Distinct networks of expressed genes are associated with neophobia in the hippocampus of male and female Eurasian tree sparrows (*Passer montanus*)

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Author contributions
CRL, KJK, MGK, and SEL conceptualized the project. CRL and SEL secured funding for the project. KJK, MGK, and EBC conducted the lab work. ABB led the data analysis, with support from CRL and SEL. ABB, CRL, and SEL prepared the original draft of the manuscript. All authors contributed to the review and editing of the finalized manuscript, approved for publication, and agreed to be accountable for the accuracy and integrity of their work performed on this project.

Data Availability
Behavioral data and R code used to analyze the datasets can be accessed from Dryad here: https://doi.org/10.5061/dryad.f4qrfj72h. R code used to analyze transcriptomic data is available upon request. Gene expression data are available at the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) repository under XXX.

Conflict of Interest
The authors declare no conflicts of interest.

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Abstract

Neophobia, avoidance of novel stimuli, is an ecologically and evolutionarily relevant behavioral trait that varies among individuals and across species. Especially among wild animals, the neuromolecular mechanisms underlying individual variation in neophobia have not been well characterized. We examined three neophobic behaviors in captive female and male Eurasian tree sparrows (*Passer montanus*) from a wild population introduced to the USA in 1870: responses towards novel objects, novel foods, and repeated presentations of the same initially novel object. We compared transcriptomic patterns associated with neophobia in three brain regions, the striatum, dorsal hippocampus, and rostral hippocampus, using differential expression and co-expression network analyses. We found that the striatum and hippocampus had distinct transcriptomic profiles, as did the rostral and caudal subregions of the hippocampus, supporting recent hypotheses that these subregions are functionally specialized. Despite the absence of sex differences in neophobic behaviors, neophobia-associated gene modules revealed sex-specific patterns within brain regions. For females, neophobic behaviors more strongly correlated with gene modules in the caudal hippocampus, a region involved in stress and anxiety, whereas for males, neophobic behaviors correlated with gene modules in the rostral hippocampus, a region that may play a larger role in spatial cognition. These modules exhibited significant overlap, suggesting that neophobic behaviors in both females and males are driven by shared neurobiological mechanisms, though they exhibit sex-specific patterns of brain region localization. Further, this work highlights the importance of examining both male and female animals in neurobiological research.

Keywords: neurogenomics, sex difference, transcriptomics, RNAseq, songbird, behavior, striatum, novelty

Introduction

Fearful behavior towards novel stimuli, or neophobia, is found in many animal species, and can be observed in response to new foods, objects, and environments (Crane & Ferrari, 2017; Kimball & Lattin, 2023b). While an aversive reaction to a dangerous new stimulus can help ensure an animal's survival, a fearful response to novelty may prevent access to new foods, nesting sites, or other beneficial resources (Greenberg & Mettke-Hofmann, 2001). Neophobia has become increasingly relevant for many wild animals as their environments undergo anthropogenic changes. Research suggests that urban animals are less neophobic than their rural counterparts (Breck, Poessel, Mahoney, & Young, 2019; Huang, St.Mary, & Kimball, 2020; Jarjour, Evans, Routh, & Morand-Ferron, 2019; Tryjanowski et al., 2016) and even within urban sites, individuals that experienced more anthropogenic disturbances were less neophobic than those encountering fewer disruptions (Grunst, Grunst, Pinxten, & Eens, 2019). Neophobia has also been tied to invasion success, where reduced neophobia may help invasive species exploit novel resources (Candler & Bernal, 2015; Cohen, Kumar, Nair, Hauber, & Dor, 2020; L. B. Martin & Fitzgerald, 2005). However, despite its ecological relevance and the wide variation in neophobia observed across individuals, populations, and species, the molecular mechanisms underlying this behavior are not well understood. Therefore, there is a clear need for more studies investigating neurobiological differences between neophobic and non-neophobic individuals.

Much of what is currently known about the neurobiological basis of neophobia comes from laboratory rodent models. Although lab-bred rats and mice typically show a preference for novel over familiar stimuli, they do exhibit neophobia towards some - but not all - novel tastes and when feeding in a novel environment (hyponeophagia) (Olszewski, Ulrich, Ling, Allen, & Levine, 2014; Roman, Lin, & Reilly, 2009; Shephard, Buxton, & Broadhurst, 1982; Stehberg, Moraga-Amaro, & Simon, 2011). Rodent research has identified several neurobiological mediators in the glutamate, serotonin, and norepinephrine systems involved in these types of feeding neophobia (Ramírez-Lugo, Zavala-Vega, Pedroza-Llinas, Núñez-Jaramillo, & Bermúdez-Rattoni, 2015; Royet, Gervais, & Araneda, 1983; Simpson et al., 2011; Steketee, Silverman, & Swann, 1992). However, because these neophobic responses are context- and stimulus-specific, it is unclear how generalizable these results are to the more widespread

neophobia typically observed in wild animals (Crane & Ferrari, 2017). Further, because lab animals tend to be highly inbred, they do not show the wide within-species variation in neophobia observed in many wild populations. Research in wild-caught or outbred animals is necessary to reveal the neuroendocrine basis for individual variation in neophobia.

Transcriptomic approaches enable the unbiased identification of gene expression patterns associated with behavior, extending the scope beyond the handful of neurotransmitters and hormones typically investigated with more targeted approaches (Bell & Robinson, 2011; Bengston et al., 2018; Rebecca M. Calisi & MacManes, 2015). Transcriptomics has been particularly useful in revealing what brain regions are involved in behaviors with wide inter-individual variation, like neophobia. For example, a recent transcriptome study in the invasive house sparrow (*Passer domesticus*) found that neophobic and non-neophobic individuals had distinct patterns of constitutive differential gene expression in brain regions that included the hippocampus (a region linked to spatial cognition, novelty responses, and memory) and the striatum (which is involved in reward and learning) (Güntürkün, 2005; Lattin, Kelly, Kelly, & Johnson, 2022; Medina & Reiner, 1995; Rose & Colombo, 2005; Shinohara & Yasoshima, 2021). Differences between neophobic and non-neophobic sparrows were especially pronounced in the hippocampus, suggesting this region may be particularly important in neophobia (Lattin et al., 2022), a conclusion further supported by a study showing high neuronal activity in the hippocampus after exposure to novel objects (Kimball et al., 2022). However, this work was performed in only one sex, leaving uncertainty about sex-specific mechanisms.

Although many species - including house sparrows - show no sex differences in neophobia (Ensminger, Westneat, & Zeh, 2012; Kelly, Kimball, Stansberry, & Lattin, 2020; Krajcir et al., 2024), it is not necessarily the case that the same neurobiological mechanisms regulate the same behaviors in males and females (McCarthy, Arnold, Ball, Blaustein, & De Vries, 2012). Many sex differences have been found in hippocampal anatomy in mammals (Brivio, Lopez, & Chen, 2020; Yagi & Galea, 2019), which may reflect divergent life history pressures and endocrine phenotypes. For instance, in laboratory mice, females have greater dendritic spine density and heightened gene expression responses to receptor knockouts in the hippocampus than males, likely driven by differences in steroid hormone levels (Oakley, Riddick, Moy, & Cidlowski, 2024; Shors, Chua, & Falduto, 2001). In birds, female brown-

headed cowbirds (*Molothrus ater*) have a larger hippocampus and greater neurogenesis than males, potentially associated with females' brood parasite life history strategy (Guigueno, MacDougall-Shackleton, & Sherry, 2016; Sherry, Forbes, Khurgel, & Ivy, 1993). However, food-caching black-capped chickadees (*Poecile atricapillus*) do not show sex differences in spatial memory or hippocampal size (Petersen & Sherry, 1996), and recent genome-wide association studies revealed little effect of sex on the genetic contribution to chickadee spatial cognition (Semenov et al., 2024). Thus, the inclusion of both female and male subjects in biological research has the potential to reveal sex-dependent biological processes (Klein et al., 2015; Shansky & Murphy, 2021).

In the present study, we examined potential links between neophobia and constitutive gene expression in the brain of Eurasian tree sparrows (*Passer montanus*), an invasive songbird introduced to the USA in 1870 (Widmann, 1889). Birds are among the taxa with the highest levels of neophobia (Crane & Ferrari, 2017), and like their congener the house sparrow, Eurasian tree sparrows have wide and repeatable inter-individual variation in neophobia (Krajcir et al., 2024). Because different types of neophobia tests can sometimes show varying results (Dusang et al., 2025; Kimball & Lattin, 2023a), neophobia was assessed using three different behavioral assays: sparrows' responses towards novel objects, novel foods, and repeated exposures of the same initially novel object. Three weeks after neophobia behavior tests were complete, we compared gene expression patterns in three different brain regions (striatum, rostral hippocampus, and caudal hippocampus) in males and females in relation to their behavioral performance using differential expression and co-expression network analyses. We divided the hippocampus into subregions because recent evidence suggests that the avian hippocampus shows specialization along a rostral-caudal axis, similar to that of the dorsal and ventral hippocampus in rodents (Fanselow & Dong, 2010; Gualtieri et al., 2019; Smulders, 2017). We predicted that neophobic and non-neophobic individuals would show differences in constitutive gene expression across the three brain regions, with distinct patterns reflecting their functional specializations. Based on past work showing novel object exposure increased neuronal activity in the caudal but not rostral portions of the house sparrow hippocampus (Kimball et al., 2022), we expected the strongest effects of neophobia on expression in the caudal hippocampus. Furthermore, although neophobia behaviors did not differ by sex, we predicted that the underlying transcriptomic patterns might show sex specificity.

