TALK ABSTRACTS

2025 ANIMAL BEHAVIOR CONFERENCE

PLENARY

THE PROXIMATE BASIS OF EVOLUTIONARY DIVERGENCE IN PATERNAL CARE IN STICKLEBACKS

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There is tremendous diversity in family life among animals, but little is known about how differences in family life arise over the course of evolution, and which mechanisms are most labile and hence likely to change during the early stages of divergence. Presumably, behavioral divergence requires coordinated changes at multiple levels of biological organization including at the genetic, molecular, neural and endocrine levels, but how and when those changes arise and are integrated together is an outstanding question. In this talk I will present insights that my lab is starting to get into the proximate basis of evolutionary divergence in family life in three-spine stickleback fish. Typically, male sticklebacks exhibit sole paternal care which is necessary for offspring survival. However, an unusual stickleback ecotype has recently evolutionarily lost paternal care and exhibits a suite of traits associated with this divergent reproductive strategy. Quantitative trait locus mapping revealed that that the white strategy evolved through at least nine different genetic changes distributed across the entire genome, each affecting distinct behavior modules. At the neural level, there were differences in the activity – but not the number – of oxytocin neurons in the preoptic area of the hypothalamus. Specifically, in the ancestral caregiving ecotype, oxytocin neurons were activated at the initiation of care behavior, suggesting that these neurons promote the transition into fatherhood. In the white ecotype, activation of these neurons did not change across stages. This suggests that a loss of activation of oxytocin neurons may have contributed to the loss of care. Finally, we observed higher hypothalamic-pituitary-gonadal activity (HPG) in the white ecotype, with evolutionary changes at multiple points along the HPG axis. Altogether these results provide insights into how mechanisms evolve to support distinct behavioral strategies.

KEYNOTE

IT'S A ZOO BENEATH OUR FEET! THE ECOLOGY OF SOIL INVERTEBRATES AND THEIR FUNCTIONS IN CHANGING ECOSYSTEM

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In less than a handful of soil, there can be millions of types of microbes and hundreds of species of invertebrate animals. The identities and natural histories of these microscopic flora and fauna, and many of the larger visible soil fauna, are the least-known biota in terrestrial ecosystems. However, new evidence is rapidly revealing the major contributions of soil organisms to the maintenance of life on Earth, opening a new frontier for exploration and a rising concern about the loss of soil biodiversity with increasing degradation of the soil habitat locally and globally. This keynote talk will present recent evidence indicating that communities of microscopic soil animals such as nematodes, and larger ones such as earthworms, affect the way ecosystems around the globe respond to the changing climate and land use regimes. The talk will showcase recent advances in the field of soil fauna ecology which can challenge aboveground-based predictions of ecosystem functioning under global change.

Remaining talks are alphabetical by presenter last name, in **bold**

HABITAT ENRICHMENT PROMOTES WELFARE BUT ALTERS DOMINANCE HIERARCHIES IN A CICHLID FISH

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Environmental enrichment improves animal welfare by promoting natural behaviors and providing shelters. However, while some studies suggest that enrichment reduces aggression, others indicate it may enhance territoriality which may negatively impact fish that are the target of territorial aggression. In this study, we investigated how habitat complexity influences aggression and markers of animal welfare in male *Astatotilapia burtoni*, a highly social cichlid fish. Within a group setting, *A. burtoni* males establish a dominance hierarchy, where dominant males defend a specific structure as mating territories, while subordinate males show submissive behaviors and refrain from defending territories. This species is used to study physiological and behavioral differences in subordinate and dominant fish. To establish a social hierarchy, we housed two size-different males with six females in one compartment with a defendable structure. We manipulated habitat complexity by placing one, two, or three flower pots close to the corners of the tanks. At the end of the 5-week experiment, subordinate males exhibited higher body condition in more complex environments. The aggression of dominant males varied with the number of defendable structures; however, they maintained territorial status across enrichment levels by sustaining high specific growth rates, gonadosomatic index, and testosterone levels. In contrast, increased habitat complexity elevated testosterone levels in subordinate males, potentially diminishing the physiological distinction between social ranks. Our findings suggest that environmental enrichment influences aggression in dominant males and enhances the welfare of subordinates at the expense of reduced physiological differences between dominant and subordinate fish.

NEST ATTRIBUTES INFLUENCE CHOICE ACCURACY, BUT NOT DECISION SPEED IN ACORN ANT NEST-SITE SELECTION

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Decision making comes with fitness consequences in different aspects of animals' life. For acorn ants *Temnothorax curvispinosus*, deciding on a new nest quickly and accurately can affect the survival and fitness of the whole colony. When evaluating potential nest sites, ants consider attributes like brightness and shapes of the cavity. More information about these attributes can only benefit ants if they can use it quickly and correctly. Previous research has shown that the number of options does not affect ant colonies' decision-making latency, but the types of attributes involved were limited. In our study, we asked whether the increasing number of attributes would affect the accuracy and latency of the colonies' decision-making process. We used pair-wised tests between artificial nests that varied in several important nest attributes. Each pair differed in either one, two, or three attributes, with one option considered less ideal. We recorded the nest chosen by the colonies and the time they spent deciding on a new nest. We found that the degree of differences between nests did not affect the decision-making latency but significantly affected whether ants chose the expected preferred option. This may suggest that ant colonies searching for new nests might be constrained temporally when making decisions, needing to make quick decisions, regardless of nest quality. Interestingly, choice accuracy increased with an increasing number of attributes. This may indicate that when the degree of difference increases, the consequence of making a poor decision might also increase. Such findings emphasize the need to account for the complexity of real-world choice scenarios in natural environments.

