED ANTIPREDATOR RESPONSES IN EMBRYONIC MINNOWS

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Antipredator behaviors are important for animals at all life stages. The embryonic stage presents a unique opportunity for animals to learn the identity of potential predators before encountering them after birth, but learning strategies at this life stage are poorly understood. In previous research, we found that embryos behave differently in the presence of predator cues based on proximity to their clutchmates, which suggests that embryos pay attention to each other. In this project, we are investigating whether embryonic fathead minnows (*Pimephales promelas*) show evidence of socially acquired recognition of a predator, bluegill sunfish (*Lepomis macrochirus*). We are using a within-clutch experimental design, with some embryos kept in control water ("naïve" embryos), and others in water with a combination of predator and alarm cue to "train" them to recognize the predator cue ("trained" embryos). This asocial learning phase takes place on days 0-4. The social learning phase, which takes place on day 4, involves pairing trained embryos with naïve control embryos and exposing them to predator cue without the presence of alarm cue. Finally, on day 5, we tested socially trained embryos in predator cue without alarm cue to determine if they would enact antipredator behaviors without the direct presence of a trained demonstrator. When assessing the differences between naïve embryos in social conditioning tests on day 4, in the social learning phase, we see no difference between those tested in control water and those tested in predator water. On day 5, the naïve embryos previously socially conditioned with predator cue show reduced activity compared to those socially conditioned with control water. This research indicates that embryos are able to learn via social mechanisms, and adds to our overall understanding of how and when social learning can develop.

## 19 HOW AND WHY FEMALE AGGRESSION VARIES WITHIN AND AMONG CONTEXTS: A LIFE HISTORY PERSPECTIVE IN THE TREE SWALLOW (*TACHYGINETA BICOLOR*)

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Aggression is a widespread behavior expressed across many contexts, from territorial defense to antipredator protection. The degree to which this behavior is consistently displayed across contexts may be impacted by life history trade-offs, the outcome of which may vary due to condition or age. We explored these issues, focused on consistency in aggression in female tree swallows (*Tachycineta bicolor*). As obligate secondary cavity-nesters and single-brooded birds, tree swallows fiercely compete with conspecifics for access to a nesting territory, and they aggressively defend their eggs and chicks from predators. We used behavioral assays to measure aggression in two contexts, i.e. in the presence of a conspecific or a nest predator. We found that females

were consistent in their degree of aggression *within* a context but not *across* contexts. Younger females were more aggressive towards a conspecific decoy, whereas older females were more aggressive towards the nest predator decoy. Morphological measures, including mass and wing length, did not relate to variation in aggression, but they did explain consistency in an age-dependent manner. Specifically, at least among younger females, structurally larger and heavier individuals were more behaviorally consistent. These relationships suggest that consistency changes over an individual's lifetime or that selection may favor deviations from consistent behavior in an age-specific way. In doing so, these findings provide insight into the causes and consequences of variable degrees of behavioral consistency.

## 20 USING REFERENTIAL ALARM CALLS TO INVESTIGATE MENTAL TIME TRAVEL IN FREE-LIVING SONGBIRDS

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Mental time travel (MTT) is a cognitive process which combines episodic memory and future planning. Although MTT is well-understood in humans, studies of MTT in animals has largely been limited to lab experiments, which lack ecological relevance. We tested the hypothesis that hearing referential “seet” calls on one day causes yellow warblers *Setophaga petechia* to alter patterns of nest vigilance at dawn the following morning when brown-headed cowbird *Molothrus ater* brood-parasitism is most likely to occur. We exposed female warblers to three treatments (by treatments do you mean conditions?): recordings of seet calls, chip calls (general alarm call), and control (silence). We presented playback recordings at warbler nests and monitored their extended vigilance patterns using remote-sensing trail cameras. Although preliminary findings suggest that warblers may alter vigilance patterns according to the type of stimulus they were exposed to the previous day, more research is necessary to obtain a larger sample.

## 21 TIME-ACTIVITY BUDGET OF THE COMMON LOON *GAVIA IMMER* DURING SUMMER

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Animals must navigate trade-offs in many areas, including how they spend their time. These trade-offs can be measured with time-activity budgets, which can vary between species and within them. In July of 2021, I collected observational data on the Common Loon *Gavia immer* at Douglas Lake in Pellston, Michigan during morning, afternoon, and evening activity time periods and analyzed differences between these periods, particularly with regards to foraging behaviors. I found that loons were far less likely to forage during the evening (7%) rather than morning (55%), and that the most common activity during the morning period was diving, while the most common activity during the evening period was resting. Knowing the activity patterns and foraging habits of common loons can help researchers protect the common loon, which is a threatened species in Michigan.