Materials and methods

Scale of Inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Sex	Sex	13 males, 11 females
Brain region	Brain region	3 brain regions: striatum, caudal hippocampus, rostral hippocampus
Behavior	Behavior	Consistently neophobic: 4 males, 3 females; Consistently non-neophobic: 4 males, 3 females

Bird capture and behavioral trials

All animal work was approved by the Louisiana State University Institutional Animal Care and Use Committee (under protocol #10-2021), and Eurasian tree sparrows were collected under an Illinois State Scientific Collecting Permit. Sparrow capture, transport, housing, and euthanasia followed bird research guidelines from the Ornithological Council (Fair, Paul, Jones, & Bies, 2023). Adult Eurasian tree sparrows (n = 13 males, 11 females) were captured from Tazewell County, Illinois, United States in March 2023 using mist nets and nest box traps on private landowners' property. Sparrows were transported to Louisiana State University overnight (to minimize stress) in a modified pet carrier with multiple food and water dishes and perches. Once birds arrived at Louisiana State University, they were placed in long-term housing cages in a vivarium facility on campus. The cages were supplied with multiple perches of differing materials, a dust bathing tray filled with sand, a plastic pine branch to use as a hide, water, and food (a mix of seeds, vitamin-supplemented food pellets, and grit). Birds were acclimated to captivity for three weeks before beginning behavior trials. Neophobia behavior trials are further detailed in Krajcir et al. (2024). Birds were doubly housed until three days before behavioral trials, after which they were singly housed to avoid any effects of social interactions on neophobia (Kelly et al., 2020). Sparrows were cared for daily by the research team and animal husbandry staff, and weighed every two weeks to ensure body mass was maintained.

Neophobia in captive Eurasian tree sparrows was quantified using the same methods

previously used in house sparrows (Kimball et al., 2022). Briefly, food was removed 30 min before lights out in the room, and trials conducted the following morning 30 min after lights on. Neophobia was quantified as latency to first approach and feed from a food dish presented either with a novel object (a purple plastic egg, white partial cover, red-painted dish, yellow pipe cleaners, or blinking light) or a novel food item (chopped kiwi, shredded cheese, peanut butter, or fruit-flavored cereal) during 1-h trials. Control approach and feeding times were determined by presenting birds with their normal food dish filled with familiar foods. Food neophobia, object neophobia, and habituation to repeated exposures of an initially novel object were tested. Object neophobia was tested during week one (n = 3 novel object trials n = 2 control trials per bird, presented in random order), food neophobia was tested during week two (n = 4 novel food trials and n = 2 control trials per bird, presented in random order), and object habituation was tested during week three (n = 4 trials using the same initially novel object and n = 1 control trial per bird, with the control trial first). All six behavioral measures of neophobia (average latency to approach the food dish when novel objects were present, average latency to feed when novel objects were present, average latency to approach the food dish when it contained novel food, average latency to eat novel food, average latency to approach the food dish during novel object habituation trials, and average latency to feed during novel object habituation trials) were highly and significantly correlated among individuals when measured as a group, as well as when males and females were examined separately, except for novel food approach and novel food feeding in females (Table S1).

RNAseq tissue preparation and sample collection

Three weeks after neophobia trials ended, sparrows were euthanized using an overdose of isoflurane anesthesia followed by rapid decapitation. After decapitation, brains were removed, flash-frozen on powdered dry ice, and stored at -80°C until sectioned coronally on a cryostat (Cryostar NX50, Thermo Fisher). Tissue was embedded in Optimal Cutting Temperature compound (VWR 95057-838) in block molds and placed in the cryostat to be sliced at -22°C. Slices were mounted directly onto slides (Superfrost Plus, VWR 48311-703) in two alternating series of 50 μm and 200 μm slices (Lattin et al., 2022). The 50 μm slides were dried overnight at 4°C, stained with thionin, and coverslipped. The 200 μm slides were chilled on dry ice and

stored at -80°C until tissue punching.

Brain tissue punches were taken from the three target regions: striatum, caudal hippocampus, and rostral hippocampus (Figure 1A). Target brain slices for punching in the 200 µm slides were determined using landmarks in the adjacent 50 µm thionin-stained slides, using published songbird brain atlases as guides (Nixdorf-Bergweiler & Bischof, 2007; Stokes, Leonard, & Nottebohm, 1974). Striatum punches came from slices that also contained the quinto-frontal tract. Two 1 mm diameter punches (Fine Science Tools 18035–01, 15 G) were taken from each hemisphere of the striatum on one target slice (i.e., four total punches), ~3 mm from the rostral tip of the brain. Rostral hippocampus was punched on slices where the septomesencephalic tract (TrSM) was evident, ~5 mm into the brain. Ten 0.5 mm diameter punches (Fine Science Tools 18035-50, 19G) were collected from both hemispheres of the rostral hippocampus across three consecutive brain slices. Caudal hippocampus was punched from slices where the cerebellum first came in, ~8 mm into the brain. Tissue was collected from both hemispheres of four consecutive slices using the 0.5 mm diameter tissue punch (10-16 punches total). All punches from the same target brain region and subject were combined in sterile D/RNAse Free 1.6 mL centrifuge tubes and stored at -80°C until RNA extraction. Tissue punching tools were sterilized between subjects and brain regions using D/RNAse Free solution (Cole-Parmer EW-78901-43) followed by submersion in 95% molecular-grade ethanol, DEPC-treated water, and 95% molecular-grade ethanol.

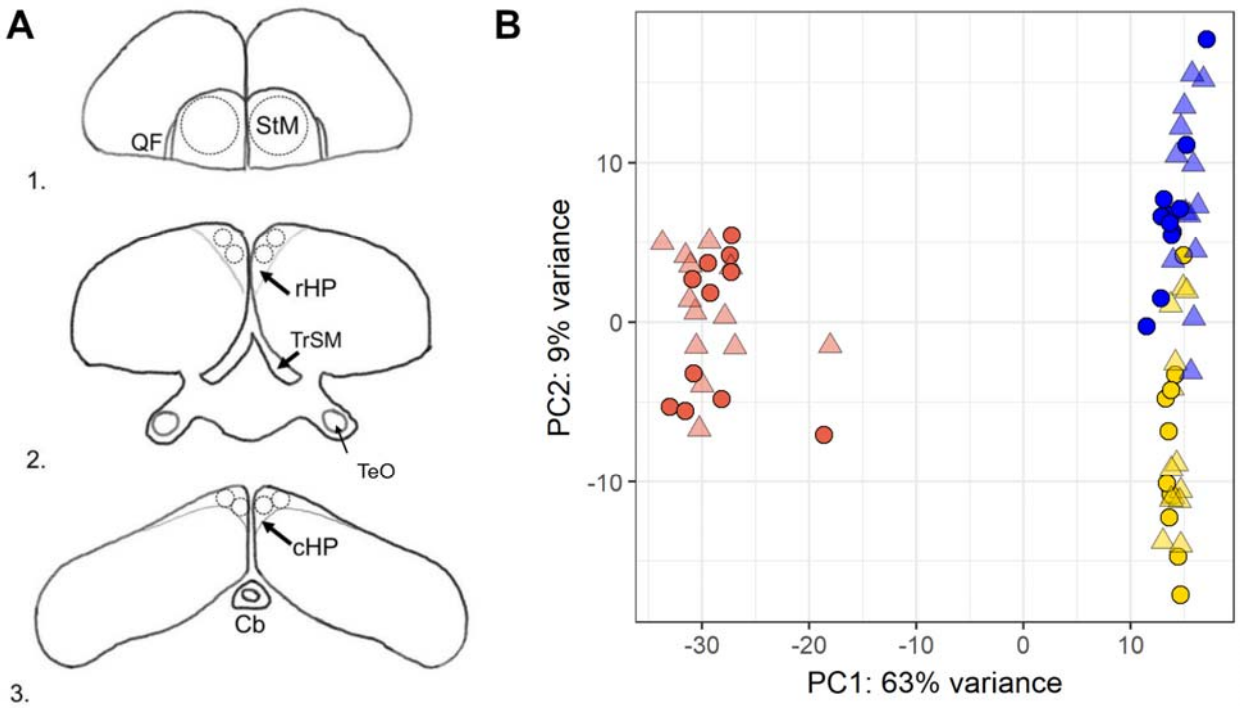

Figure 1. (A) Anatomical landmarks in hand-drawn coronal sections of Eurasian tree sparrow brains depicting the three target regions as well as the relative size and location of the punches. The top image (1) is the most anterior slice and depicts the striatum (StM) and its landmark, the quinto-frontal tract (QF). The middle image (2) depicts the rostral hippocampus (rHP) along with its target landmark, the septomesencephalic tract (TrSM), as well as a rough depiction of the optic tectum (TeO). The bottom image (3) is the most posterior of the drawings, and depicts the caudal hippocampus (cHP), along with its target landmark, the cerebellum (Cb) at its first emergence. (B) Principal component analysis of gene expression patterns, showing the first two principal components (PC), which explain 72% of the variance. Points represent male (triangles) and female (circles) sparrows across three brain regions: striatum (red), caudal hippocampus (yellow), and rostral hippocampus (blue). Sample size = 13 males and 11 females per region.

RNA extraction and library preparation

RNeasy Lipid Tissue Mini Kits (Qiagen; 1023539) were used to extract RNA from brain tissue and RNA concentrations and quality assessed using a Bioanalyzer system (Agilent 5400). Some samples initially resulted in RNA concentrations too low for library preparation, most

likely due to the smaller brain mass of Eurasian tree sparrows compared to house sparrows. Therefore, 13 samples were evaporated down in a sterile incubator until target concentrations for library preparation were achieved. The incubator was sterilized with D/RNase free and 100% molecular-grade ethanol before being brought to 45°C. Samples were evaporated for no more than 3 h. Final sample quality was assessed using the Bioanalyzer, and average total RIN score for all samples was 8.6 (range: 6.7-9.7).

RNA sequencing, alignment, and mapping

We submitted total RNA to Novogene for sequencing using 150 bp paired-end sequencing (NovaSeq X Plus system) to generate an average of ~52.4 million reads per sample (Table S2). After filtering to remove adapters and low-quality nucleotides, average reads per sample was ~49.7 million. The error rate of sequencing for all samples was 0.01 and the G-C percentage was 48%.We used the zebra finch genome (GCA_003957565.4) as a reference (Rhie et al., 2021), as it is the most closely related species with a well-annotated genome (Hauber, Louder, & Griffith, 2021; Kumar et al., 2022). We aligned reads using HISAT2 (Mortazavi, Williams, McCue, Schaeffer, & Wold, 2008), for a 28.6% (range: 25.7-30.7%) mapping rate, with roughly ~14 million reads per sample. We used StringTie to assemble transcripts (Pertea et al., 2015). We ran a principal component analysis (PCA) to visualize transcriptomic patterns across brain regions and sexes (Figure 1B).