ANTIPREDATOR BEHAVIORAL SYNDROMES AND NEST-SITE CHOICE IN A FRESHWATER TURTLE

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Behavioral syndromes, correlated suites of behavior across time or contexts, lend insight into ecological and evolutionary causes and consequences of animal behavior. While descriptive studies documenting the presence of behavioral syndromes across taxa have become more common, fewer studies seek to determine how behavioral syndrome expression may drive broader ecological and evolutionary processes, especially in wild populations. In this study, we leverage the complex nesting ecology of a free-ranging population of painted turtles (*Chrysemys picta*) to determine if and how behavioral syndromes alter nest-site choice in the field. We quantify an anti-predator behavioral syndrome in this species and demonstrate, for the first time, that one measure of behavioral boldness predicts nesting behavior. We document the presence of behavioral syndromes by demonstrating a correlation between behaviors displayed in a simulated predation trial and a novel environment assay in the field. Turtles that displayed more active defenses under simulated predation were faster to explore a novel environment, signaling a consistent behavior type across contexts. Emergence latency, one measure of boldness and exploratory behavior, predicted the extent of canopy cover at nest sites; specifically, bolder turtles chose nest sites with less canopy cover, which has well-documented consequences for nest thermal environments and, subsequently, offspring phenotypes. Together, these results provide further evidence for the presence of behavioral syndromes in this taxon and demonstrate that behavior type should be considered when elucidating the factors that influence nest-site choice and its associated ecological and evolutionary consequences.

MATERNAL BEHAVIOR AND VARIATION IN THE CENTRAL HIMALAYAN LANGUR

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The monkeys of Mandal, India live in a mixed landscape of fragmented forests, agriculture fields, human communities, and pilgrimage paths with an array of other species. *Semnopithecus schistaceus*, the Central Himalayan Langur, are a unique colobine that live in large multi-male multi-female groups and spend a significant amount of time moving between landscapes to meet their social and energetic needs. Mothers are the foundations of life in primates and uniquely shape the development experiences and trajectory of offspring, which can have a profound impact on group success. Mothers exhibit different parenting behaviors, which can be classified into styles based on intra-variation, and respond to stimuli differently, which consequentially shapes their offsprings livelihood and behavior. In this study, I examined the variation of maternal behaviors of 20 *S. schistaceus* mothers from January – June 2024 across landscapes (forest, field, town, road) and in response to anthropogenic events (dog attack, human attack). From this, parenting style profiles can be developed, and we can understand how anthropogenic influences impact maternal behavior, as well as further appreciate the breadth and complexity of motherhood.

EXPLORING THE ROLE OF GLUCOCORTICOID SIGNALING IN THE MOLECULAR, SYNAPTIC, AND BEHAVIORAL EFFECTS OF NORBAEOCYSTIN

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Norbaeocystin is a tryptamine produced by several varieties of *Psilocybe* spp. mushrooms, which has been shown to promote adaptive behaviors in rodents without inducing head-twitch responses. This suggests that norbaeocystin may be non-hallucinogenic at relevant doses, which would present distinct clinical advantages over other tryptamines such as psilocybin. In this context, it is essential to better

understand the molecular and synaptic mechanisms that underlie its behavioral effects. Recent studies have implicated glucocorticoid (GC) signaling, a critical regulator of neuroplasticity, in the behavioral aspects of different tryptamines. Consistent with prior studies we found that a single dose of norbaeocystin (1 mg/kg) promoted exploratory behavior in the open field task and improved discrimination in the temporal object recognition task. Further studies revealed that norbaeocystin stimulates corticosterone concentration within blood plasma 30 minutes after dosing. In addition, 1 hour following dosing, we observe an increase in the activation and nuclear trafficking of the glucocorticoid receptor (GR) in prefrontal neuronal nuclei. To determine if GC signaling leads to synaptic changes, we used Thy1-GFP(M) mice to examine dendritic spine density on pyramidal neurons in the PFC at 24 hours after norbaeocystin administration. Interestingly, we found no change in the synaptic density in the PFC, contrasting reported effects of psilocybin. Ongoing studies are examining additional times points to assess protracted synaptic effects as well as potential physiological changes using patch-clamp electrophysiology. To directly test the role of GC signaling in the effects of norbaeocystin, planned studies will use metyrapone, an inhibitor of GC release. These studies are the first to examine the molecular and cellular pathways underlying the effects of norbaeocystin. In addition, these studies are the first to explore the potential role of GR signaling following norbaeocystin.

DO YOU HEAR WHAT I HEAR? USING PLAYBACKS TO ATTRACT POLLINATORS TO AN ENDANGERED PLANT

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The Hawaiian Islands are home to a myriad of species that are heavily endangered or threatened. *Hesperomannia oahuensis* is an endangered plant native to Hawaii, of which fewer than ten remain in the wild. In order to attract local pollinators, we played calls and songs of native pollinators over the course of three years to examine which birds approached the flower, pollinated, and for how long. Despite previous work showing that the primary pollinators in these forests are invasive birds, we found that the primary pollinator of the *Hesperomannia* was an endangered honeycreeper native to Oahu.

REDUCTION IN AMBIENT LIGHT ALTERS SOCIAL INTERACTIONS AND WEAKENS DOMINANCE HIERARCHIES IN A COOPERATIVELY BREEDING FISH

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The transmission and collection of pertinent social information is a key component of social behavior. However, effective communication between groupmates depends on the properties of organisms' sensory environment. Therefore, human induced changes to the sensory environment can strongly impact communication between members of a social group, potentially altering their social behavior. We varied light levels in cooperatively breeding groups of *Neolamprologus pulcher* to examine how a purely visual change in the sensory environment would impact individuals' social behavior, and the structure of the group. We found that under low light conditions agonistic interactions between dominant females and large subordinates were reduced, which may be due to a reduced ability of dominants to detect nearby subordinates. Related to this, dominance relationships were more poorly defined under low light, which could impact group stability. After light levels were returned to normal, dominance relationships did not fully recover to their original states and agonistic interactions between dominant pairs increased, suggesting long-term effects of the manipulation. If groups of *N. pulcher* have low resilience to fluctuations in the visual landscape, they could be vulnerable to future

perturbations. Our findings show that a relatively weak change in the sensory environment is sufficient to alter communication and social behavior in groups of a highly social fish, highlighting the vulnerability of such groups to anthropogenic disturbances.