## 22 RATS' RELIANCE ON EPISODIC MEMORY AND FAMILIARITY

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Episodic memory refers to the ability of an individual to recall an earlier event and/or episode. It has traditionally been thought to be unique to humans, however, new research suggests that it may also be evident in nonhuman animals. Exploration of episodic memory in varied species can be used as a novel tool to gain a better understanding of the neural circuitry involved in memory and possibly aid in the development of new treatments for memory disorders. In previous studies that claimed to study episodic memory, familiarity was present. Familiarity is the vague sense that a stimulus was presented earlier. Familiarity is devoid of recollection, which means the stimulus is remembered, but without certainty of details. Due to previous studies neglecting to separate episodic memory and familiarity, it is possible that high accuracy was achieved through familiarity in the absence of episodic memory. Thus, our objective was to evaluate accuracy in diagnostic (familiarity and episodic memory unconfounded) and non-diagnostic (familiarity and episodic memory confounded) memory assessments in order to investigate the contributions of episodic memory and familiarity. It was hypothesized that, in respect to episodic memory, when both episodic memory and familiarity are available, rats will rely on familiarity during non-diagnostic trials. To test this hypothesis, rats were trained using an item in context approach, which dissociates episodic memory from familiarity cues. We concluded that both diagnostic and non-diagnostic trials have above chance and equivalent levels of accuracy. This data suggests that when episodic memory and familiarity are confounded, they contribute equally to performance.

## 23 FRAMING EFFECTS IN PIGEONS' PREFERENCE FOR RISKY OR SAFE ALTERNATIVES

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We examined pigeons' preferences when given the choice between one safe (constant) outcome vs. a risky (variable) outcome, in which each alternative resulted in the same average reward. When both outcomes were framed in terms of loss, pigeons showed a modest preference for the risky outcome. However, when both outcomes were framed as a gain, pigeons did not show a significant preference for either alternative. When neither outcome predicted a net gain or loss, pigeons showed a strong preference for the risky alternative. Finally, when the safe alternative was framed as a gain, but the risky alternative framed as neither a gain nor a loss (despite both outcomes resulting in the same average reinforcement), pigeons preferred the safe alternative. These results suggest that framing effects play an important role in pigeons' preferences for risky vs. safe alternatives. These results have important implications for economic and optimal foraging theories

**24 SEX DIFFERENCES IN PROGRESSIVE RATIO CHOICE TASK IN RATS: FEMALE RATS SHOW SENSITIVITY TO DOPAMINERGIC AND GLUTAMATERGIC DRUGS**

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Motivation is defined as energizing behavior in pursuit of a goal and as such, is an integral component for all behaviors (Simpson et. al., 2016). Motivational dysfunction is associated with several mental disorders, including major depressive disorder and schizophrenia. Previous studies suggest that the neurotransmitter dopamine is important in motivation and blocking the dopamine receptor disrupts motivated behavior (Errante et al., 2021). The neurotransmitter glutamate regulates dopamine pathways in the brain and the glutamate antagonist ketamine reduces depression in humans but may result in neurocognitive deficits associated with schizophrenia when given to rodents. The current study determines whether motivation for a sucrose reward in a choice decision-making task was altered by either the dopamine antagonist haloperidol or the glutamate antagonist ketamine. Male and female Sprague Dawley rats were trained to lever press in an operant chamber using increased fixed ratio over five days. Rats were then trained in a progressive ratio task with a choice of food: sucrose pellet (high reward) provided for lever pressing or traditional rat chow (low reward) available with no effort. After behavior stabilized, rats were injected with saline, ketamine (10mg/kg), or haloperidol (0.1mg/kg), then tested in the progressive ratio choice task. There were significant differences between males and females with females lever pressing more than males during baseline and following a saline injection. Moreover, female rats showed significantly lower lever presses and earned fewer rewards following ketamine or haloperidol injections compared to following saline injections. Rewards earned in males was not changed between conditions. These data suggest that female rats are more sensitive to the effects of ketamine and haloperidol in the progressive ratio effort task with choice. Further research is needed to understand the pharmacology behind these behavioral effects.