Identification of differentially expressed genes

We first identified sex- and region-specific genes that were differentially expressed between individuals showing the most consistent behavioral traits. We categorized individuals as “neophobic” or “non-neophobic” based on whether their latency to approach or feed was above (more neophobic) or below (less neophobic) the median for novel object, novel object habituation, and/or novel food trials. There were 7 individuals (n = 4 males; n = 3 females) that were consistently neophobic across all 6 behavioral trials and 7 individuals (n = 4 males; n = 3 females) that were consistently non-neophobic. This more conservative binary approach focused on the most pronounced differences in gene expression associated with neophobia. However, although latencies across trials were highly correlated, behavioral responses may still differ in

meaningful ways; therefore, we also used gene co-expression networks to analyze the full spectrum of individual variation in each trial (see below).

Differentially expressed genes (DEGs) and normalized values were determined using DESeq2 v.1.42.0 in R (Love, Huber, & Anders, 2014). Transcripts with < 10 counts in the smallest group sample size (n = 3 for females and n = 4 for males) were filtered out (n = 8,888 transcripts retained for female caudal hippocampus; n = 8,976 male caudal hippocampus; n = 8,957 female rostral hippocampus; n = 9,180 male rostral hippocampus; n = 8,869 female striatum; n = 8,772 male striatum). We performed Wald tests for each gene comparing non-neophobic vs neophobic (reference) individuals by sex within each brain region. Log2 fold change estimates were shrunk using the *apeglm* function in DESeq2. P-values were corrected using Benjamini-Hochberg corrections and FDR ≤ 0.05 were considered differentially expressed.

DEGs were assessed for enrichment of biological process Gene Ontology (GO) terms using PANTHER (Mi, Muruganujan, Ebert, Huang, & Thomas, 2019) with a Fisher's Exact test and a cut-off of FDR ≤ 0.05. GO terms were further summarized with REVIGO (http://revigo.irb.hr/), which clusters GO terms based on semantic similarity (similarity threshold = 0.7).

Construction of weighted gene co-expression networks

Next, we performed a weighted gene co-expression network analysis (WGCNA) in each brain region (n = 24 individuals; 11 females and 13 males) to determine how whole networks of putatively co-regulated genes, rather than individual genes, relate to the full range of variation in latency to approach and feed during trials (Langfelder & Horvath, 2008). We created networks within brain regions because gene expression patterns were more region- than sex-specific (Figure 1B). Using the normalized counts from DESeq2, we filtered out genes with low counts (< 10 norm counts in the smallest group size; n = 11 for females) and low variability (25% least variable transcripts) within the hippocampus and striatum (n = 6,640 transcripts retained in cHp and rHp; n = 6,528 in striatum). Sample clustering was performed to detect sample-level outliers. We then generated a signed hybrid network by selecting a soft threshold power (β) = 3 for the caudal hippocampus, β = 9 for the rostral hippocampus, and β = 3 for the striatum in accordance with scale-free topology (signed $R^2 > 0.80$; Figure S1). We used a biweight mid-correlation (bicor) function and modules were calculated using a minimum module size of 30. A threshold

of 0.25 was used to merge modules in Dynamic Tree Cut (Figure S2). Genes not assigned to a module were classified to the color gray.

Identifying gene networks of interest

Expression levels for each module were summarized by the first principal component (module eigengene), which we used to test for an association between modules and the average time to approach or feed in each behavioral assay using the bicor function. We examined these relationships across and within sexes. We additionally included sex and body size (wing chord, tarsus length, and post-captivity mass) to account for these potentially confounding variables. We assessed each trait-associated module (defined as $p < 0.05$ and bicor > |0.60|) for enrichment of biological process GO terms using PANTHER (Mi et al., 2019). We only assessed genes with a significant module membership (MM; the correlation between the gene expression profile and module eigengene), defined as $p < 0.05$ and MM > 0.60.

We identified intramodular hub genes in trait-associated modules, as these genes are potential drivers of the patterns highlighted in these modules. We defined intramodular hub genes as those with a high trait-based gene significance (GS > |0.60|) for at least one module-related trait of interest and high MM (> 0.80), with a threshold of $p < 0.05$ for both (Horvath & Dong, 2008). We additionally looked for overlap between intramodular hub genes and DEGs as these genes have strong trait-related expression, making them core candidate hub genes. We identified whether these genes were found in the most enriched GO terms and visualized networks using Cytoscape (Shannon et al., 2003). Networks were filtered to only show edges supported by protein-protein interaction (PPI) data from the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) online database (https://string-db.org/) (Szklarczyk et al., 2019), with a combined score threshold of 0.4. We calculated the degree score for each gene, and those in the top 10% were classified as PPI hub genes.

To assess functional similarity across the two hippocampal subregions, we looked for consensus between the caudal and rostral hippocampus network modules by assessing similarity in module structure and gene membership. We additionally examined whether neophobia-related expression patterns emerged across brain regions by performing a permutational multivariate analysis of variance (PERMANOVA). We compared sample-level module eigengenes from all behavioral trait-related modules across sex, neophobia phenotype, and their interaction in

consistently neophobic and non-neophobic sparrows (n = 3 neophobic and 4 non-neophobic males; n = 3 neophobic and 3 non-neophobic females). Module eigengenes summarize the expression pattern of a module and reflect how strongly a sample aligns with the overall module profile, allowing comparisons of co-expression patterns across networks within samples. We used the "adonis2" function in the R package *vegan* (Oksanen et al., 2019), with Euclidean distance, and visualized sample differences using PCA.

Results

Distinct transcriptomic profiles by brain region

One of our goals was to examine whether Eurasian tree sparrows showed distinct patterns of constitutive gene expression in the striatum as well as in different parts of the hippocampus. Out of 8,935 genes, the majority (89.1%) were expressed in all three brain regions, though each region had ~100-200 uniquely expressed genes (Figure S3). A PCA revealed that the three brain regions had distinct transcriptomic profiles (Figure 1B). PC1 explained 63% of the variance and partitioned the striatum from both hippocampal regions, while PC2 explained 9% of the variance and partitioned the hippocampal regions. Overall, these data suggest that the caudal and rostral hippocampus had distinct transcriptomic profiles, but were more similar to each other than to the striatum.

Differentially expressed genes in neophobic and non-neophobic individuals by region and sex

Another goal was to compare sex- and region-specific differences in constitutive gene expression in consistently neophobic and non-neophobic individuals. The greatest differences were observed in the caudal hippocampus of females, followed by the rostral hippocampus of males (Figure S4). In females, there were n = 257 DEGs between non-neophobic and neophobic individuals in the caudal hippocampus (Figure S5A), n = 2 DEGs in the rostral hippocampus, and n = 4 DEGs in the striatum. In males, there were n = 2 DEGs between non-neophobic and neophobic individuals in the caudal hippocampus, n = 64 DEGs in the rostral hippocampus (Figure S5B), and n = 6 DEGs in the striatum. Only four DEGs were present in multiple contrasts, including neurotensin (NTS) in the male striatum and female caudal hippocampus, LOC100225836 in female striatum and caudal hippocampus, NBEAL2 in female striatum and caudal hippocampus, and NAPG in male rostral hippocampus and female caudal hippocampus

(for all DEGs see Table S3).

Significantly enriched biological processes were only identified for DEGs in the female caudal hippocampus (Table S4). DEGs upregulated in neophobic females relative to non-neophobic females were most significantly enriched for “cytoplasmic translation” and “ribosome biogenesis”, while DEGs upregulated in non-neophobic females were most enriched for “neuron cellular homeostasis” and “learning or memory”, as well as other processes like “cellular responses to BDNF” and “Gamma-aminobutyric acid (GABA) metabolic processes”. While no GO biological processes were found, several of the DEGs in other regions are involved in neuroendocrine processes (e.g., SCG2 and NTS in the male striatum and RIT2 in the male caudal hippocampus), neuron signaling (e.g., GRM2 in the female striatum and GRIK2 in male rostral hippocampus), and stress responsiveness (e.g., OPRK1 in female rostral hippocampus).

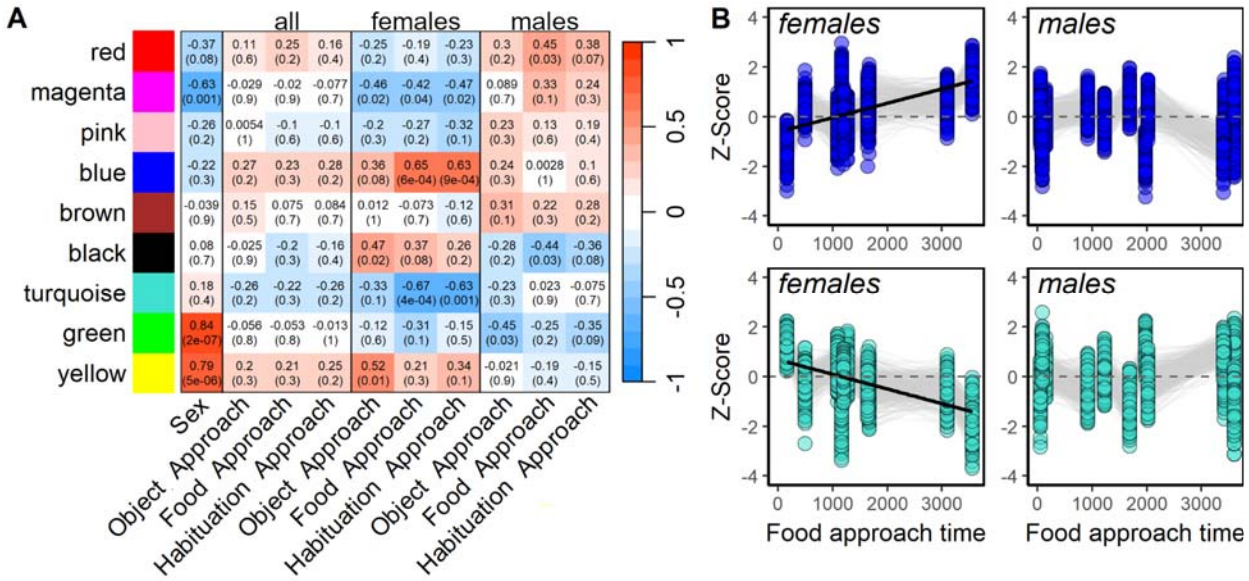

Figure 2. (A) Module-trait relationships in the sparrow caudal hippocampus were identified using bicor tests in WGCNA. Associations are shown for sex (females as reference) and latency to approach in behavioral trials across and within sexes. “Object Approach” refers to the latency to approach a food dish with a novel object; “Food Approach” a food dish containing a novel food; and “Habituation Approach” a food dish with the same (initially novel) object over four days. Correlation coefficients are shown with p-values in parentheses. (B) Relative expression of genes with MM > 0.8 in the blue (top) and turquoise (bottom) modules is shown across food approach times (in seconds) for females and males. Gray lines connect genes across samples. Z-

scores reflect standardized gene expression: $Z = (x - \mu)/\sigma$, where μ and σ are the mean and standard deviation across all samples and x is the expression of the gene in a specific sample.