WINTER MEANS MEANER: EFFECTS MELATONIN ADMINISTRATION ON OFFENSIVE AND DEFENSIVE AGGRESSION IN SIBERIAN HAMSTERS

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Many animals show seasonal changes in physiology and behavior, including non-reproductive social behaviors (e.g., aggression). Previous studies from our lab suggest that melatonin acts via steroid hormones to regulate seasonal aggression in Siberian hamsters *Phodopus sungorus*, a species where both sexes display increased aggression during winter. Our lab identified a role for dehydroepiandrosterone (DHEA) in regulating territorial aggression via a "seasonal switch", which hypothesizes a shift from gonadal to adrenal regulation of agonistic behaviors across seasons. Here, we test the hypothesis that MEL influences seasonal aggression via DHEA and explore the associated neuroendocrine mechanisms underlying a seasonal switch. Adult males and females were singly housed in long- (LD) or short-day (SD) photoperiods and received daily timed MEL or saline two hours before dark for 2 or 10 weeks. Aggression was assessed using resident-intruder and neutral cage tests to measure defensive and offensive behaviors. We hypothesized that long-term MEL and SD conditions would induce physiological and behavioral changes. Blood samples were collected pre- and post-behavior, and gonads were weighed to quantify reproductive status. SD and long-term MEL inhibited reproduction and increased defensive aggression in both sexes. Melatonin, however, did not alter offensive aggression, suggesting context-specific neuroendocrine regulation of aggressive behaviors. Additionally, DHEA levels increased after agonistic behavior in long-term MEL-treated animals. We measured neural activation by quantifying c-Fos expression in brain regions that have been implicated in aggressive behavior (e.g., lateral septum), predicting territorial aggression circuits are distinct from offensive aggression. Collectively, these findings highlight the role of MEL in coordinating a seasonal switch in defensive aggression and suggest novel neuroendocrine mechanisms underlying aggression in both sexes.

CAN'T YOU BE MORE LIKE YOUR BROTHER? AN EXPERIMENTAL HEAT CHALLENGES REVEALS SUBSTANTIAL VARIATION IN NESTLING THERMOREGULATORY BEHAVIOR

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Temperatures are rising worldwide, killing adult endotherms and their altricial young. Behavior can shield organisms from intense heat, with strategies like shuttling or panting to avoid overheating. However, it is unclear if *all* individuals are prepared to meet this challenge - how much do individuals vary in their use of thermoregulatory behaviors? This knowledge gap is particularly wide for endotherms and their altricial young, for whom heat may be inescapable. Using an experimental heat challenge, we quantified variation in heat dissipation behaviors of individually marked tree swallow (*Tachycineta bicolor*) nestlings. We elevated the ambient temperature of nestboxes by 4°C and scored the behaviors of twelve-day old chicks, namely panting and proximity to cool air from the nestbox entrance. Consistent with prior studies, heat-exposed broods perform more thermoregulatory behaviors. And yet, within these broods, we uncover 4-fold individual variation in behavioral strategies; when confronted with the same heat challenge, some nestlings pant almost constantly while their siblings hardly pant at all. We also uncover 32-fold variation across nests in time spent panting. This suggests that some individuals may be more equipped to mount a behavioral shield against heat than

others. Our future research will explore the underlying causes of this variation – and its consequences – to understand how behavior may alter the pace of adaptation to heat.

MEASURING THE IMPACT OF ARTIFICIAL LIGHT AT NIGHT ON THE BEHAVIOR AND COGNITION OF DARK-EYED JUNCOS (*JUNCO HYEMALIS*)

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Light pollution, or Artificial Light at Night (ALAN), has rapidly increased throughout the world as a consequence of urbanization. The presence of ALAN is a significant and recent development, since life on earth evolved during billions of years of light days and dark nights. Birds are particularly susceptible to ALAN, as most species are diurnal and accustomed to being inactive during dark nights, so exposure to ALAN marks a potentially highly disruptive environmental change. Research to date has largely focused on the impact of ALAN on avian physiology, whereas much less is known about ALAN's potential impact on cognition. Here, across two experiments, we examined the impact of exposure to dim levels of ALAN (3 lux) on the exploratory behavior and inhibitory control of Dark-eyed Juncos (*Junco hyemalis*). In Experiment 1, we captured 40 birds from the wild between November 2023 and January 2024, exposed half the birds to ALAN, and measured birds' behavior on a novel environment test in March 2024. In Experiment 2, we captured 22 birds from the wild in December 2024, exposed half the birds to ALAN, and tested birds' performance on a detour reach task in January 2025. We hypothesized that exposure to ALAN would impair birds' inhibitory abilities, causing them to explore more in the novel environment and to perform more poorly on the detour reach task. While we found no significant effect of ALAN on birds' performance on the novel environment test, there was a marginally significant effect of ALAN on birds' performance on the detour reach task, such that birds exposed to ALAN were less successful at inhibiting their pecking response when presented with a food item in a clear feeding tube. These findings suggest that exposure to ALAN can potentially negatively affect the cognitive performance of wild birds and provide insight into the impact of urbanization on animals.

IMPLICATIONS OF IRON OXIDE NANOPARTICLE EXPOSURE ON THE AUDITORY FUNCTION AND PHYSIOLOGY OF HOUSE SPARROWS (*PASSER DOMESTICUS*)

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Urbanization is a growing phenomenon. With an increase in urbanization, increases in pollution follow. Air pollution is composed of two parts, gasses and solids. Both the gaseous matter and the solid matter are cause for concern, but our work focuses on the solid matter. Particulate matter (PM) is broken into categories by size; the smallest category recognized in the United States is $PM_{2.5}$ including particles 2.5 micrometers and smaller. The nanoparticle is in this category and is small enough to bypass the blood-gas barrier and subsequently the blood-brain barrier. Such barriers are not permeable to most molecules; many are constrained to the outside of the barriers due to size. They are a mechanism the body uses to protect important "highways" within the body; essentially, they do not allow entrance into vital systems to keep the body clear of foreign material. Nanoparticles bypass these protective barriers but the mechanism for this is unknown. Possible explanations for this bypass are through sensory nerves, namely olfactory but potentially through the auditory nerve. Current observational studies in model organisms (e.g., canines and humans) have linked living in urban locations to reduced auditory sensitivity. However, as this is correlational data, there is no experimental evidence to confidently link air pollution to hearing deficits. The aim of this project was to determine the impact of chronic iron oxide nanoparticle (IONP) exposure on the auditory sensitivity of the house sparrow (*Passer domesticus*). House sparrows are songbirds that inhabit the entire urbanization gradient.