**25 ADULT RHESUS MONKEYS DO NOT COPY THE CHOICES OF A CONSPECIFIC SHOWN IN VIDEOS**

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There is substantial evidence of group-specific behaviors in wild animals that are thought to be socially transmitted. Yet experimental studies with monkeys have reported conflicting evidence on the extent to which monkeys learn by observing their conspecifics. In this study, we tested the feasibility of using pre-recorded video demonstrations to investigate social learning from conspecifics in rhesus monkeys. With training, monkeys gradually learned to respond correctly following videos of a demonstrator, however, follow-up experiments revealed that this was not due to copying the behavior of the demonstrator monkey. In generalization tests with videos that were horizontally reversed, monkeys continued responding to the location they had associated with each video, rather than matching the new choice location shown in the mirrored video. When the task was changed to make location irrelevant, such that monkeys could choose correctly only by selecting the same image selected by the demonstrator in the video, observer monkeys did not exceed

chance in 12,000 training trials. Because monkeys readily learn to follow nonsocial visual cues presented on a monitor to guide image choice, their inability to copy a demonstrator here indicates substantial limitations in the capacity for social learning from videos. Furthermore, these findings encourage deeper consideration of what monkeys perceive when presented with video stimuli on computer screens.

## 26 REVERSE CONTINGENCY AND PERCEIVED LOSS IN PIGEONS

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Chimpanzees struggle to learn the reverse contingency task, a task in which two choices are presented to the subject, one a small reward and the other a larger reward. To receive the larger reward, they must point to the smaller one. We tested a version of this task with pigeons. In Experiment 1, pigeons were presented with a choice between 2 pellets or 1 pellet, if the 2-pellet alternative was chosen, they would be prevented from eating the second pellet. If the 1 pellet was chosen, they would be given a second pellet. We found that pigeons learned this task easily. We made the alternatives salient and delayed the choice by 5 s. There are three components to the reverse contingency task: a loss when choosing one alternative, a gain when choosing the other, and the contrast between the reward amount expected and the actual outcome obtained. In Experiment 2, we asked if the pigeons were sensitive to a perceived loss. Pigeons were offered 2 pellets or 1 pellet. If they chose the two pellets, they received only one. If they chose the one pellet, they received it. The pigeons preferred the single pellet offered – the one that did not represent a loss. In Experiment 3, both alternatives were associated with 2 pellets. Choice of single pellet gave the pigeon an additional pellet, while choice of 2 pellets gave the pigeon both pellets. In this case the pigeons also preferred the single pellet offered, the one that gave them a gain. We explain the results in terms of contrast, the difference between what is expected and what is obtained. Although the choice in Experiment 2 as well as in Experiment 3 resulted in equal outcomes, in Experiment 2, the pigeons expected a loss and they rejected it, whereas in Experiment 3, the pigeons expected a gain and they selected it.

## 27 THE BITE FORCE AWAKENS: SEXUAL DIMORPHISM IN HEAD MORPHOLOGY AND BITE FORCE IN *SCELOPORUS* LIZARDS

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Across territorial polygamous reptiles, head shape represents a balance between the forces of natural and sexual selection; larger heads are linked to increased bite force which is important for sexual selection, but may hinder the animal's ability to hide in crevices from predators. Additionally, males and females may be subject to different selective pressures which can lead to sexual size dimorphism. For example, a larger size in males would give them an advantage in male combat, whereas a larger body size in females may be linked to higher fecundity. *Sceloporus* lizards are found across North America, and many species exhibit sexual dimorphism in morphology and behavior. The eastern fence lizard, *S. undulatus hyacinthinus* is found commonly in the eastern United States, and exhibits female-biased sexual size dimorphism. Here we examine the variation and degree of sexual

dimorphism in head shape and determine the biomechanical consequences of differences in head dimension using bite performance as a proxy.