Trait-associated modules in the caudal hippocampus

We also used co-expression network analyses to examine which genes in the hippocampus and striatum were most associated with the full range of neophobic or non-neophobic behaviors. Within the caudal hippocampus, WGCNA constructed 9 modules (Table S5), several of which demonstrated trait-related patterns in females but not males (Figure 2A). The blue module was positively associated with novel food and novel object habituation approach times in females, while the turquoise module was negatively associated with these traits (Figures 2A, 2B). Genes in the blue module were most enriched for terms linked to translation (e.g., "cytoplasmic translation" and "ribosome assembly") and energy production (e.g., "aerobic respiration" and "proton motive force-driven mitochondrial ATP synthesis") (Figure 3A; Table S6), suggesting co-regulated genes involved in these processes are linked to slower approach times to novel food items and slower habituation to novel objects in females. Several of the intramodular hub genes in the blue module were differentially expressed between consistently neophobic and non-neophobic females (n = 38 genes), of which 12 genes were also PPI hub genes (Figure S6A). These core candidate hub genes in the blue module were linked to some of the most enriched pathways (Figure 3A), suggesting they could be drivers of these processes.

Genes in the turquoise module were enriched for terms like "learning or memory", "chemical synaptic transmission", and "insulin receptor signaling pathway" (Figure 3B; Table S6), suggesting co-regulated genes involved in these processes are linked to faster approach times to novel food items and repeated presentations of the same initially novel object in females. Several of the intramodular hub genes in the turquoise module were also differentially expressed between consistently neophobic and non-neophobic females (n = 78 genes), of which 14 genes were also PPI hub genes (Figure S6B). These strong candidate hub genes were again linked to some of the most enriched biological processes (Figure 3B). Additional trait-related modules included the green and yellow modules that had genes expressed higher in males compared to females, and the magenta module that had genes with elevated expression in females relative to males (Figure S7). The magenta module was also negatively correlated with

novel food feeding time (Figure S7). Functional enrichment results for all trait-associated modules can be found in Table S6.

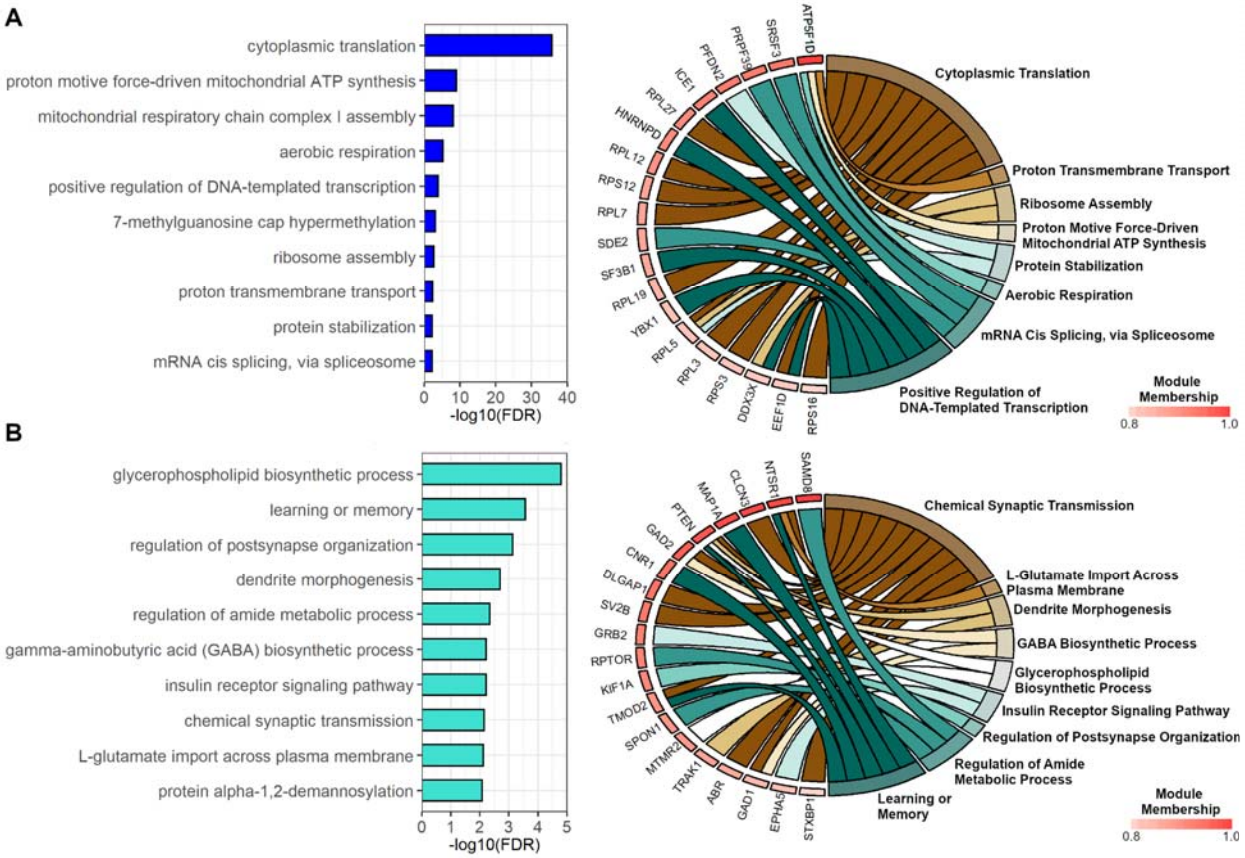

Figure 3. Barplot of the 10 most significant Gene Ontology (GO) biological processes and GOChord plot of associated genes in the (A) blue and (B) turquoise modules of the caudal hippocampus of Eurasian tree sparrows. Genes are linked to their assigned GO biological processes via colored ribbons and ordered according to their module membership. All genes shown are intramodular hubs and are differentially expressed between consistently neophobic and non-neophobic individuals.

Trait-associated modules in the rostral hippocampus

In the rostral hippocampus, WGCNA constructed 8 modules (Table S7), several of which demonstrated trait-related patterns in males but not females (Figures 4A, S8). In males, the greenyellow module was positively associated with novel food approach time, while the black module was negatively associated with this behavior (Figure 4B). The purple module was

negatively associated with novel object approach time and time to feed in the presence of a novel object in males (Figures 4A, S8). Genes in the greenyellow module were most enriched for terms related to cholesterol biosynthesis, steroid metabolism, and neurotransmitters ("GABA metabolic process" and "glutamate secretion") (Figure 5A; Table S6), suggesting co-regulated genes involved in these processes are associated with slower approach times to novel food items in males. One intramodular hub gene in the greenyellow module was differentially expressed between consistently neophobic and non-neophobic males (SLC1A3), and it was also a PPI hub gene (Figure S9A). SLC1A3 is linked to the following enriched GO terms: "transepithelial transport", "GABA metabolic process", and "behavior". Genes in the black module were enriched for terms related to transcription and post-transcriptional modifications, specifically mRNA splicing (Figure 5B; Table S6), suggesting co-regulated genes involved in these processes are linked to faster approach times to novel food items in males. Four intramodular hub genes in the black module were differentially expressed between consistently neophobic and non-neophobic males (MED13, SRSF7, RBBP6, and LOC100229022), of which SRSF7 was also a PPI hub gene (Figure S9B). SRSF7 is linked to the following enriched GO terms: "regulation of mRNA splicing, via spliceosome" and "mRNA cis splicing, via spliceosome". Genes in the purple module were most enriched for a post-translational modification (i.e., "peptidyl-serine phosphorylation"; Table S6), suggesting this process could be linked to faster novel object approach and feeding times in males. One intramodular hub gene in the purple module was also differentially expressed between consistently neophobic and non-neophobic males (MACF1). Additional trait-related modules included the green and brown modules that had genes expressed higher in males and females, respectively (Figure S8). Of note, several critical stress-related genes, including CRH, NR3C1, and CRHR2 were assigned to the brown module. Functional enrichment results for all trait-associated modules can be found in Table S6.

