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Sex Differences in Forebrain Monoaminergic Response to Song Performance

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Keywords

Auditory telencephalon · Catecholamine · Dopamine · Monoamine · Norepinephrine · Pallium · Serotonin · Sex differences · Songbird · Song performance

Abstract

In many species, successful reproduction is dependent on the ability to adjust social behavior in response to an ever-changing social environment. Because a sexual signal's value and meaning can differ between females and males, responses to those signals should also differ. One way individuals can modulate social behavior is through experience-dependent modulation of the sensory systems that process social signals. Central monoamines (norepinephrine, dopamine, serotonin) modulate neural sensitivity to social stimuli and are key regulators of experience-dependent neuroplasticity in vertebrate sensory systems. However, few studies have examined how exposure to different sexual signals influences monoaminergic activity in female compared to male sensory systems. We used Lincoln's sparrows *(Melospiza lincolnii)* to examine sex differences in how variation in the trill performance of song influences central monoaminergic activity in the auditory telencephalon. Trill performance measures the rate at which a song syllable is produced relative to the syllable's frequency bandwidth and is thought to reflect the difficulty with which songs are produced. High-performance trills are more threatening to males but more attractive to females. We found that the effects of trill performance on monoaminergic activity were sex-dependent. Relative to the response to low-performance songs, exposure to high-performance songs decreased noradrenergic activity in the caudomedial nidopallium, and tended to decrease serotoninergic activity in the caudomedial mesopallium and caudomedial nidopallium of the auditory telencephalon in females, but in males, the monoamine measurements were indistinguishable between song treatments. These results suggest that the mechanisms underlying sensory processing of male sexual signals differ between the sexes.

Introduction

A sexual signal from a male can have profoundly different effects depending on whether the receiver is a male or female. Signals that are high quality or challenging to

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produce might be attractive to females, who may be prospecting for high-quality mates. However, the same high-quality, challenging signals might be threatening to males, who may encounter the signal in competition for resources [Searcy, 1992; Nowicki and Searcy, 2004; Searcy and Beecher, 2009]. These sex differences in perception of the valence of a sexual signal suggest sex differences in the underlying neural systems that regulate receiver responses to social signals.

Neural differences between the sexes in response to a sexual signal could manifest in perceptual, motor, or motivational centers of the brain. Within perceptual centers of the brain, monoamines are implicated in modulating aspects of perception and sensory processing. Monoamines, which include serotonin, dopamine, and norepinephrine, function by integrating information about the internal state of the individual with information about external stimuli [Bao et al., 2001; Berridge and Waterhouse, 2003; Hurley et al., 2004; Castelino and Schmidt, 2010; Hurley and Hall, 2011]. Serotonin facilitates sensory encoding in mammalian auditory systems [Hurley and Hall, 2011]. Dopamine is involved in regulating learning and neuroplasticity [Bao et al., 2001] and in encoding aspects of stimulus reward [Berridge and Robinson, 1998; Maney, 2013]. Norepinephrine regulates attention and memory formation throughout sensory systems by increasing the signal-to-noise ratio of neuronal responses to sensory stimuli [Oades, 1985; Aston-Jones and Cohen, 2005; Sara, 2009; Castelino and Schmidt, 2010]. One or all of these monoamines may be involved in modulating sensory perception in response to experience with the value of a sexual signal [Sockman, 2007; Salvante et al., 2009; Sewall et al., 2013]. However, because the valence of sexual signals differs between the sexes, we predicted that the monoaminergic responses to sexual signals would also differ.

In this study, we examined sex differences in the effects of bird song quality on monoaminergic activity in perceptual, auditory-processing regions of songbird brains. We first exposed male and female Lincoln's sparrows *(Melospiza lincolnii)* to one morning of songs of either high or low trill performance. Trill performance is a sexually selected component of bird song that reflects a biomechanical constraint between the rate with which individuals produce trilled syllables (trill rate) and the frequency bandwidth of those syllables [Podos, 1997; Wilson et al., 2014]. It is difficult for males to produce high-performance songs compared to low-performance songs [Podos, 1996; Podos, 1997; Podos, et al. 1999], and studies have found a positive association between aspects of male quality and trill performance [Ballentine, 2009; Vehrencamp et al., 2013]. Furthermore, the valence of high-performance songs differs for females and males. Studies in multiple species of songbirds, including 2 studies in Lincoln's sparrows, indicate that females find high-performance songs more attractive than low-performance songs [Ballentine et al., 2004; Caro et al., 2010; Lyons et al., 2014], while male songbirds, including Lincoln's sparrows, are more threatened by and aggressive towards high-performance songs than low-performance songs [Illes et al., 2006; Sewall et al., 2010; DuBois et al., 2011; Moseley et al., 2013; Lyons, 2016].