**28 CARRY-OVER EFFECTS AND REPRODUCTIVE READINESS IN A COMMON MIGRATORY SONGBIRD**

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With a net loss of 3 billion North American birds since 1970, and 2.5 billion being native migratory birds, it is critical to investigate population dynamics and threats to species across the full annual cycle of reproduction, molt, and migration. Full annual cycle models are useful tools for better understanding selective pressures both among and between populations, which leverage individual fitness and overall population persistence. Exceptional advances in biologging technologies have made full annual cycle data more accessible than ever before, supporting trends of carry-over effects (COEs), which arise when conditions experienced by an individual in one season influence performance at a subsequent time. This technology has also revealed a great diversity in migration distance, generating questions about whether migration distance may dilute or even counteract COEs. Few studies have addressed the relationship between winter habitat quality and migratory distance in generating beneficial COEs. Here, we propose to investigate COEs by quantifying the relationship between non-breeding habitat quality and migration timing, body condition, and reproductive readiness upon arrival to breeding grounds. We also aim to explore how migration distance and age demographics may interact with COEs. We will explore a new imaging technique for quantifying habitat quality using biologgers and Geographic Information Systems (GIS), validated by carbon isotopic signatures. This research will contribute to the growing knowledge of full annual cycle models, inform changes in population dynamics, and has the potential to explain notable among-population variation in fitness.

**29 THE EFFECT OF A SEROTONIN-MODULATING DRUG ON SOCIAL BEHAVIOR AND AGGRESSION IN A SPECIES OF WEAKLY ELECTRIC FISH**

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Aggression is a ubiquitous behavior across animals, and underlying mechanisms of aggression and other social behaviors are conserved across vertebrate species.

*Parapteronotus hasemani*, the pig-duck knifefish, is a species of weakly electric fish that can generate and perceive electric signals. The fish's electric organ, composed of neural tissue, sends out electric organ discharges (EODs) to communicate information between fish and better navigate murky waters. Serotonin, or 5HT, is a neurotransmitter that has been associated with reduced aggression and the inhibition of dominant social interactions in vertebrates. 8OH-DPAT, a 5HT<sub>1A</sub> receptor agonist, causes an increase in high-frequency EOD modulations called chirps in males in sexually dimorphic knifefish species. However, the effect of 8OH-DPAT has not been explored in *P. hasemani*, a fish that is sexually monomorphic in its EOD frequency (EODf), or in live behavioral dyadic interactions. Little is known about the effect of pharmacological manipulations of serotonin on behavioral



interactions between apteronotid knifefish, as prior studies have focused on artificial playbacks for experiments. This project aims to increase understanding of serotonin's effects on behavior in *P. hasemani* by asking the question: How does the modulation of serotonin with 8OH-DPAT impact aggressive behaviors and EOD modulations in *P. hasemani* fish? Two male fish (n=18 males) were placed in an agonistic dyad interaction after one fish was injected with saline or 8OH-DPAT. Aggressive and dominant behaviors were recorded and analyzed. We quantified lunges, nips, wrestling, and shelter occupancy. Electric signals, including chirps and rises, were analyzed using Adobe Audition. Based on preliminary results, fish treated with 8OH-DPAT appear to engage in fewer aggressive and dominant behaviors than those given saline.

**30 IMPACTS OF TESTOSTERONE AND HAEMOSPORIDIAN PARASITES ON SONG SPARROW SPERM TELOMERE LENGTH**

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Haemosporidians are common vector-borne blood parasites found in songbird populations throughout the world and are the causative agents of avian malaria. Some studies suggest that haemosporidians can negatively affect the reproductive success of their hosts. However, it's unknown if infections in parents can impact the health of their offspring. Sperm telomere length is one possible mechanism that could connect parental infection to offspring quality. Telomeres are the regions of DNA located on the ends of chromosomes that provide protection and stability to the genome. In humans, longer telomeres in sperm are associated with increased fertilization rates and higher embryo quality. Previous studies in mammals have also shown that sperm count and motility are reduced in hosts infected with haemosporidian blood parasites, and that higher levels of testosterone are associated with higher parasite loads. Therefore, we investigated the relationship between circulating testosterone levels, haemosporidian infection status, and sperm telomere length in adult Song Sparrows (*Melospiza melodia*). We predicted that higher levels of circulating testosterone and parasite presence would be associated with shorter telomere length. We collected blood and sperm samples from Song Sparrows captured near Bloomington, Indiana. We then used qPCR to determine telomere length in DNA extracted from sperm, enzyme-linked immunosorbent assays to quantify circulating testosterone levels in plasma, and PCR to determine parasite presence. We did not detect a difference in sperm telomere length between infected and uninfected birds. We also did not detect a difference in testosterone levels between infected and uninfected birds, nor did we find a relationship between testosterone levels and sperm telomere length. We will discuss possible reasons why our predictions were not supported and the potential implications of these results. We will also discuss future steps planned for a follow-up project.