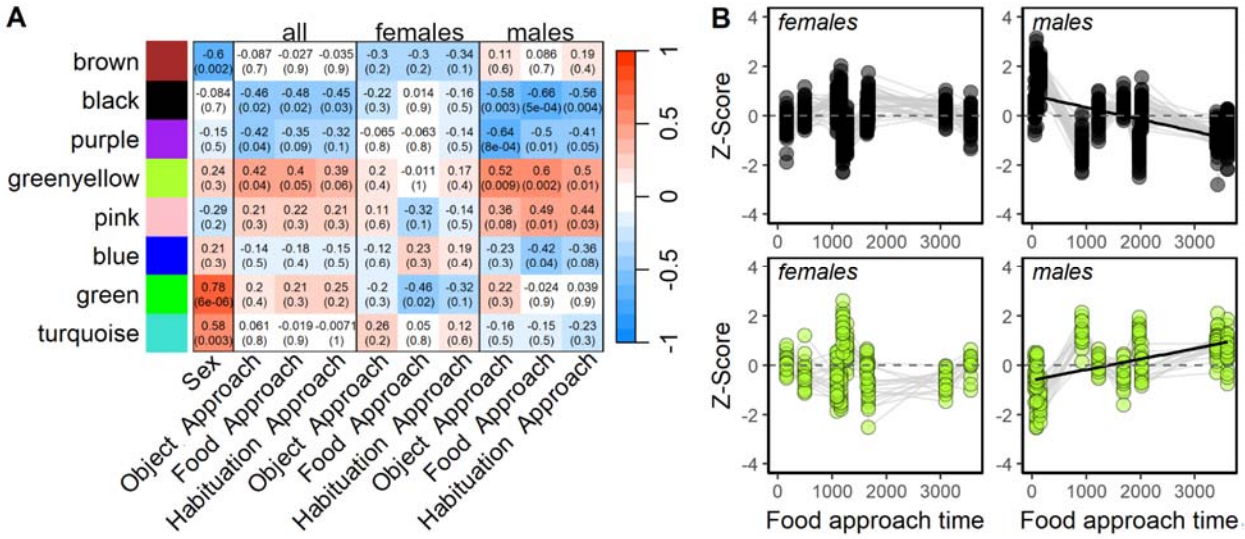

Figure 4. (A) Module-trait relationships in the sparrow rostral hippocampus were identified using bicor tests in WGCNA. Associations are shown for sex (females as reference) and latency to approach in behavioral trials across and within sexes. "Object Approach" refers to the latency to approach a food dish with a novel object; "Food Approach" a food dish containing a novel food; and "Habituation Approach" a food dish with the same (initially novel) object over four days. Correlation coefficients are shown with p-values in parentheses. (B) Relative expression of genes with MM > 0.8 in the greenyellow (top) and black (bottom) modules is shown across food approach times (in seconds) for females and males. Gray lines connect genes across samples. Z-scores reflect standardized gene expression: Z = (x − μ)/σ, where μ and σ are the mean and standard deviation across all samples and x is the expression of the gene in a specific sample.

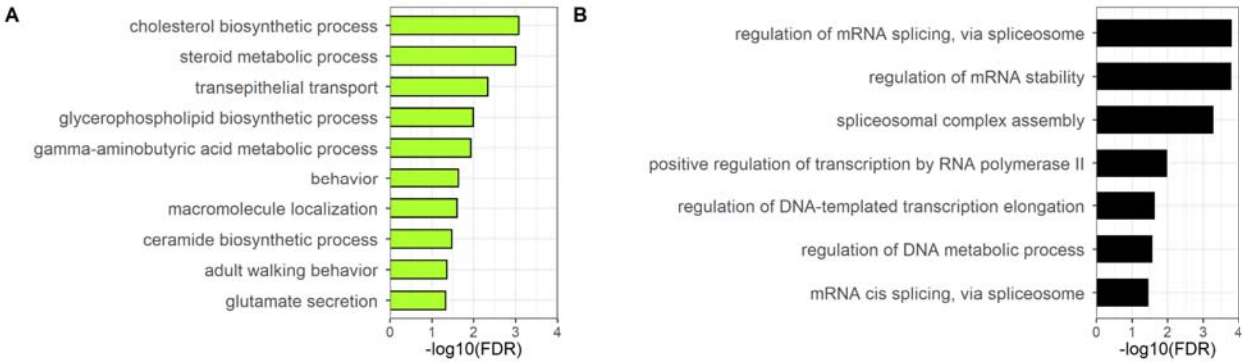

Figure 5. Barplot of the most significant Gene Ontology biological processes in the (A) greenyellow and (B) black modules of the rostral hippocampus of Eurasian tree sparrows.

Trait-associated modules in the striatum

One outlier sample was removed from the striatum dataset (Figure S10). WGCNA constructed 12 modules in the striatum (Table S8), several of which demonstrated trait-related patterns in both males and females (Figures S11A, S12). The lightcyan module was negatively associated with multiple behavioral metrics in males, while the midnight blue, purple, and black modules were positively associated with neophobia metrics in males. The brown and red modules were positively associated with neophobia behavioral metrics in females. The most significant relationships were between the brown module and female novel object habituation approach time and the lightcyan module and male novel food approach time (Figure S11B).

Genes in the brown module were most enriched for terms linked to cellular transport and organization (i.e., "regulation of vesicle-mediated transport", "positive regulation of organelle organization", and "regulation of catabolic processes"; Table S6), suggesting co-regulated genes involved in these processes are linked to greater neophobia in females. There was no overlap between DEGs and intramodular hub genes in the brown module. Genes in the lightcyan module had no enriched terms, but one intramodular hub gene (HTT) was also differentially expressed between consistently neophobic and non-neophobic males, and it was also a PPI hub gene (Figure S13). Genes in the midnight blue module were enriched for terms related to sensory development (i.e., "visual system development" and "inner ear auditory receptor cell differentiation") and signaling (i.e., notch and BMP signaling pathway terms), suggesting genes linked to these processes are elevated in more neophobic males, but there was no overlap between DEGs and intramodular hub genes. The black module was also most enriched for sensory system terms (i.e., "trigeminal nerve structural organization", "branchiomotor neuron axon guidance", "G protein-coupled opioid receptor signaling pathway", "sensory perception of pain", "adult behavior", and "sensory system development") and signaling processes (i.e., "neuropeptide signaling pathway" and "regulation of neuronal synaptic plasticity"), but there was no overlap between DEGs and intramodular hub genes. Genes in the purple module had no enriched terms, but one intramodular hub gene was also differentially expressed between consistently neophobic and non-neophobic males (SCG2). The red module was most enriched for mRNA splicing, suggesting genes linked to this process are elevated in more neophobic females, but there was no overlap between DEGs and intramodular hub genes. Additional trait-related modules included the yellow module that had genes expressed higher in males than

females. Functional enrichment results for all trait-associated modules can be found in Table S6.

Patterns across brain regions

To better understand patterns of similarity and difference across the two subregions of the hippocampus, and their sex-specific associations, we examined consensus between rostral and caudal hippocampal modules. We found significant gene overlap between neophobia-associated modules in the female caudal hippocampus and male rostral hippocampus, but relationships were often reversed in the two sexes. Significant overlap in trait-associated modules included the blue module in the caudal hippocampus and the black module in the rostral hippocampus, the turquoise module in the caudal hippocampus and the greenyellow module in the rostral hippocampus, and the yellow module in the caudal hippocampus and green module in the rostral hippocampus (Figure 6). The blue-black module overlap involved 278 genes (constituting 77.9% of the black module genes) that were positively correlated with novel food approach times in the female caudal hippocampus (Figure 2B), but negatively correlated with this trait in the male rostral hippocampus (Figure 4B). Genes found in both modules that had a MM > 0.80 and were significantly associated with behavioral traits were linked to transcription and post-transcriptional (e.g., mRNA splicing) processes (Figure S14A), suggesting that these processes may be elevated in the rostral hippocampus of less neophobic males and in the caudal hippocampus of more neophobic females. The turquoise-greenyellow overlap involved 212 genes (constituting 88.3% of the greenyellow module genes) that were negatively correlated with novel food approach times in the female caudal hippocampus (Figure 2B), but positively correlated with this trait in the male rostral hippocampus (Figure 4B). Trait-related genes with high MM found in both modules were linked to chemical synaptic transmission and processes related to GABA and dopamine (Figure S14B), suggesting these processes may be elevated in the caudal hippocampus of less neophobic females and in the rostral hippocampus of more neophobic males. The yellow-green overlap involved 184 genes that were elevated in males in both brain regions.

We also examined whether neophobia-related expression patterns emerged across brain regions using the most consistently neophobic and non-neophobic individuals. We found that module eigengene profiles across brain regions significantly differed by behavioral phenotype. PERMANOVA results showed a significant main effect of neophobia ($F_{1,9} = 3.44$, $p = 0.006$),

but not sex ($F_{1,9}$ = 0.98, p = 0.45). There was a trend for the neophobia-by-sex interaction to be significant (neophobia by sex: $F_{1,9}$ = 1.96, p = 0.08), thus we analyzed the principal component scores within each sex separately. Among males, more neophobic sparrows had significantly lower PC1 scores ($F_{1,5}$ = 13.04, p = 0.015), but no difference in PC2 ($F_{1,5}$ = 1.87, p = 0.23). Among females, more neophobic sparrows had significantly lower PC2 scores ($F_{1,4}$ = 10.71, p = 0.03), but not PC1 ($F_{1,4}$ = 0.003, p = 0.96). These differences were evident in the PCA, where the first two PCs explained 61.1% of the total variance in trait-related module eigengenes (Figure S15A). Lower PC1 scores in neophobic males reflected relatively lower expression of genes in the lightcyan module (striatum) and black module (rostral hippocampus), and higher expression in the greenyellow module (rostral hippocampus) (Figure S15B). In contrast, lower PC2 scores in neophobic females reflected higher expression in the brown module (striatum) and blue module (caudal hippocampus), and lower expression in the turquoise module (caudal hippocampus) (Figure S15B). These results suggest that region-specific co-expression patterns are not isolated but instead co-occur within individuals in a behavior- and sex-dependent manner, likely reflecting coordinated transcriptional patterns across multiple brain regions.