Following song exposure, we measured monoaminergic activity in 2 regions of the songbird auditory telencephalon: the caudomedial mesopallium (CMM) and the caudomedial nidopallium (NCM). The CMM and NCM are analogous to regions of the mammalian secondary auditory cortex [Vates et al., 1996; Pinaud and Terleph, 2008], and they process information about variation in conspecific song in both female and male songbirds [Gentner et al., 2001; Gentner et al., 2004; Sockman, 2007; Knudsen and Gentner, 2010]. Due to the sex differences in the valence of song performance, we predicted that the effect of song performance on monoaminergic activity in perceptual, auditory regions of the brain would differ between the sexes.

Methods

Animals and Housing

We performed the research reported here according to guidelines established by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee (protocol 05-138.0-A). In June and July 2008 and 2010, we collected 8-day-old Lincoln's sparrow nestlings near Molas Pass, CO (37.74°N, 107.69°W), molecularly sexed them, and reared them in outdoor aviaries on natural photoperiods at the University of North Carolina (Chapel Hill, NC, USA) in a manner identical to a previously published study [Caro et al., 2010]. On November 15, 2012, we moved 31 Lincoln's sparrows (aged 2.5–4.5 years) into an indoor testing facility and housed them individually in cages with ad libitum access to food and water on an 8-h light:16-h dark photoperiod. Starting January 13, 2013, we switched 8 birds to a 16-h light:8-h dark photoperiod for 4 weeks in order to stimulate the development of reproductive behavior and physiology [Nicholls et al., 1988]. Previous studies have used similar photoperiod schedules to test sexual behavior in female [Caro et al., 2010; Lyons et al., 2014] and male [Sewall et al., 2010; Lyons, 2016] Lincoln's sparrows. Furthermore, photostimulation with long days has stimulated the development of reproductive physiology in other species of sparrows [e.g., Wingfield et al., 1997]. Every 3 days, we switched another 8 birds to the 16-h light:8-h dark photoperiod for a total of 4 experimental sessions. The first 3 sessions contained 4 females and 4 males each. The last session contained 1 female and 6 males.

Song Treatments

From 2005 to 2011 at Molas Pass, CO, our lab recorded 6,866 Lincoln's sparrow songs as described previously [Sockman, 2009]. For each of the more than 20,000 trills in these songs, we determined trill rate and frequency bandwidth using the software Raven Pro version 1.5 (Cornell Laboratory of Ornithology, Ithaca, NY, USA). Specifically, the software calculates the duration and frequency bandwidth of the middle 90% of sound energy of the area of the trill we specified, which, for each trill, was all except the final syllable. Excluding the final syllable circumvents the problem of uncertainty about where a trill ends and the next phrase of the song begins. We then calculated trill rate as 1 less than the number of syllables in the trill divided by the duration described above (in s). We then calculated the upper bound regression of frequency bandwidth as a function of trill rate, and determined each trill's performance as the orthogonal distance of the trill from an upper bound regression line. Trills that fell farther below the regression line had more negative trill performance values and indicated a lower trill performance [Podos, 1997; Sockman, 2009].

From the recorded songs, we selected 3 songs from each of 6 different males, with each song consisting of 4 trills with similar mean trill rates and performances. We generated treatment song files containing all 18 songs by generating 2 identical digital copies of each song. From one copy, we cut 15 ms of silence between each syllable of each trill (high-performance treatment) and pasted it into the corresponding inter-syllable space in the other copy (low-performance treatment; Fig. 1). Manipulated trills differed from each other (trill rate mean ± SEM: low performance 8.04 ± 0.23; high performance 9.76 ± 0.35; paired *t* test $t = 12.81$, $p < 0.001$; trill performance: low performance –0.41 ± 0.014; high performance –0.37 ± 0.15; paired *t* test $t = 12.81$, $p < 0.001$) and fell within the natural range of variation in trill rate and performance recorded for the study population.