**31 EFFECTS OF FOOD AVAILABILITY AND IMMUNE ACTIVATION ON REPRODUCTION IN HOUSE CRICKETS (*ACHETA DOMESTICUS*)**

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Although investments in reproduction and immune function can both increase an organism's fitness, these two systems often compete for limited supplies of energy. The role that food intake plays in determining these energetic trade-offs in insect populations is still unknown. This study examines how food intake affects the reproductive investment of male *Acheta domesticus* crickets following a lipopolysaccharide (LPS) challenge. We assigned males to food and LPS treatment groups. Following treatment, we extracted each male's spermatophores and determined spermatophore number, mass, and protein concentration. We found that males with food access were significantly more likely to produce at least one spermatophore. LPS did not affect reproductive investment, possibly because the dose was not high enough to fully activate the immune system. These findings suggest that *A. domesticus* populations have evolved a strategy of delayed reproduction to conserve energy under starvation conditions. Future studies should use a higher LPS dose to determine whether crickets also delay reproduction after a severe infection and whether food intake affects that strategy.

**32 DOES IMMUNE PRIMING OCCUR THROUGH MATING IN ACHETA DOMESTICUS?**

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<sup>1</sup>Program in Animal Behavior, <sup>2</sup>Program in Neuroscience, <sup>3</sup>Human Biology Program and Department of Gender Studies, and <sup>4</sup>Department of Biology, Indiana University

Immune priming is a process that allows invertebrates to better react to known immune challenges. We were particularly interested in whether or not male *Acheta domesticus* crickets can pass immune priming information to females during mating, allowing the females to respond more effectively to pathogens present in their environment. While transgenerational immune priming happens primarily through the transfer of genetic material, it is also possible that immune priming would occur non-genetically via components of the seminal fluid. If this is the case, we expect that females who mate with an immune-challenged male will up-regulate their immune response, relative to those who mate with a male who is not undergoing an immune-response. Over a three-day period, we presented male *A. domesticus* crickets with an immune challenge, mated them with female *A. domesticus* crickets, and drew hemolymph samples from the females. We then performed a lysozyme assay on the hemolymph samples by exposing the samples to bacteria and comparing the growth curves. We did not see more antimicrobial activity in hemolymph samples from females mated with immune challenged males than from females mated with non-immune challenged males, indicating that immunological information is not passed from males to females during mating.

**33 SEASONAL PLASTICITY IN NEURAL STEROID SENSITIVITY AND TERRITORIAL AGGRESSION IN SIBERIAN HAMSTERS**

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Many species display pronounced seasonal changes in physiology and behavior, including fluctuations in steroid hormones and social behaviors, such as territorial aggression. Previous studies from our lab suggest that the hormone melatonin, which serves as a

biochemical signal for changes in photoperiod (i.e., day length), is important in regulating the neuroendocrine mechanisms underlying seasonal aggression in Siberian hamsters *Phodopus sungorus*, a species in which both males and females display increased aggression during the non-breeding seasons. The neural actions of melatonin on steroids and aggressive behavior, however, are relatively understudied, and it is unclear whether these processes differ between the sexes. In this study, we investigated how melatonin influences neural sensitivity to sex steroids and aggression in male and female hamsters. Hamsters were housed in long-day photoperiods (LDs, characteristic of breeding season) or short-day photoperiods (SDs, characteristic of non-breeding season) and given timed melatonin (M) or control injections. Following 10 weeks of treatment, aggressive behavior was quantified using a resident-intruder paradigm, and the relative mRNA expression of 3 $\beta$ -hydroxysteroid dehydrogenase, aromatase, and estrogen receptor  $\alpha$  was measured using quantitative PCR in four brain regions associated with aggression or reproduction [the medial preoptic area (MPOA), anterior hypothalamus (AH), arcuate nucleus (ARC), and periaqueductal gray (PAG)]. We expect that LD-m and SD hamsters will have higher levels of aggression than LD hamsters, regardless of sex. In contrast, we predict that males and females will exhibit differences in the expression of genes related to steroid sensitivity in brain regions associated with aggression (i.e., AH, PAG), but not reproduction (i.e., MPOA, ARC). Together, these results will enhance our understanding of how melatonin modulates steroid hormones and aggression in seasonally breeding animals.