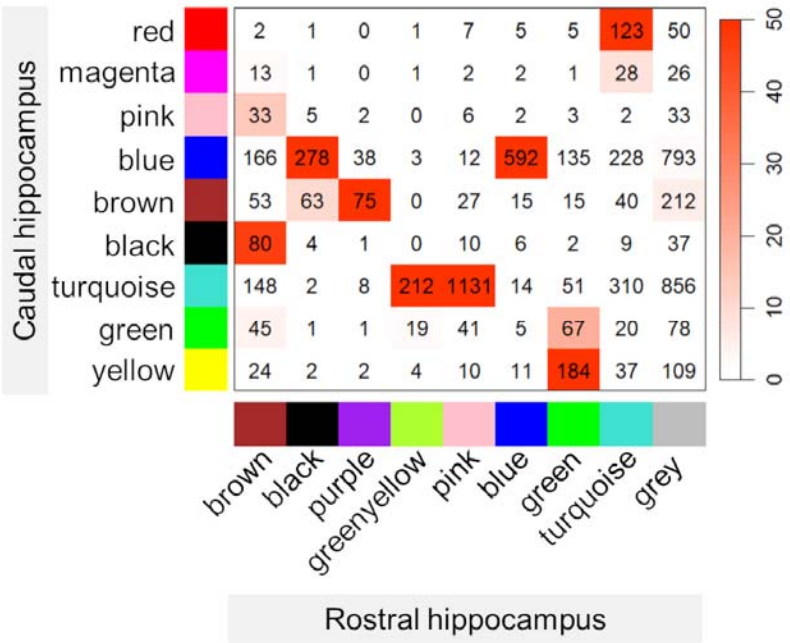

Figure 6. Correspondence between gene co-expression modules in the caudal and rostral hippocampus of Eurasian tree sparrows. Each row represents a module from the caudal hippocampus, and each column represents a module from the rostral hippocampus. Cell values

indicate the number of transcripts shared between modules. Color intensity reflects the significance of module overlap; darker red color indicates a more significant overlap as calculated using Fisher's exact test and shown as -log10(p-value).

Discussion

We identified region-specific, constitutive transcriptomic patterns associated with neophobia in female and male Eurasian tree sparrows (*Passer montanus*). The striatum and hippocampus had distinct transcriptomic profiles, as did the rostral and caudal subregions of the hippocampus. The number of differentially expressed genes between neophobic and non-neophobic individuals varied across brain regions and sexes. However, the full complexity of the molecular mechanisms underlying neophobic behaviors was better captured using WGCNA, which revealed that neophobia was associated with similar gene modules between sexes, but their expression was localized to different hippocampal subregions in males and females. In females, neophobic behaviors were associated with gene modules in the caudal hippocampus that were enriched for synaptic transmission, steroid metabolism, and post-transcriptional processes, whereas in males, neophobic behaviors were associated with similar gene modules in the rostral hippocampus. Altogether, these findings suggest that although neophobic behaviors are similar between sexes, they may be driven by region-specific neurobiological pathways. Below, we discuss these results and their implications for our understanding of the neurotranscriptomic mechanisms related to neophobia.

Brain regions had distinct transcriptomic profiles, differentiating the caudal and rostral hippocampus

Brain region was a major driver of variation in gene expression. Though most genes were expressed in the striatum, caudal hippocampus, and rostral hippocampus, each region had ~100-200 uniquely expressed genes. We found that the caudal and rostral hippocampus had distinct transcriptomic profiles, supporting the hypothesis that the avian hippocampus may show subregional specialization along a rostral-caudal axis, similar to the dorsal-ventral axis of the rodent hippocampus (Fanselow & Dong, 2010; Gualtieri et al., 2019; Smulders, 2017). Specifically, the rostral hippocampus may play a larger role in spatial cognition, while the caudal hippocampus may be more involved in anxiety, stress, and avoidance responses (Madison,

Bingman, Smulders, & Lattin, 2023). A recent study in black-capped chickadees also supports the functional specialization of these regions in songbirds, as the caudal hippocampus received more inputs from amygdalar regions involved in emotional responses, whereas the rostral hippocampus received more inputs from the thalamus, which plays a key role in relaying sensory and motor information (Applegate, Gutnichenko, & Aronov, 2023). To our knowledge, our study is the first to confirm that the rostral and caudal hippocampus express different transcriptomic profiles in birds. Future transcriptomic comparisons between the rostral and caudal subregions can further clarify whether different parts of the hippocampus are specialized for different functions in birds as well as mammals.

The striatum was most differentiated from the caudal and rostral hippocampus, as would be predicted based on its distinct structure and functions. Though subregional specializations of the striatum have not been well-explored in birds (Mezey & Csillag, 2002), the striatum of mammals is typically divided into the dorsal striatum, encompassing the caudate and putamen, and the ventral striatum, which includes the nucleus accumbens and the olfactory tubercle (Voorn, Vanderschuren, Groenewegen, Robbins, & Pennartz, 2004). Future work could explore the transcriptomic profiles of striatal structures in birds as a means to better characterize potential striatal subregions.

Neophobia-associated gene modules differed by brain region and sex

While prior work with this same group of Eurasian tree sparrows (as well as studies of the congeneric house sparrow) found no sex difference in neophobic behaviors (Ensminger et al., 2012; Kelly et al., 2020; Krajcir et al., 2024), we identified sex-specific patterns of neophobia-associated gene expression. Specifically, neophobic behaviors correlated with gene expression in the caudal hippocampus of females and the rostral hippocampus of males. Both categorical and continuous comparisons of gene expression related to neophobia revealed this sex-by-region interaction. Though more work is needed to understand the drivers of sex-specific gene expression in Eurasian tree sparrows, one hypothesis is that not only does the hippocampus show subregional specialization in birds, but that neural processes in these subregions vary by sex. Notably, our finding in birds aligns with numerous studies from rodents that cognitive performance-related behaviors are associated with neurogenesis in the ventral hippocampus of

females, and in the dorsal hippocampus of males (Dalla et al. 2009, Yagi et al. 2016, Yagi & Galea 2019). In rats, there are numerous sex differences in hippocampal anatomy that include dendritic spine density in the CA1 subregion (Shors et al., 2001; Woolley, Gould, Frankfurt, & McEwen, 1990), morphology of CA3 pyramidal neurons (Juraska, Fitch, & Washburne, 1989), and dendritic branching in the dentate gyrus (Juraska, Fitch, Henderson, & Rivers, 1985). Similar differences may exist in the hippocampus of male and female birds, but this remains to be determined. This rodent work, together with the present study, suggest that sex differences in hippocampal processes may be conserved across vertebrates.

Our findings highlight that neophobia is regulated by sex-specific patterns of gene expression, and provide testable hypotheses for genes and processes that may play a conserved role in neophobia across species. In the caudal hippocampus, the most neophobic females had higher expression of genes involved in energy production and transcription/mRNA splicing/translation (blue module). The latter processes could increase transcriptomic diversity and functional complexity (Mauger & Scheiffele, 2017), yielding more diverse molecular routes to respond to novel stimuli. In contrast, non-neophobic females had higher expression of genes involved in various forms of signaling activity (turquoise module). This included GO terms related to learning or memory, synaptic transmission, and GABA biosynthesis. GABA is a key inhibitory neurotransmitter, and its synthesis depends on glutamic acid decarboxylases (GAD1 and GAD2), both of which were differentially expressed hub genes. Expression of these enzymes is highly correlated with GABA levels and inhibitory synaptic neurotransmission (Dicken, Hughes, & Hentges, 2015; Lee, Lee, & Lee, 2019), which shape neuronal activity and contribute to contextual learning in the hippocampus (Lovett-Barron et al., 2014). Additional differentially expressed hub genes included NTSR1 and CNR1, which are implicated in hippocampal learning and memory (Li et al., 2011; Ruiz-Contreras et al., 2013), as well as in modulating stress responses and anxiety-like behaviors (Kyriatzis, Khrestchatisky, Ferhat, & Chatzaki, 2024; M. Martin, Ledent, Parmentier, Maldonado, & Valverde, 2002). Thus, differences in the constitutive expression of these genes may influence how individuals respond to novel and potentially threatening stimuli. In the rostral hippocampus, the most neophobic males showed higher expression of genes involved in signaling activity, including GABA and steroids (greenyellow module), but lower expression of genes linked to transcription/mRNA splicing (black module). Note that although we measured gene expression three weeks after behavioral trials ended, it is

possible that exposure to novel stimuli initiated lasting effects on neuroplasticity that were still ongoing at the time of tissue collection. Regardless, these patterns suggest that neophobic males may rely on specific signaling pathways rather than broad transcriptional flexibility in a hippocampal subregion involved in spatial processing, whereas non-neophobic females demonstrate a similar strategy but in a subregion associated with stress and anxiety.

Overall, our results suggest that basic neurobiological processes vary among individuals with different behavioral phenotypes, and that some parallel mechanisms may regulate neophobia in both sexes but in different brain regions and in opposite directions. This supports so-called 'Type III' sex differences (sensu (McCarthy et al., 2012)), where convergent behaviors are underlain by divergent neurobiological processes in females and males. What remains to be determined is how these individual and sex-specific differences in gene expression arise; is variation driven by innate neurobiological processes, or is it shaped by flexibly interacting with the environment? Activational and/or organizational effects of sex steroids could underlie these sex differences (Adkins-Regan, 2005). From an evolutionary standpoint, life-history differences in parental investment between male and female birds and differential vulnerability to stress-induced reproductive suppression also likely shape the patterns we observed (R. M. Calisi, Austin, Lang, & MacManes, 2018; Hirschenhauser, Winkler, & Oliveira, 2003; Ouyang, Quetting, & Hau, 2012; Schmidt, MacDougall-Shackleton, Soma, & MacDougall-Shackleton, 2014). As more studies (finally!) include both males and females, we encourage sex contextualist approaches (Pape et al., 2024; Richardson, 2022; Smiley et al., 2024) that assess the possibility of sex-related variation in neurobiology even in the absence of behavior differences, as we would not have discovered these sex-specific associations between neophobia and neural gene expression otherwise.

References

Adkins-Regan, E. (2005). *Hormones and Animal Social Behavior*. Princeton, NJ: Princeton University Press.