Experimental Procedure

We used 8 sound-attenuation chambers each containing a cage with food cups, water bottles, and 2 perches, as well as a functioning speaker on one end of the chamber and a nonfunctioning speaker on the other end (Pioneer Corp. TS-G1040R). We balanced the side with the functioning speaker across the chambers and experimental treatments. We attached individual speakers to monoblock-amplifiers (Audiosource Amp 5.1A, Portland, OR, USA) that we interfaced (M-Audio Delta 1010, Irwindale, CA, USA) to a central computer (Apple Inc., Cupertino, CA, USA) simultaneously broadcasting low-performance and high-performance songs to their respective chambers. We spatially interspersed the treatments and sexes among the 8 chambers, which were contained in one room, with one sex (one individual) and treatment per chamber. We switched treatment and sex assignments of the chambers between sessions.

Starting with the first session on February 10, 2013, we exposed all birds to their treatment songs (low- or high-trill performance) for one period that lasted a total of 5.25 h. During the 5.25 h, the treatment songs played for 20-min intervals with 10-min silent cycles between each interval. The treatment songs played in random order. An individual song lasted 2–3 s, and we interspersed songs with approximately 10 s of silence. Approximately 85 songs played per 20-min interval, and approximately 890 songs played over the entire 5.25 h of song exposure. Songs played at a peak amplitude of 70 dB 5 cm from the speaker [Sewall et al., 2013; Lyons et al., 2014].

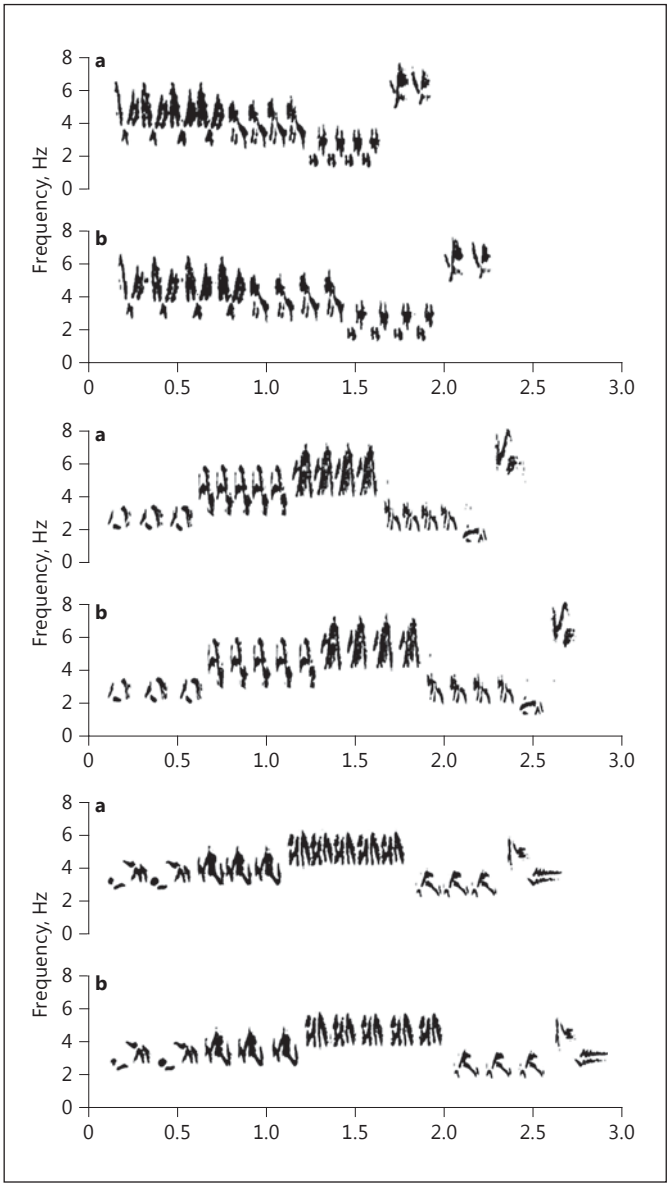

Fig. 1. Exemplars of 3 (of 18 total) treatment songs that were recorded from wild, free-ranging Lincoln's sparrows *(Melospiza lincolnii)* and digitally manipulated to be high performance (**a**) and low performance (**b**) by removing 15 ms silence between the intersyllabic space of each trill of one digital copy of the song and pasting it in the corresponding space of the other copy of the song.