Songbirds have particularly thin blood-gas barriers and are sensitive to air contaminants. Together, these factors make them an ideal model for this study. We use an auditory brainstem response (ABR) test to measure auditory sensitivity; these give an index of the response of the auditory nerve to the onset of a sound stimulus. ABRs are performed by inserting subdermal electrical probes and recording electrical potential changes during sound stimuli. The ABR response is quantified based on the response time, the intensity of the response, and the lowest possible intensity of sound that still elicits a response. Sparrows (n=48) were wild caught from locations surrounding Holland, MI, USA and divided between control and treatment (i.e., IONP exposed) animals. We examined ABR latency, amplitude, and thresholds in a pre-test versus post-test design examining the effect of IONP exposure on auditory processing. We predicted that exposure to IONPs would decrease auditory sensitivity. Changes in sensitivity could underlie communication deficits in animals living across an urbanization gradient. This disruption has the potential to interfere with natural anti-predator and mating behaviors, which may impact survival and fitness of avian species. We expect that this work will have the potential to inform air pollution standards for both wildlife and humans alike.

VIP-EXPRESSING MIDBRAIN PATHWAY CONTROLS POTENTIATION OF HIPPOCAMPAL INPUTS TO THE CENTRAL AMYGDALA DURING CONTEXTUAL FEAR LEARNING

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Contextual fear learning is a mental process, during which an originally neutral context becomes associated with a threat. Previous data have shown that one of the main controlling units of the defensive system, the central amygdala (CeA) is an active participant of contextual fear learning. Recently we showed that vasoactive intestinal polypeptide (VIP)-expressing neurons in the periaqueductal gray and dorsal raphe that innervate the CeA play a role in contextual fear acquisition, but not in the recall. As the primary source of contextual information is the hippocampus, we aimed to uncover the synaptic mechanisms underlying midbrain VIP neuron-driven changes at ventral hippocampal (vHC) fibers in the CeA. We hypothesized that VIP axon terminals from the midbrain would potentiate vHC inputs on CeA neurons thus inducing LTP. In line with this prediction, we found that in 60% of slices, LTP was successfully induced by stimulating the vHC and midbrain VIP axons subsequently. Furthermore, we compared the AMPA/NMDA current ratio at vHC afferents in CeA neurons between fear conditioned and control animals and found a significant increase after fear learning. However, reducing the activity of midbrain VIP neurons using chemogenetics could prevent the increase in the AMPA/NMDA ratio induced by fear learning. Our data thus show that the midbrain VIP inputs on CeA neurons is necessary for contextual fear learning by potentiating the vHC synapses by increasing the AMPA/NMDA ratio.

STARVATION RISK AND ENVIRONMENTAL STABILITY INFLUENCE THE USE OF SOCIAL INFORMATION IN A FORAGING MODEL

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Social learning is an effective way an individual can gather information about its environment and is utilized by many animal taxa. However, gathering information socially can be costly and the information can become outdated if environmental quality is temporally variable. Given these costs, when should an individual use social learning instead of gathering information directly from the environment? I created a stochastic dynamic programming model to find conditions under which an individual maximizes its fitness by watching another, given its current information about the environment and its physical state. The presence of another animal can indicate a lack of predators, help identify the quality of a potential foraging patch, or both. However, because time or attention is

spent on watching others, this reduces food detected directly by the observer while watching. Thus, an individual must balance long-term gains in information versus short-term food gains. The historic environmental stability, or consistency in food availability, in which a social learning strategy has evolved influences the use of social learning. Strategies that have evolved in stable environments involve using social learning over a wider range of conditions than those that evolved in unstable environments. This supports the hypothesis that a higher chance of information being outdated selects against using information from others. I also found that an individual's physical condition influences its decision to use social information. I found that individuals in poor conditions do not gather information from others over a wider range of conditions. I suggest that, for individuals in poor condition, the short-term gains of acquiring food outweigh the longer-term benefits of increasing information about relative patch quality and the presence of predators. The costs of social learning can overcome the benefits if an individual is in too poor a condition to effectively use the information gained.

POSTPARTUM LIMITED BEDDING AND NESTING ALTERS MATERNAL AGGRESSION AND HABENULAR CFOS EXPRESSION IN LONG EVANS RATS

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Stress is a major risk factor for development of postpartum disorders, which can be mimicked using a limited bedding and nesting (LBN) manipulation. LBN dams and pups reside on a metal grate from postnatal day (PND) 2-10. Previously, we found that LBN increases dam's pup-directed behavior and decreases self-care. To determine if LBN impacts additional postpartum behaviors, we randomly assigned Long Evans dams (100-150 days old) to standard (n = 15) or LBN (n = 12) housing, then ran a resident-intruder task on PND10 with a male intruder to elicit aggression. LBN dams have lower total attack durations ($p = 0.01$), which is driven by significant decreases in offensive (pin, wrestle, shove; $p = 0.01$), but not defensive (kick, box, bite; $p = 0.36$) durations. LBN consistently decreases attack duration across all offensive measures, though only pin ($p = 0.008$) and wrestle ($p = 0.03$) are significant (shove; $p = 0.49$). We conducted whole-brain cFos to determine regions underlying these behavioral shifts. LBN dams show decreased cFos in the medial ($p = 0.04$) and lateral habenula ($p = 0.06$) after aggressing. Ongoing work examines additional regions that may be differentially activated following aggression and discerns the functional role of these regions in our stress-aggression effect.