Applegate, M. C., Gutnichenko, K. S., & Aronov, D. (2023). Topography of inputs into the hippocampal formation of a food-caching bird. *Journal of Comparative Neurology, 531*(16), 1669-1688. doi:https://doi.org/10.1002/cne.25533

Bell, A. M., & Robinson, G. E. (2011). Behavior and the dynamic genome. *Science, 332*(6034), 1161. doi:10.1126/science.1203295

Bengston, S. E., Dahan, R. A., Donaldson, Z., Phelps, S. M., van Oers, K., Sih, A., & Bell, A. M. (2018). Genomic tools for behavioural ecologists to understand repeatable individual differences in behaviour. *Nat Ecol Evol, 2*(6), 944-955. doi:10.1038/s41559-017-0411-4

Breck, S. W., Poessel, S. A., Mahoney, P., & Young, J. K. (2019). The intrepid urban coyote: a comparison of bold and exploratory behavior in coyotes from urban and rural environments. *Sci Rep, 9*(1), 2104. doi:10.1038/s41598-019-38543-5

Brivio, E., Lopez, J. P., & Chen, A. (2020). Sex differences: Transcriptional signatures of stress exposure in male and female brains. *Genes, brain and behavior, 19*(3), e12643.

Calisi, R. M., Austin, S. H., Lang, A. S., & MacManes, M. D. (2018). Sex-biased transcriptomic response of the reproductive axis to stress. *Horm Behav, 100*, 56-68. doi:10.1016/j.yhbeh.2017.11.011

Calisi, R. M., & MacManes, M. D. (2015). RNAseq-ing a more integrative understanding of animal behavior. *Current Opinion in Behavioral Sciences, 6*, 65-68. doi:https://doi.org/10.1016/j.cobeha.2015.09.007

Candler, S., & Bernal, X. E. (2015). Differences in neophobia between cane toads from introduced and native populations. *Behavioral Ecology, 26*(1), 97-104. doi:10.1093/beheco/aru162

Cohen, T. M., Kumar, R. S., Nair, M., Hauber, M. E., & Dor, R. (2020). Innovation and decreased neophobia drive invasion success in a widespread avian invader. *Animal Behaviour, 163*, 61-72. doi:10.1016/j.anbehav.2020.02.012

Crane, A. L., & Ferrari, M. C. O. (2017). Patterns of predator neophobia: a meta-analytic review. *Proc Biol Sci, 284*(1861). doi:10.1098/rspb.2017.0583

Dicken, M. S., Hughes, A. R., & Hentges, S. T. (2015). Gad1 mRNA as a reliable indicator of altered GABA release from orexigenic neurons in the hypothalamus. *Eur J Neurosci, 42*(9), 2644-2653. doi:10.1111/ejn.13076

Dusang, B. A., Henry, M. S., Kimball, M. G., Cochran, E. B., Wilson, M. B., & Lattin, C. R. (2025). Spatial neophobia is still not correlated with object neophobia in wild-caught house sparrows (Passer domesticus). *Royal Society Open Science, 12*(5), 250220.

Ensminger, A. L., Westneat, D. F., & Zeh, D. (2012). Individual and sex differences in habituation and neophobia in house sparrows (Passer domesticus). *Ethology, 118*(11), 1085-1095. doi:10.1111/eth.12009

Fair, J. E., Paul, E., Jones, J., & Bies, L. (2023). *Guidelines to the Use of Wild Birds in Research*. Washington D.C.: Ornithological Council.

Fanselow, M. S., & Dong, H. W. (2010). Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron, 65*(1), 7-19. doi:10.1016/j.neuron.2009.11.031

Greenberg, R., & Mettke-Hofmann, C. (2001). Ecological aspects of neophobia and neophilia in birds. In V. Nolan & C. F. Thompson (Eds.), *Current Ornithology* (Vol. 16). Boston, MA: Springer.

Grunst, A. S., Grunst, M. L., Pinxten, R., & Eens, M. (2019). Personality and plasticity in neophobia levels vary with anthropogenic disturbance but not toxic metal exposure in urban great tits: Urban disturbance, metal pollution and neophobia. *Sci Total Environ, 656*, 997-1009. doi:10.1016/j.scitotenv.2018.11.383

Gualtieri, F., Armstrong, E. A., Longmoor, G. K., D'Eath, R. B., Sandilands, V., Boswell, T., & Smulders, T. V. (2019). Unpredictable chronic mild stress suppresses the incorporation of new neurons at the caudal pole of the chicken hippocampal formation. *Sci Rep, 9*(1), 7129. doi:10.1038/s41598-019-43584-x

Guigueno, M. F., MacDougall-Shackleton, S. A., & Sherry, D. F. (2016). Sex and seasonal differences in hippocampal volume and neurogenesis in brood-parasitic brown-headed cowbirds (Molothrus ater). *Dev Neurobiol, 76*(11), 1275-1290. doi:10.1002/dneu.22421

Güntürkün, O. (2005). The avian ‘prefrontal cortex’ and cognition. *Current Opinion in Neurobiology, 15*(6), 686-693. doi:http://dx.doi.org/10.1016/j.conb.2005.10.003

Hauber, M. E., Louder, M. I., & Griffith, S. C. (2021). Neurogenomic insights into the behavioral and vocal development of the zebra finch. *Elife, 10*. doi:10.7554/eLife.61849

Hirschenhauser, K., Winkler, H., & Oliveira, R. F. (2003). Comparative analysis of male androgen responsiveness to social environment in birds: the effects of mating system and paternal incubation. *Horm Behav, 43*(4), 508-519. doi:10.1016/s0018-506x(03)00027-8

Horvath, S., & Dong, J. (2008). Geometric interpretation of gene coexpression network analysis. *PLoS Comput Biol, 4*(8), e1000117. doi:10.1371/journal.pcbi.1000117

Huang, P., St.Mary, C. M., & Kimball, R. T. (2020). Habitat urbanization and stress response are primary predictors of personality variation in northern cardinals (Cardinalis cardinalis). *Journal of Urban Ecology, 6*(1), juaa015. doi:10.1093/jue/juaa015

Jarjour, C., Evans, J. C., Routh, M. l., & Morand-Ferron, J. (2019). Does city life reduce neophobia? A study on wild black-capped chickadees. *Behavioral Ecology, 31*(1), 123-131. doi:10.1093/beheco/arz167

Juraska, J. M., Fitch, J. M., Henderson, C., & Rivers, N. (1985). Sex differences in the dendritic branching of dentate granule cells following differential experience. *Brain research, 333*(1), 73-80.

Juraska, J. M., Fitch, J. M., & Washburne, D. L. (1989). The dendritic morphology of pyramidal neurons in the rat hippocampal CA3 area. II. Effects of gender and the environment. *Brain research, 479*(1), 115-119.

Kelly, T. R., Kimball, M. G., Stansberry, K. R., & Lattin, C. R. (2020). No, you go first: phenotype and social context affect house sparrow neophobia. *Biology Letters, 16*, 20200286.

Kimball, M. G., Gautreaux, E. B., Couvillion, K. E., Kelly, T. R., Stansberry, K. R., & Lattin, C. R. (2022). Novel objects alter immediate early gene expression globally for ZENK and regionally for c-Fos in neophobic and non-neophobic house sparrows. *Behav Brain Res, 428*, 113863. doi:10.1016/j.bbr.2022.113863

Kimball, M. G., & Lattin, C. R. (2023a). Exploration of A novel environment is not correlated with object neophobia in wild-caught house sparrows (Passer domesticus). *Behav Processes, 210*, 104913. doi:10.1016/j.beproc.2023.104913

Kimball, M. G., & Lattin, C. R. (2023b). The “Seven Deadly Sins" of Neophobia Experimental Design. *Integr Comp Biol*. doi:10.1093/icb/icad127

Klein, S. L., Schiebinger, L., Stefanick, M. L., Cahill, L., Danska, J., De Vries, G. J., . . . Woodruff, T. K. (2015). Sex inclusion in basic research drives discovery. *Proceedings of the National Academy of Sciences, 112*(17), 5257-5258.

Krajcir, K. J., Kelly, T. R., Kimball, M. G., Cochran, E. B., Stansberry, K. R., Dusang, B. A., . . . Lattin, C. R. (2024). Eurasian tree sparrows are more food neophobic and habituate to novel objects more slowly than house sparrows. *Biological Invasions*. doi:10.1007/s10530-024-03403-5

Kumar, S., Suleski, M., Craig, J. M., Kasprowicz, A. E., Sanderford, M., Li, M., . . . Hedges, S. B. (2022). TimeTree 5: An Expanded Resource for Species Divergence Times. *Mol Biol Evol, 39*(8). doi:10.1093/molbev/msac174

Kyriatzis, G., Khrestchatisky, M., Ferhat, L., & Chatzaki, E. A. (2024). Neurotensin and neurotensin receptors in stress-related disorders: Pathophysiology & novel drug targets. *Current neuropharmacology, 22*(5), 916-934.

Langfelder, P., & Horvath, S. (2008). WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics, 9*, 559. doi:10.1186/1471-2105-9-559

Lattin, C. R., Kelly, T. R., Kelly, M. W., & Johnson, K. M. (2022). Constitutive gene expression differs in three brain regions important for cognition in neophobic and non-neophobic house sparrows (Passer domesticus). *PLoS One, 17*(5), e0267180. doi:10.1371/journal.pone.0267180

Lee, S. E., Lee, Y., & Lee, G. H. (2019). The regulation of glutamic acid decarboxylases in GABA neurotransmission in the brain. *Arch Pharm Res, 42*(12), 1031-1039. doi:10.1007/s12272-019-01196-z

Li, J., Chen, C., Chen, C., He, Q., Li, H., Li, J., . . . Dong, Q. (2011). Neurotensin receptor 1 gene (NTSR1) polymorphism is associated with working memory. *PLoS One, 6*(3), e17365.

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol, 15*(12), 550. doi:10.1186/s13059-014-0550-8

Lovett-Barron, M., Kaifosh, P., Kheirbek, M. A., Danielson, N., Zaremba, J. D., Reardon, T. R., . . . Losonczy, A. (2014). Dendritic inhibition in the hippocampus supports fear learning. *Science, 343*(6173), 857. doi:10.1126/science.1247485

Madison, F. N., Bingman, V. P., Smulders, T. V., & Lattin, C. R. (2023). A bird's eye view of the hippocampus beyond space: Behavioral, neuroanatomical, and neuroendocrine perspectives. *Horm Behav, 157*, 105451. doi:10.1016/j.yhbeh.2023.105451

Martin, L. B., & Fitzgerald, L. (2005). A taste for novelty in invading house sparrows, Passer domesticus. *Behavioral Ecology, 16*(4), 702-707. doi:10.1093/beheco/ari044

Martin, M., Ledent, C., Parmentier, M., Maldonado, R., & Valverde, O. (2002). Involvement of CB1 cannabinoid receptors in emotional behaviour. *Psychopharmacology, 159*(4), 379-387.