In order to standardize the amount of time exposed to songs and allow time to collect brains at the conclusion of song exposure, we staggered the onset of song exposure for pairs of birds from opposite treatments by 30 min. Therefore, within each group of birds, songs played from 5:35 to 10:50 a.m. for the first pair of birds and from 7:05 a.m. to 12:05 p.m. for the last pair of birds. We rapidly decapitated pairs of birds and collected their brains at the conclusion of the 5.25 h of song playback. We fresh froze one hemisphere

Table 1. Effects of the experimental treatment, sex, and their interaction on levels of monoamines and their metabolites (ng/mg protein) in the caudomedial mesopallium of Lincoln's sparrows *(Melospiza lincolnii)*

Term	Estimate	SEM	Denom. df	*F*	*p* value
Serotonin					
Intercept	9.97	0.17			
Treatment	–0.78	0.25	8.95	6.46	0.031
Sex	–0.39	0.29	6.81	0.06	>0.2
Treatment × sex	0.65	0.27	19.96	5.69	0.027
5-HIAA (serotonin metabolite)					
Intercept	7.62	0.41			
Treatment	0.06	0.43	6.65	0.12	>0.2
Sex	0.30	0.37	5.89	1.24	>0.2
Treatment × sex	0.15	0.29	15.88	0.27	>0.2
Dopamine[a]					
Intercept	34.60	4.37			
Treatment	–7.57	6.77	7.24	1.61	>0.2
Sex	–4.50	6.60	7.64	0.38	>0.2
Treatment × sex	2.06	7.26	13.29	0.08	>0.2
DOPAC (dopamine metabolite)					
Intercept	4.14	1.37			
Treatment	–0.39	1.48	8.22	0.001	>0.2
Sex	0.98	1.93	6.84	0.61	>0.2
Treatment × sex	0.73	1.78	17.64	0.17	>0.2
Norepinephrine[a]					
Intercept	48.96	6.99			
Treatment	12.18	8.17	22.29	3.05	0.10
Sex	–5.09	9.26	7.49	1.12	>0.2
Treatment × sex	–6.20	10.17	21.20	0.37	>0.2
MHPG (norepinephrine metabolite)					
Intercept	3.86	1.52			
Treatment	0.78	1.38	8.68	0.27	>0.2
Sex	2.26	1.58	6.68	0.49	>0.2
Treatment × sex	–2.61	1.45	17.87	3.26	0.09

Females and the low-performance treatment were coded as 0, males and the high-performance treatment were coded as 1. [a] Square root transformed.

on dry ice and held it at –80°C until we measured monoaminergic activity in it using high-performance liquid chromatography with electrochemical detection (HPLC-ECD). We alternated between sex and treatments in the use of the hemisphere.

Quantification of Monoamines, Metabolites, and Protein

We used a cryostat to section the fresh frozen hemisphere from each brain into 300-µm sections in the sagittal plane. From the 300-µm sections, we used micropunches to collect tissue in CMM and NCM, using Field L as a guide as described previously [Sewall et al., 2013]. We collected a 0.5-mm diameter section from CMM and a 1-mm diameter section from NCM. Upon collection, we stored tissue samples in 1.9 mL polypropylene microcentrifuge tubes at –80°C until analysis.

When a monoamine is secreted from a pre-synaptic neuron, it may be metabolized [Moore, 1986; Eisenhofer et al., 2004; Meiser et al., 2013]. Therefore, in addition to quantifying serotonin, dopamine, and norepinephrine, we quantified their respective primary metabolites, 5-hydroxyindolacetic acid (5-HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC), and 3-methoxy-4-hydroxyphenylglycol (MHPG), as indicators of secretion. However, since some of the secreted, non-metabolized monoamine can be taken back into the pre-synaptic neuron, and since monoamines can be metabolized intraneuronally [Moore, 1986; Eisenhofer et al., 2004; Meiser et al., 2013], these measurements may not capture the total amount of monoamine secreted.