#### 34 OXYGEN REDUCES APOPTOSIS IN RETINAL CELLS FOLLOWING TRAUMATIC OPTIC NEUROPATHY BUT DOES NOT REVERSE IMPAIRED OPTOKINETIC RESPONSES

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Vision impairment after head trauma can be mediated by traumatic injury to the optic nerve, termed traumatic optic neuropathy (TON), which has few effective options for treatment. We performed a blunt, closed head injury on 8-week-old, male C57BL/6 mice and investigated a behavioral measure of visual impairment via the optokinetic response (OKR), an involuntary response to moving visual stimuli. In previous studies, we noted post-injury apnea that often led to death after head trauma. Brief exposure to 100% oxygen immediately following injury significantly improved the mortality rate. We then hypothesized brief oxygen exposure may have an effect on the degree of injury in the optic nerve. In the current study, oxygen was administered for a total of five minutes immediately after TON to investigate possible beneficial effects to visual acuity. Data was collected for protein expression analyses (western blotting) and the aforementioned OKR behavior. Visual impairment was tested in an optomotor machine, which utilizes varying black and white bars (visual gratings) to assess OKR function and approximate visual acuity. After injury, mice were allowed to recover for one day, then underwent OKR testing for three days. Mice were subjected to three different gratings, 0.12, 0.26, and 0.39 cycles per degree. We found decreased optokinetic responses in TON mice compared to shams 7 days post injury. A significant decrease in OKR function was found at the widest grating, 0.12,

between TON and TON with oxygen. We have shown that Endoplasmic Reticulum (ER) stress may be a contributing mechanism of retinal cells death, and used Western blotting to examine ER stress and apoptosis markers 7 days post-injury. We found that TON mice given oxygen had reduced levels of Caspase3 and CHOP (both apoptosis markers) in retinal cells compared to TON mice given room air. Ultimately, even when apoptosis in retinal cells was reduced, OKR impairment persisted.

**35 HEAT-INDUCED THERMOREGULATORY BEHAVIORS OF FREE-LIVING TREE SWALLOW (*TACHYGINETA BICOLOR*) NESTLINGS**

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Within the last 25 years, tree swallow (*Tachycineta bicolor*) populations have begun shifting further south into warmer climates, unlike many other species that are shifting north into cooler climates. Nevertheless, how tree swallows behaviorally respond to heat remains unclear. Nestlings are particularly interesting because they are undergoing a critical period of development while confined to the thermal environment of the nest cavity. Our project tests the hypothesis that nestling thermoregulatory behaviors change in response to a sudden increase in nest temperatures. To do this, we experimentally heated nest boxes in southern Indiana, part of the southern half of the tree swallow breeding range. Nest temperatures were elevated 4°C above controls, for 4 hours, using air-activated warmers on the 12th day post-hatch. To assess experimental efficacy, we measured three temperature locations: ambient air, nest box air, and nest cup surface. Nestling behavior during the second hour of experimental heat was recorded and scored. To determine how thermoregulatory behaviors are affected by the immediate environment, we then assessed the relationship between panting, nestling space use, and temperature across the thermal gradient of the nest box. Our results show that birds pant more and move toward the nest box entrance as nest box temperatures rise. We have also begun to explore how thermoregulatory behaviors may trade off with begging behaviors. These data further our understanding of how nestling birds respond to heat within the nest cavity.

**36 DOSE-RESPONSE OF CLOMIPRAMINE ON ZEBRAFISH (*DANIO RERIO*) BEHAVIOR**

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Anxiety disorders are increasingly prevalent in the United States. Although chronic anxiety disorders are often managed with serotonin-targeting antidepressant drugs, acute treatment has been associated with paradoxical anxiogenic effects. Therefore, more research in preclinical models is needed to better understand the role of serotonin modulation in acute anxiety-like states. Zebrafish (*Danio rerio*) are an emerging model for studying anxiety due to their homologous neuroendocrine stress systems. Although antidepressants do not elicit overt anxiogenic responses in zebrafish, previous research indicates that zebrafish display unique surfacing behaviors in response to acute treatment with serotonin-modulating drugs. Surfacing is characterized by alterations in motor activity and increased top-dwelling in the novel tank test (NTT), although whether surfacing reflects