DEVELOPMENT OF FACE RECOGNITION IN NEWBORN CHICKS AND EMBODIED COMPUTATIONAL MODELS

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Face recognition is a necessary skill for survival in social species such as humans, chimpanzees, and chickens, but it remains unclear how face recognition emerges in the brain. Did specialized circuitry for face recognition evolve over phylogenetic time, or does it emerge from domain-general learning mechanisms? To address this debate, we developed a new benchmark—a Newborn Embodied Turing Test (NETT) for face recognition. We raised newborn animals and "newborn" machines in the same environments and tested them with the same tasks, to directly compare their learning abilities. First, we raised newborn chicks in controlled environments containing a single rotating face, then tested their ability to recognize that face versus a set of novel faces. Then, we performed parallel controlled-rearing experiments with artificial chicks by rearing and testing artificial agents in virtual versions of the rearing and testing conditions of the biological chicks. When creating the artificial chicks, we used deep neural network "brains" that varied by visual sensors (monocular vs. binocular vision), imprinting reward, and visual encoder to assess if these features led to face recognition that was more like the

biological chicks. Biological chicks successfully distinguished the familiar face from most of the unfamiliar faces, but their performance varied widely across the different test faces. Notably, most of the artificial chicks produced a very similar pattern of performance, with strong performance where biological chicks were most successful and weaker performance where biological chicks were least successful. These findings indicate that complex face discrimination can emerge from general-purpose learning mechanisms that exploit the statistical structure of the visual environment. Our study challenges the necessity of specialized, innate face-processing modules and suggests that recognition can emerge from neural computations that directly fit to their visual environment.

IT'S COMPLICATED: SOCIAL DENSITY AND SPATIAL COMPLEXITY IN THE LIVING ENVIRONMENT INTERACT TO INFLUENCE MOUSE COURTSHIP BEHAVIOR ACROSS CONTEXTS

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Environmental enrichment (EE) allows researchers to provide complex living conditions and promote naturalistic behaviors for laboratory animals. Enrichment can be social, by group-housing animals, and it can be physical, by providing structures in the cage that allow animals to hide, nest, and climb. EE induces positive effects on wellbeing and cognitive behavior, but little attention has been paid to EE's influences on courtship behavior, particularly communication. Here, we leverage the robust and context-dependent vocal behaviors of male house mice to investigate how both physical and social EE influence courtship motivation, social competence, and sexual behaviors in a variety of contexts. Mice were housed either socially (S+) or isolated (S-) and were either physically enriched (P+) or physically deprived (P-) for a month, creating four distinct groups with varying levels of social and physical EE: S+P+, S+P-, S-P+, and S-P-. We then employed a battery of social behavioral assays to investigate ultrasonic vocalization production and nonvocal courtship behaviors interactions with female stimulus mice. We found that EE influenced only some of the courtship behaviors we measured, including how often mice called, the average duration of their calls, and how long it took them to start calling. Overall, physical enrichment tended to promote courtship behavior, and social enrichment tended to decrease courtship behavior. Importantly, the effect that one type of enrichment had on a behavior was often dependent on whether or not the other type of enrichment was present. These results emphasize that mouse courtship behavior is influenced by the social, sensory, and motor stimulation that an individual experiences in their living environment, stressing the importance of considering these factors in studies that evaluate social behavior in laboratory animals.

HOW BEHAVIORAL STRATEGIES AND SEX SHAPE BRAIN TRANSCRIPTOME EVOLUTION

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The degree of sex differences varies among species, especially in relation to behavior. As we seek to understand how behavior evolves, we naturally turn to the brain, yet it is unclear how neuromolecular sex differences evolve. We address this question via new algorithms in the software, CAGEE (Computational Analysis of Gene Expression Evolution). We measure gene expression in the ventromedial telencephalon in 10 songbird species, half of which have convergently evolved obligate cavity-nesting, a reproductive strategy that is associated with multiple behavioral phenotypes. We focus on sex differences in gene expression in the brain, and model how it varies in its rate of evolution. The results show that sex bias evolves more rapidly on the Z sex chromosome and in association with cavity nesting, but each gene's ancestral state does not predict its sex bias evolution. Furthermore, this work provides a broadly applicable framework for understanding macro-evolutionary

patterns of gene expression across levels of analysis, from cells to networks, associated with any continuously varying trait.

MICROGLIAL *NR3C1* DEPLETION ALTERS STRESS EFFECTS ON SYNAPTIC DENSITY AND BEHAVIOR IN A SEX-DEPENDENT MANNER

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Chronic stress affects neuron-microglia interactions in the prefrontal cortex (PFC) differently based on sex, impacting synaptic plasticity and behavior. Male mice, not female, display cognitive impairment and reduced PFC synapses after 14 days of chronic unpredictable stress (CUS). Glucocorticoid receptor (GR; *Nr3c1*) signaling is crucial for stress-induced synapse loss and associated behaviors, yet the cell type-specific role of GR in stress neurobiology and its contribution to sex differences remain unexplored. Male and female mice with microglia-specific GR depletion were subjected to 14 days of CUS or handled intermittently. CUS reduced body weight gain in both sexes, unaffected by genotype. To define the neurobiological effects of microglial GR depletion, we systemically injected AAV(PHP.eB)-hSyn1-tdTomato into mice. As expected, CUS caused synaptic deficits and impaired working memory in male mice, but microglial GR depletion did not affect these endpoints. Similar to prior work, genotype control females did not show any CUS-induced changes in synaptic density or working memory function. However, microglial GR depletion in female mice led to synaptic deficits in the PFC and reduced discrimination in temporal object recognition following CUS exposure. To explore molecular mechanisms underlying female stress susceptibility after GR depletion, we performed RNA sequencing on sorted microglia. This revealed significant stress-induced gene expression changes, including immune-related transcripts like *H2-T9*, neuroplasticity-associated genes such as *Fam3c* and *Naca*, and clock genes *Nr1d1* and *Nr1d2*. In conclusion, these findings highlight microglial GR signaling plays a crucial role in chronic stress responses, with disrupted regulation rendering female mice more susceptible to stress. Further research is needed to elucidate underlying mechanisms and their relevance to human stress responses and microglial regulation.