We used methodology described previously [Sewall et al., 2013] to quantify monoamines and their metabolites by HPLC-ECD. We added 100 µL of mobile phase to each tissue sample tube. We sonicated tissue samples in the mobile phase solution, centrifuged them at 16,000 *g* at 4°C for 16 min, and then injected 10 µL of the supernatant into an HTEC-500 stand-alone HPLC-ECD system (Eicom, San Diego, CA, USA) using a Midas autosampler (Spark Holland, Netherlands). The mobile phase solution was at pH 3.5 and was composed of citric acid (8.84 g), sodium acetate (3.10 g), sodium octyl sulfonate (215 mg), EDTA (5 mg), methanol (200 mL), and ultra-pure water (800 mL; Sigma-Aldrich, St. Louis, MO, USA). Monoamines and their metabolites were separated on an

Eicompak SC-30DS column (Eicom, San Diego, CA, USA) and were then passed over an electrode. The electrode maintained a potential of 750 mV against an Ag/AgCl reference electrode. The HPLC-ECD system determined the amount of each compound relative to a 2-point standard curve. The 2 standards were concentrated at 1 pg/μL and 10 pg/μL of each of the 6 compounds of interest. We included with each standard and sample an internal standard (1 pg/μL isoproterenol, added prior to sample processing) to control for sample loss during preparation. We used PowerChrom software (eDAQ, Colorado Springs, CO, USA) to compare the area under the curve for each compound within each sample to the area generated by the 2 standards. In all cases, we used the peak area ratio function in order to account for variation in isoproterenol across samples.

After determining the amount of each compound in the 10 μL of injected supernatant, we needed to account for variation in the amount of tissue from which the compounds were obtained. Therefore, we dissolved the remaining tissue sample in 0.2 M NaOH (20 μL for 0.5-mm diameter samples, 50 μL for 1-mm diameter samples) and used a Bradford protein-dye binding assay (Quickstart Bradford Protein Assay, BioRad, Hercules, CA, USA) to measure the amount of protein in each tissue sample. We used bovine serum albumin as a standard and performed all analyses on an UQuant microplate spectrophotometer (BioTek, Winooski, VT, USA). In situations in which the protein assay was unreliable and we were not able to repeat it due to low sample volume, we followed the protocol established previously [Sewall et al., 2013], and estimated the amount of protein as the average of the amount in the other tissue samples from the same brain region (CMM = 1 male, NCM = 3 females).

Analyses

The main goal of the study was to understand the influence of trill performance and sex on monoaminergic activity in the auditory system. We therefore ran separate analyses for each monoamine and for each metabolite in each region of the auditory telencephalon. For these analyses of monoamine levels, we used linear mixed effects models and included compound concentration as the dependent variable and log-transformed the values for normality unless otherwise noted. We included treatment, sex, and their interaction as the independent variables. We included chamber as a random intercept and as a random coefficient for sex and treatment. We were unable to target CMM and NCM in tissue from one female. We fit all models using the R package lme4 [Bates et al., 2015], and used Satterthwaite Approximations for degrees of freedom for *F* tests of linear mixed effects models. We used R version 3.2.2 for all analyses [R Core Team, 2015].

Results

The major findings in this study were an interaction between the effects of song treatment and sex on levels of serotonin in CMM and on levels of serotonin metabolite (5-HIAA), norepinephrine, and norepinephrine metabolite (MHPG) in NCM. Post hoc analyses showed that females in the high-performance treatment had lower levels of both norepinephrine and MHPG in NCM compared to females in the low-performance treatment. There was no effect of treatment on any of the monoamines or metabolites in males. Below are the statistical details supporting these claims.

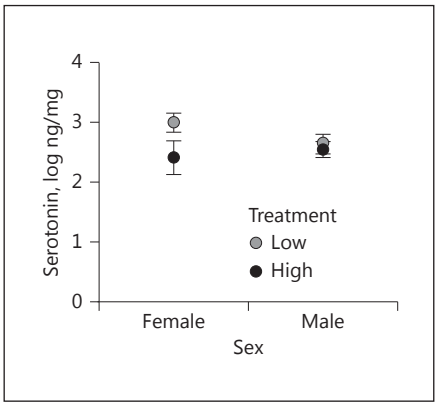

Fig. 2. Effects of sex, the experimental treatment, and their interaction on the amount of serotonin in the caudomedial mesopallium in Lincoln's sparrows *(Melospiza lincolnii)*. The experimental treatment involved the digital manipulation of the trills in playback songs to be of either low or high performance. In statistical models, the interaction term and the treatment effect were significant; sex was not significant. In post hoc analyses, there were no significant differences between treatments within either sex nor were there significant differences between the sexes within either treatment.