anxiety is not fully understood. Clomipramine is a tricyclic antidepressant commonly used to treat depression and anxiety disorders; however, the effects of clomipramine in zebrafish are unknown. To determine the dose-response of clomipramine on surfacing and anxiety-like behaviors, 55 adult, mixed-sex, wild-type zebrafish were randomly exposed to clomipramine-treated water (0.0, 0.125, 0.25, 0.5 or 1.0 ml/L), followed by the NTT. Clomipramine significantly and dose-dependently reduced the total distance traveled, mean speed, and the number of top zone entries, while time spent in the top, proportion of distance traveled in the top, and immobility increased. Similar to previous research on serotonergic-modulating drugs, our results indicate that clomipramine increases surfacing behaviors in zebrafish. The impact of clomipramine treatment on whole-body cortisol levels and whether surfacing behavior may reflect anxiolytic responses in zebrafish will also be discussed.

**37 CAN FLATWORMS LEARN? AN ASSESSMENT OF MULTIPLE LEARNING MODALITIES IN FLATWORMS (PHYLUM: PLATYHELMINTHES; *DUGESIA TIGRINA*)**

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An organism's ability to learn characteristics of its environment in the presence or absence of certain stimuli is a vital aspect of its ability to survive. However, the level at which certain species can learn is thought to vary along the animal phylogenetic tree. We performed a series of experiments to investigate the learning capabilities of free-living flatworms (*Dugesia tigrina*). Specifically, we tested three forms of learning. First, flatworms' ability to learn via classical conditions was assessed using an electric shock. Second, we tested the potential for operant conditioning by pairing a negative stimulus (shock) with visual stimulus (red light) in an arena. Finally, we tested whether flatworms utilize a form of social learning by exposing flatworms to a substrate in which conspecifics had previously utilized. Our results shed light on the learning abilities of flatworms and contribute to the understanding of the evolution of learning in species with simple neural mechanisms.

**38 LARVAL DEVELOPMENT COSTS OF ADULT BEHAVIORAL RESISTANCE AGAINST ECTOPARASITIC MITES**

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Costs of parasite resistance can serve as important constraints on resistance evolution, while also contributing to the maintenance of genetic variation for this ecologically important trait. We tested for costs of resistance in an insect host in the form of trade-offs between adult ectoparasite resistance and larval feeding and survival. Artificial selection for increased behavioral defensive ability against an ectoparasitic mite, *Gamasodes pachysetis*, was applied in replicate lines of the fruit fly, *Drosophila melanogaster*. After achieving significant responses to selection after 16 generations, we contrasted replicate selected and control (unselected) host lines in the absence of parasitism in larval feeding rate. It was found that feeding rate, measured as the number of cephalopharyngeal retractions per minute, was reduced in selected lines relative to controls. Next, we contrasted larva-to-adult survival between selected and control lines under different experimentally applied

feeding constraints. Here, third instar larvae from the different lines were allowed to feed for five experimental periods of time (49h, 53h, 56h, 61h, and up to the wandering stage of larval development) on a high-quality diet (a 42.5% w/v yeast slurry), and then transferred to a standard low nutrient (agar-only) substrate until eclosion to adulthood. Larva-to-adult survival was reduced among selected lines, but only under the shortest feeding durations, suggesting that these fitness costs are mediated by the fly's nutritional environment. This integrative research provides evidence for the existence evolutionary important costs of ectoparasite resistance in pre-adult traits.

**39 THE BOYS IN BLUE: INFORMATION CONTENT OF COLOR SIGNALS IN THE EASTERN FENCE LIZARD**

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Color patches frequently convey useful information during aggressive male-male encounters, with the patch size often signaling dominance or aggression. Most male lizards in the genus *Sceloporus* have paired blue belly patches rimmed with black borders that are actively displayed during territorial disputes. Territorial disputes will often escalate to fighting, where lizards will bite one another, potentially resulting in injuries. Though males display these two colors simultaneously, previous behavioral work indicates that they may act as separate signals and are interpreted differently by receivers. Here, using territorial male *Sceloporus undulatus* lizards in the field, we examine the relationship between color patch size and bite force, in order to tease apart the information content of these two color signals. We find that blue belly patches do not predict bite force, but the combination of black throat and black belly borders is predictive of bite force. This indicates that in this species, multiple color traits are used to convey different types of information.