CANNABINOID REGULATION OF MURINE EXOCRINE SECRETION

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Tearing and salivation are wholly dependent on the activity of exocrine (lacrimal and salivary) glands, whereas vaginal moisture and secretion rely on a combination of exudation and exocrine secretion. Exocrine gland disorders impact millions, and women with Sjogren's Syndrome often experience dry eye and mouth as well as vaginal dryness. Cannabis users' complaints of dry eye and 'cottonmouth' are well-known, but some female cannabis users also report vaginal dryness. Given that more and more states are legalizing marijuana it is important to understand the potential health impacts. The regulation of vaginal secretion by the cannabinoid signaling system is essentially unstudied. We recently reported that despite their small size and nocturnal nature, laboratory mice have measurable vaginal moisture and stimulated secretory responses that are regulated by circadian and estrous factors. Using this model, we tested the regulation of vaginal moisture by cannabinoid receptors in awake and behaving mice. We now report that the cannabinoid receptor agonist CP55940 does not alter baseline vaginal moisture when administered acutely, but prevents a secretory response normally induced by exposure to bedding scented by male secretions. Chronic intermittent CP55940 reduces basal vaginal moisture and unmasks a potentiating action for CP55940. Both the effects appear to be CB1 receptor dependent, suggesting multiple sites of action. THC, a partial agonist, has no acute, chronic intermittent or chronic effects. In summary, we report complex cannabinoid regulation of vaginal moisture and secretion in a murine model. Strongly activated cannabinoid CB1 receptors largely

impair vaginal secretory responses both with acute and prolonged treatment. The extent to which these findings translate to humans remains to be determined.

NUTRITIONAL NICHE SEPARATION AMONGST SYMPATRIC THREATENED LEMURS: THE ROLE OF MICRONUTRIENTS IN FOOD SELECTION AND IMPLICATIONS FOR PHYSIOLOGIC HEALTH

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Madagascar's biodiversity is heavily affected by anthropogenic change, and conservation management plans need to reflect current research strategies to aid in the protection of biodiversity. Few primatology studies have focused on micronutrients, and even less consider a multi-species comparison. My research provides a novelty approach to studying community ecology and niche separation among mineral consumption between three sympatric lemur species, *Hapalemur griseus*, *Propithecus diadema*, and *Eulemur fulvus*. I collected behavioral data and plant samples across two seasons and two sites varying in habitat disturbance on *H. griseus* in Tsinjoarivo, Madagascar. From the diet of *H. griseus*, I assessed 60 plant samples for macronutrients and 27 plant samples for 11 minerals. I combined my dataset with the pre-existing datasets of *P. diadema* and *E. fulvus* diets to explore how minerals drive food selection, assess differences in plant part mineral concentrations, and evaluate the influence of seasonality to reveal interspecific dietary differences that likely limit health and growth. Generally, leaves and young stems were the highest in mineral concentrations across the three lemur species. However, the diet of *H. griseus* reflects greater mineral concentrations (Ca, Cu, Fe, K, Mg, Mn, Mo, P, & Zn) than *P. diadema* & *E. fulvus* and challenges previous hypotheses suggesting rainforest habitats are deficient in minerals, specifically calcium. Additionally, I compared results to the National Resource Council's (NRC) dietary recommendations for nonhuman primates to evaluate health; while comparisons should be interpreted with caution, most plants fed on by the lemurs fell below NRC requirements for several minerals (Ca, Cu, Fe, Na, P & Zn).

EXPERIMENTS IN SYNTHETIC SENSORY ECOLOGY

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Sensory systems shape how organisms perceive and respond to their environments, influencing behavioral strategies and ecological adaptation. In this study, we analyze and compare the performance of three virtual robots, each employing a distinct sensory modality—(1) an auditory system detecting sound cues, (2) a gradient-based system responding to olfactory intensity, and (3) a distal system integrating information from a distance. These models are optimized using evolutionary computation techniques to refine their responses in a taxis task, allowing for systematic comparisons of efficiency, robustness, and adaptability across sensory strategies. Our findings reveal trade-offs between sensory resolution, processing demands, and environmental predictability, offering insight into the functional constraints that shape sensory evolution. By situating these results within the framework of sensory ecology, we explore how different sensory modalities structure perception-action loops in both biological and artificial agents. This work provides a theoretical perspective on how organisms leverage information in their environments, with implications for understanding both natural and engineered navigation systems.

UNCOVERING THE TIMING AND ORIGINS OF DE NOVO MUTATIONS IN THE OFFSPRING AND PLACENTA OF RHESUS MACAQUES

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De novo mutations are critical for introducing genetic diversity. These mutations can arise in both the parental germline and during embryonic development as somatic mutations. However, distinguishing between the two is challenging, as both types of mutation can be unique to a single offspring and occur at high frequencies within the offspring's cells. Ideally, comparing the genome of the early zygote to that of the fully developed individual would allow us to pinpoint the origin of these mutations. While direct access to the zygote is not feasible, an effective alternative is to examine an early diverging lineage in development, such as the placenta. To assess the contribution of germline mutations and those arising at different developmental stages to the total number of de novo mutations in an individual, we compare whole-genome sequences from offspring and their matched placental samples (as well as their parents) in rhesus macaques. Mutations shared between the offspring and placenta likely occurred prezygotically or very early in embryonic development, whereas tissue-specific mutations reflect later stages of development. Additionally, we investigate potential sex-specific biases in mutation acquisition across developmental stages. De novo mutations are known to exhibit a male bias, primarily attributed to the greater number of cell divisions in the male germline compared to the female. By comparing male and female offspring, we aim to determine whether this male bias extends to prezygotic mutations and uncover potential differences in the timing and frequency of mutations, enhancing our understanding of how and when de novo mutations arise.