Monoamines and Metabolites in CMM

We found an effect of treatment and an interaction between the effects of treatment and sex on serotonin levels in CMM (Table 1; Fig. 2). Post hoc analyses of serotonin levels did not detect a significant difference between the treatments within females ($F_{1,10}$ = 3.28, p = 0.10) or males ($F_{1,8.66}$ = 0.26, p > 0.2). We also did not detect a significant difference between the sexes for either the high ($F_{1,13}$ = 0.24, p > 0.2) or low ($F_{1,13}$ = 2.30, p = 0.15) treatments.

We did not detect an effect of treatment, sex, or an interaction between the effects of treatment and sex on the levels of 5-HIAA, norepinephrine, dopamine or their metabolites in CMM (Table 1). We also did not detect a correlation between any monoamine and its metabolite within CMM (p > 0.2).

Monoamines and Metabolites in NCM

We found a trend for an interaction between the effects of treatment and sex on serotonin levels. There was

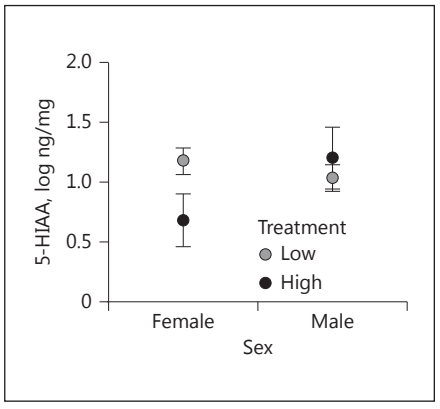

Fig. 3. Effects of sex, the experimental treatment, and their interaction on the amount of 5-HIAA in the caudomedial nidopallium in Lincoln's sparrows *(Melospiza lincolnii)*. The experimental treatment involved the digital manipulation of the trills in playback songs to be of either low or high performance. In statistical models, only the interaction term was significant; sex and treatment main effects were not significant. In post hoc analyses, there was a trend for females exposed to high-performance songs to have lower levels than females exposed to low-performance songs. There was not a significant effect of treatment in males, nor were there significant differences between the sexes within either treatment.

also an interaction between the effects of treatment and sex on levels of the serotonin metabolite, 5-HIAA (Table 2; Fig. 3). However, 1 male in the high-performance treatment had 5-HIAA levels greater than 2 SD above the mean. When this data point was omitted from the analysis there was no longer a significant interaction (treatment X sex: $F_{1,21.93} = 1.92$, $p = 0.18$, Table 2). We performed post hoc analyses on 5-HIAA. Post hoc analyses investigating the effects of treatment within each sex for 5-HIAA revealed that there was a trend for females in the high-performance treatment to have lower levels of 5-HIAA compared to females in the low-performance treatment ($F_{1,5.6} = 4.80$, $p = 0.074$; Fig. 3). Within males, we did not detect a significant effect of treatment on 5-HIAA ($p > 0.2$). We also did not detect a significant effect of sex on 5-HIAA for either the high ($F_{1,7.72} = 2.56$, $p = 0.15$) or low ($F_{1,7.49} = 0.75$, $p > 0.2$) treatments.

There was also a significant interaction between the effects of treatment and sex on norepinephrine levels (Table 2; Fig. 4a) as well as levels of the norepinephrine metabolite, MHPG (Table 2; Fig. 4b). There was one male in the high-performance treatment with norepinephrine levels greater than 2 SD above the mean, but after removal of this data point, the interaction between treatment and sex remained significant (treatment X sex: $F_{1,14.51} = 4.82$, $p = 0.045$).