Mauger, O., & Scheiffele, P. (2017). Beyond proteome diversity: alternative splicing as a regulator of neuronal transcript dynamics. *Curr Opin Neurobiol, 45*, 162-168. doi:10.1016/j.conb.2017.05.012

McCarthy, M. M., Arnold, A. P., Ball, G. F., Blaustein, J. D., & De Vries, G. J. (2012). Sex differences in the brain: the not so inconvenient truth. *J Neurosci, 32*(7), 2241-2247. doi:10.1523/JNEUROSCI.5372-11.2012

Medina, L., & Reiner, A. (1995). Neurotransmitter organization and connectivity of the basal ganglia in vertebrates: implications for the evolution of basal ganglia. *Brain Behav Evol, 46*(4-5), 235-258. doi:10.1159/000113277

Mezey, S., & Csillag, A. (2002). Selective striatal connections of midbrain dopaminergic nuclei in the chick (Gallus domesticus). *Cell and tissue research, 308*(1), 35-46.

Mi, H., Muruganujan, A., Ebert, D., Huang, X., & Thomas, P. D. (2019). PANTHER version 14: more genomes, a new PANTHER GO-slim and improvements in enrichment analysis tools. *Nucleic Acids Res, 47*(D1), D419-D426. doi:10.1093/nar/gky1038

Mortazavi, A., Williams, B. A., McCue, K., Schaeffer, L., & Wold, B. (2008). Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nature Methods, 5*(7), 621-628. doi:10.1038/nmeth.1226

Nixdorf-Bergweiler, B. E., & Bischof, H.-J. (2007). *A stereotaxic atlas of the brain of the zebra finch, Taeniopygia guttata, with special emphasis on telencephalic visual and song system nuclei in transverse and sagittal sections. Bethesda (MD).*

Oakley, R. H., Riddick, N. V., Moy, S. S., & Cidlowski, J. A. (2024). Imbalanced glucocorticoid and mineralocorticoid stress hormone receptor function has sex-dependent and independent regulatory effects in the mouse hippocampus. *Neurobiology of stress, 28*, 100589.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., . . . Wagner, H. (2019). vegan: Community Ecology Package. https://CRAN.R-project.org/package=vegan.

Olszewski, P. K., Ulrich, C., Ling, N., Allen, K., & Levine, A. S. (2014). A non-peptide oxytocin receptor agonist, WAY-267,464, alleviates novelty-induced hypophagia in mice: insights into changes in c-Fos immunoreactivity. *Pharmacology Biochemistry and Behavior, 124*, 367-372.

Ouyang, J. Q., Quetting, M., & Hau, M. (2012). Corticosterone and brood abandonment in a passerine bird. *Animal Behaviour, 84*(1), 261-268. doi:10.1016/j.anbehav.2012.05.006

Pape, M., Miyagi, M., Ritz, S. A., Boulicault, M., Richardson, S. S., & Maney, D. L. (2024). Sex contextualism in laboratory research: Enhancing rigor and precision in the study of sex-related variables. *Cell, 187*(6), 1316-1326. doi:https://doi.org/10.1016/j.cell.2024.02.008

Pertea, M., Pertea, G. M., Antonescu, C. M., Chang, T. C., Mendell, J. T., & Salzberg, S. L. (2015). StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat Biotechnol, 33*(3), 290-295. doi:10.1038/nbt.3122

Petersen, K., & Sherry, D. F. (1996). No sex difference occurs in hippocampus, food-storing, or memory for food caches in black-capped chickadees. *Behavioural brain research, 79*(1-2), 15-22.

Ramírez-Lugo, L., Zavala-Vega, S., Pedroza-Llinas, R., Núñez-Jaramillo, L., & Bermúdez-Rattoni, F. (2015). Effects of glutamate and its metabotropic receptors class 1 antagonist in appetitive taste memory formation. *Behavioural Brain Research, 284*, 213-217.

Rhie, A., McCarthy, S. A., Fedrigo, O., Damas, J., Formenti, G., Koren, S., . . . Jarvis, E. D. (2021). Towards complete and error-free genome assemblies of all vertebrate species. *Nature, 592*(7856), 737-746. doi:10.1038/s41586-021-03451-0

Richardson, S. S. (2022). Sex contextualism. *Philosophy, Theory, and Practice in Biology, 14*, 2. doi:https://doi.org/10.3998/ptpbio.2096

Roman, C., Lin, J.-Y., & Reilly, S. (2009). Conditioned taste aversion and latent inhibition following extensive taste preexposure in rats with insular cortex lesions. *Brain research, 1259*, 68-73.

Rose, J., & Colombo, M. (2005). Neural correlates of executive control in the avian brain. *PLoS Biol, 3*(6), e190. doi:10.1371/journal.pbio.0030190

Royet, J.-P., Gervais, R., & Araneda, S. (1983). Effect of local 6-OHDA and 5, 6-DHT injections into the rat olfactory bulb on neophobia and learned aversion to a novel food. *Behavioural brain research, 10*(2-3), 297-309.

Ruiz-Contreras, A. E., Carrillo-Sánchez, K., Gómez-López, N., Vadillo-Ortega, F., Hernández-Morales, S., Carnevale-Cantoni, A., . . . Prospéro-García, O. (2013). Working memory performance in young adults is associated to the AATn polymorphism of the CNR1 gene. *Behavioural brain research, 236*, 62-66.

Schmidt, K. L., MacDougall-Shackleton, E. A., Soma, K. K., & MacDougall-Shackleton, S. A. (2014). Developmental programming of the HPA and HPG axes by early-life stress in male and female song sparrows. *General and Comparative Endocrinology, 196*, 72-80. doi:https://doi.org/10.1016/j.ygcen.2013.11.014

Semenov, G. A., Sonnenberg, B. R., Branch, C. L., Heinen, V. K., Welklin, J. F., Padula, S. R., . . . Taylor, S. A. (2024). Genes and gene networks underlying spatial cognition in food-caching chickadees. *Current Biology, 34*(9), 1930-1939. e1934.

Shansky, R. M., & Murphy, A. Z. (2021). Considering sex as a biological variable will require a global shift in science culture. *Nature neuroscience, 24*(4), 457-464.

Shephard, R., Buxton, D., & Broadhurst, P. (1982). β-adrenoceptor antagonists may attenuate hyponeophagia in the rat through a serotonergic mechanism. *Pharmacology Biochemistry and Behavior, 16*(5), 741-744.

Sherry, D. F., Forbes, M., Khurgel, M., & Ivy, G. O. (1993). Females have a larger hippocampus than males in the brood-parasitic brown-headed cowbird. *Proceedings of the National Academy of Sciences, 90*(16), 7839-7843.

Shinohara, K., & Yasoshima, Y. (2021). Inactivation of the ventral hippocampus facilitates the attenuation of odor neophobia in rats. *Behavioural Brain Reseach, 401*, 113077. doi:10.1016/j.bbr.2020.113077

Shors, T. J., Chua, C., & Falduto, J. (2001). Sex differences and opposite effects of stress on dendritic spine density in the male versus female hippocampus. *Journal of Neuroscience, 21*(16), 6292-6297.

Simpson, K. L., Weaver, K. J., de Villers-Sidani, E., Lu, J. Y.-F., Cai, Z., Pang, Y., . . . Lin, R. C. (2011). Perinatal antidepressant exposure alters cortical network function in rodents. *Proceedings of the National Academy of Sciences, 108*(45), 18465-18470.

Smiley, K. O., Munley, K. M., Aghi, K., Lipshutz, S. E., Patton, T. M., Pradhan, D. S., . . . Sun, S. D. (2024). Sex diversity in the 21st century: Concepts, frameworks, and approaches for

the future of neuroendocrinology. *Hormones and Behavior, 157*, 105445. doi:https://doi.org/10.1016/j.yhbeh.2023.105445

Smulders, T. V. (2017). The avian hippocampal formation and the stress response. *Brain Behav Evol, 90*(1), 81-91. doi:10.1159/000477654

Stehberg, J., Moraga-Amaro, R., & Simon, F. (2011). The role of the insular cortex in taste function. *Neurobiology of learning and memory, 96*(2), 130-135.

Steketee, J. D., Silverman, P. B., & Swann, A. C. (1992). Noradrenergic receptor mechanisms in neophobia. *Psychopharmacology, 106*(1), 136-142.

Stokes, T. M., Leonard, C. M., & Nottebohm, F. (1974). The telencephalon, diencephalon, and mesencephalon of the canary, Serinus canaria, in stereotaxic coordinates. *Journal of Comparative Neurology, 156*, 337-374.

Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., . . . Mering, C. V. (2019). STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res, 47*(D1), D607-D613. doi:10.1093/nar/gky1131

Tryjanowski, P., Moller, A. P., Morelli, F., Biadun, W., Brauze, T., Ciach, M., . . . Zduniak, P. (2016). Urbanization affects neophilia and risk-taking at bird-feeders. *Sci Rep, 6*, 28575. doi:10.1038/srep28575

Voorn, P., Vanderschuren, L. J., Groenewegen, H. J., Robbins, T. W., & Pennartz, C. M. (2004). Putting a spin on the dorsal–ventral divide of the striatum. *Trends in neurosciences, 27*(8), 468-474.

Widmann, O. (1889). History of the House Sparrow Passer domesticus and the European Tree SparrowPasser montanus at Saint Louis, Mo. *Washington DC: US Department of Agriculture, Division of Economic Ornithology and Mammology*.

Woolley, C. S., Gould, E., Frankfurt, M., & McEwen, B. S. (1990). Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. *Journal of neuroscience, 10*(12), 4035-4039.

Yagi, S., & Galea, L. A. (2019). Sex differences in hippocampal cognition and neurogenesis. *Neuropsychopharmacology, 44*(1), 200-213.