THE VISUAL INFLUENCE OF SWAYING BEHAVIOR IN PRAYING MANTISES

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Among Mantodea, most species exhibit remarkable body camouflage, helping them evade predators and ambush prey. These mantises often resemble structures in their habitats, such as leaves, grasses, branches, flowers, or tree trunks. Mantises mimicking leaves, grasses, and branches sometimes display a rhythmic, pendulous swaying movement, moving their bodies side to side, particularly in response to wind. In contrast, this behavior is absent in species mimicking stationary elements like tree trunks. We hypothesized that swaying functions as motion camouflage, blending mantises with a moving background environment. To test this, we used visual moving backgrounds to elicit swaying and compared the swaying patterns among four mantis species with different mimicry types. Our findings reveal that visual cues are sufficient to stimulate swaying behavior and that species with different mimicry types exhibit distinct swaying frequencies.

SOCIAL BUFFERING AMELIORATES LONG-TERM IMPACTS OF ACUTE EARLY LIFE STRESS IN A SEX-DEPENDENT MANNER

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Posttraumatic stress disorder (PTSD) is a common psychiatric disorder with severe symptoms; notably, symptoms may take time to emerge. Early life stress is a strong predictor of PTSD later in life, and factors that reduce the impacts of early adversity must be further studied. Social buffering has been shown to reduce the impacts of stress in both sexes. Here, we focused on the impact of social buffering following acute early life stress (aELS) on the long-term maladaptive incubation of fear in adulthood. First, we assessed whether aELS would induce fear incubation in adult mice. Animals exposed to aELS (15 footshocks) showed incubation of fear memory in adulthood, while no-aELS (0 footshock) animals did not. Second, we investigated whether social interaction with the dam and/or the littermates immediately following the aELS experience would reduce its impact on adult fear memory (i.e., incubation). We identified a double dissociation where males demonstrated buffering only if the dam was present, and females demonstrated buffering only if the littermates were present. Finally, we assessed whether social buffering alters consolidation of the aELS memory. Animals received 0 or 15 footshocks on PND17 immediately followed by isolation or social interaction with the dam plus

littermates. Over the next four consecutive days, they received daily 30-minute exposures to the context (extinction). There were no differences between isolation and social interaction on the consolidation of contextual fear memory or its extinction. These results indicate that social buffering reduces the long-term impacts of aELS on adult fear conditioning in a sex-dependent manner, and further supports social buffering as an important intervention method following early trauma experiences.

PREDATORY BEHAVIOR BY STRIGIFORM OWLS IN RESPONSE TO BIOFLUORESCENT SKIN FROM THE VIRGINIA OPOSSUM (*DIDELPHIS VIRGINIANA*)

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Biofluorescence occurs when an external light source interacts with a biomolecular substance in a living organisms and converts the absorbed light into a new, often vivid wavelength. Biofluorescence has been observed in a wide variety of species, including plants, corals, insects, spiders, scorpions, crustaceans, mollusks, fish, amphibians, reptiles and mammals. In some animals, this fluorescence may be used to signal potential mates, to alert toxicity to potential predators, or to communicate with conspecifics. Despite more than 125 species of mammals displaying biofluorescence, its purpose in mammalians is still mysterious. One of these biofluorescent mammals is one of the most common backyard creatures found throughout North and Central America: the Virginia opossum (*Didelphis virginiana*). The fur of the Virginia opossum glows vibrant pink when exposed to ultraviolet light. For this investigation, we sought to gain a better understanding of how the biofluorescence of opossums affected their appeal to nocturnal predators, specifically owls. We exposed captive owls to a choice test involving fluorescent fur and non-fluorescent fur paired with their normal prey (rodents). The initial choice of the owl and interactions with each patch of skin were recorded with trail cameras. Our results help fill one small piece of the puzzle regarding a potential ecological function for mammalian biofluorescence.

PERINATAL EXPOSURE TO ANTI-AGING SUPPLEMENTS AFFECTS BRAIN DEVELOPMENT AND BEHAVIOR IN RATTUS NORVEGICUS

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As human lifespans increase, so does the number of individuals suffering from age-induced cognitive decline. In response, several companies have profited from the sale of “anti-aging” supplements. Two supplements receiving attention include nicotinamide mononucleotide (NMN) and resveratrol. Previous animal studies report both NMN and resveratrol mitigate cognitive decline by reducing apoptotic cell death in various brain regions including the prefrontal cortex and hippocampus. While these supplements are most notable for their use in cognitive-aging models, both supplements are marketed to pregnant mothers due to their anti-inflammatory properties. However, the possible consumption of these supplements by pregnant mothers raises concerns as both supplements reportedly cross the placenta. Perinatal exposure to these supplements may alter apoptotic levels in the developing brain, which is essential for the organization of neural circuits. In the present study, we divided Long Evans dams into four exposure groups: NMN-exposed animals, resveratrol-exposed animals, animals exposed to both supplements, and control animals. Dosing occurred from embryonic day 0 (E0) until postnatal day (P)10. Neonatal behavioral tests occurred on P5, P7, and P10 to assess the sensorimotor capabilities of exposed offspring. In littermates, adult behavioral testing occurred on P80 to observe possible long-lasting effects on hippocampal and PFC regulated behaviors. Total brain and body weights were collected during the neonatal period and in middle adulthood (P110). Additional data on maternal behavior, body weights, and reproductive success was also collected. This research may have implications for supplement recommendations during pregnancy and may enhance our understanding of the mechanisms mediating perinatal brain development.

THE EFFECTS OF PERIODICAL CICADA EMERGENCE ON ANT FORAGING

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Periodical cicada emergences happen once every 13 to 17 years in the eastern United States. These emergences result in a large pulse of resources for predators and decomposers. Currently, there is almost nothing known about the effect of these large resource pulses on ant communities. Ants are a dominate member of every terrestrial habitat in the eastern United States and provide multiple important ecosystem services. The nutritional preferences and foraging rates of five ant communities in Lake County Illinois were measured in the summer of 2024, in conjunction with the emergence of the 17-year periodical cicadas (*Magicada spp.*). The relationship between the status of the emergence, the genus and size of ants using nutrient baits, the type of nutrients that were targeted, and the rate at which foraging ants would remove prey items were quantified for that summer and compared with preliminary foraging and nutritional data from ant communities in the summer of 2023. Ants with an intermediate body size were most represented across nutrient baits, and those from the *Myrmica* genus were likewise most numerous across all nutrient baits. Foraging rates were found to be significantly higher in the weeks after the periodical cicada emergence, compared to those during cicada emergence. Conclusions as to the extent of the impact of periodic cicada emergences will require subsequent summers of sampling.

SOCIAL COMPLEXITY PREDICTS *CHICK-A-DEE* CALL COMPLEXITY OF MOUNTAIN CHICKADEES *POECILE GAMBELI*

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The *chick-a-dee* call of chickadees, tits, and titmice is a vocal system used in a wide range of social contexts by both sexes throughout the year and is one of the more structurally complicated vocal systems outside of human language. However, relatively little is known about the *chick-a-dee* calls of mountain chickadees, *Poecile gambeli*. This is an important species for increasing our comparative understanding of variation in *chick-a-dee* calls as they are one of the chickadee species with the largest naturally occurring flock sizes. Flock size relates to the social complexity of flocks, and the Social Complexity Hypothesis for Communication predicts that individuals in more complex social groups should communicate with greater complexity than individuals in simpler social groups. Correlational and experimental evidence in support of the hypothesis has been found in the calls of a wide range of species, including Carolina chickadees, *P. carolinensis*. Here we provide the first description of the variation in note composition and note ordering rules in calls from mountain chickadee flocks in California and Colorado. California flocks were found to be significantly larger than Colorado flocks. Analysis of note type usage and transition probabilities between note types found that calls of California birds were more complex than calls of Colorado birds, supporting a key prediction of the Social Complexity Hypothesis for Communication. We also found relatively high rates of reversals of note ordering rules in mountain chickadee calls, which might help explain the complexity of the *chick-a-dee* calls of this species. Additionally, birds in flight produced calls with different note compositions when compared to perched birds. Generally, the note type ordering and transition probabilities of calls of mountain chickadees seem comparable to other better-studied chickadee species, although their frequent note order reversals suggest potential syntax-like properties.

EVALUATING THE IMPACTS OF A TOTAL SOLAR ECLIPSE ON MIDWEST PRAIRIE SOUNDSCAPE

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Solar eclipses represent rare natural experiments. They provide researchers opportunities to explore the effects of sudden changes in ambient light on animal behavior. In this study, we examined the impact of the April 8, 2024 total solar eclipse on animal acoustic signals in Midwestern United States prairie communities. We recorded changes in acoustic diversity, complexity, and intensity before, during, and after the eclipse at three prairie sites. To quantify shifts in soundscape patterns, we used several acoustic indices: the acoustic entropy index, the normalized difference soundscape index, the acoustic complexity index, and root mean squared amplitude. We hypothesized that the calling behavior of animals during the eclipse would mimic periods of low light, such as post-dusk, and that a measurable shift in soundscape activity would occur during the eclipse period. Our results showed significant variation in some metrics between days, with the highest biophonic activity being observed on the day of the eclipse. However, we did not find differences between the time of the eclipse and the days before and after with respect to acoustic complexity. Although there are many popular narratives about the effects of total eclipses on animal behavior, most have not been experimentally confirmed. Our study is one of the few to have systematically quantified the effects of a total eclipse on animal sound. Our findings provide insights into how light fluctuations influence prairie ecosystems and have broader implications for understanding rapid environmental change and anthropogenic light disturbance.

AVIAN PHYSIOLOGICAL AND BEHAVIORAL RESPONSES TO AN ANNUAL, NIGHTTIME EVENT AT ZOO ATLANTA

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Light is a powerful and consistent environmental signal that communicates important daily and seasonal changes to an organism. In recent decades, the combination of population growth, economic development, and urbanization have increased the density and distribution of artificial light at night (ALAN) in both natural and urban settings, causing circadian disruption to wildlife, particularly in avian species Our goal was to determine how ALAN affects avian behavior and glucocorticoid production. We used Zoo Atlanta's annual lantern holiday event called "IllumiNights," as an opportunity to study the effects of ALAN on four avian species: the Chilean flamingo (*Phoenicopterus chilensis*), milky eagle owl (*Ketupa lactea*), blue-throated macaw (*Ara glaucogularis*), and tawny frogmouth (*Podargus strigoides*). Our objectives were to collect avian behavior before, during and after the event from June 2023 through March 2024 using a species-specific ethogram programmed into ZooMonitor®. Feces and discarded feathers were collected opportunistically to evaluate glucocorticoid production. A corticosterone enzyme immunoassay was used to quantify glucocorticoid metabolites. Findings will elucidate the effects of ALAN on avian behavior and glucocorticoid project to help ensure the best husbandry practices for managing wildlife in zoos. Furthermore, understanding how ALAN impacts avian circadian rhythms can offer insights into its effects on human circadian health and well-being.

URBANIZATION EFFECTS ON STRESS AND MULTIMODAL SENSORY PROCESSING IN HOUSE SPARROWS *PASSER DOMESTICUS*

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Urban expansion introduces various anthropogenic challenges for wildlife, such as habitat fragmentation, novel predators, and pollution. These challenges may disrupt animal communication and contribute to increased stress levels. This study explores the physiological consequences of urban stressors on house sparrows *Passer domesticus*. Specifically, we examine how urbanization affects the anti-stress hormone (i.e., corticosterone, CORT) levels and its relationship to auditory and visual processing. Sparrows were collected from rural, suburban, and urban areas around Holland, MI. Blood samples were analyzed for baseline and elevated CORT levels to assess stress responses. Auditory

processing was evaluated using auditory brainstem responses, which assess the ability to detect sounds at varying frequencies and intensities. Visual processing was assessed through electroretinogram tests, which indicate motion detection ability by measuring flicker fusion frequency–the threshold at which individual light flashes can no longer be distinguished. We predicted that (1) urban birds would show higher chronic CORT levels than rural birds and (2) differences in sensory processing may be due to differing CORT concentrations in urban versus rural birds. Our results will contribute to an understanding of animal communication in a growing urban environment and the overall impact of anthropogenic disturbances on wildlife. Ultimately, this research aims to inform conservation strategies to mitigate human impacts on natural populations.