Table 2. Effects of the experimental treatment, sex, and their interaction on levels of monoamines and their metabolites (ng/mg protein) in the caudomedial nidopallium of Lincoln's sparrows *(Melospiza lincolnii)*

Term	Estimate	SEM	Denom. df	*F*	*p* value
Serotonin					
Intercept	9.75	0.25			
Treatment	–0.39	0.35	12.97	0.03	>0.2
Sex	–0.47	0.35	8.96	0.02	>0.2
Treatment × sex	0.86	0.43	18.94	4.03	0.06
5-HIAA (serotonin metabolite)					
Intercept	8.09	0.17			
Treatment	–0.14	0.23	11.77	1.65	>0.2
Sex	–0.57	0.29	7.85	0.84	>0.2
Treatment × sex	0.72	0.31	17.12	5.50	0.031
Dopamine					
Intercept	8.25	0.46			
Treatment	–0.88	0.65	20.49	2.66	0.12
Sex	0.004	0.59	22.77	0.24	>0.2
Treatment × sex	0.40	0.83	21.53	0.23	>0.2
DOPAC (dopamine metabolite)					
Intercept	7.19	0.57			
Treatment	–0.51	0.85	9.73	1.02	>0.2
Sex	0.04	0.82	9.51	0.02	>0.2
Treatment × sex	–0.25	0.99	19.01	0.06	>0.2
Norepinephrine					
Intercept	8.46	0.24			
Treatment	–0.72	0.36	7.55	0.49	>0.2
Sex	–0.72	0.28	10.72	0.82	>0.2
Treatment × sex	1.06	0.37	14.29	8.33	0.012
MHPG (norepinephrine metabolite)					
Intercept	6.34	0.36			
Treatment	–1.51	0.67	7.96	1.58	0.24
Sex	–0.19	0.55	8.51	2.03	0.19
Treatment × sex	1.69	0.66	21.63	6.49	0.01

Females and the low-performance treatment were coded as 0; males and the high-performance treatment were coded as 1.

We performed post-hoc tests to investigate the within-sex effects of treatment on norepinephrine and MHPG in NCM. Females in the high-performance treatment had lower levels of norepinephrine ($F_{1,5.6} = 6.84$, $p = 0.042$; Fig. 4a) and MHPG (square-root transformed): $F_{1,10} = 5.67$, $p = 0.039$; Fig. 4b) than females in the low-performance treatment. Within males, we did not detect a significant effect of treatment on norepinephrine or MHPG ($p > 0.2$). We also performed post hoc tests to investigate the within-treatment effects of sex on norepinephrine and MHPG in NCM. For norepinephrine, females in the low-performance treatment had higher levels of norepinephrine than males in the low-performance treatment

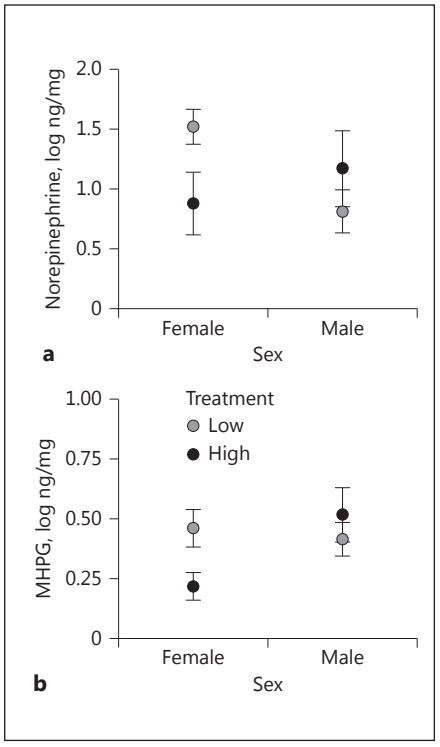

Fig. 4. Effects of sex, the experimental treatment, and their interaction on levels of norepinephrine (**a**) and the norepinephrine metabolite MHPG (**b**) in the caudomedial nidopallium in Lincoln's sparrows *(Melospiza lincolnii)*. The experimental treatment involved the digital manipulation of the trills in playback songs to be of either low or high performance. In statistical models, only the interaction term was significant; sex and treatment main effects were not significant. In post hoc analyses, norepinephrine and MHPG levels differed between treatments for females but not males. Norepinephrine levels differed between females and males from the low-performance treatment, but not the high-performance treatment. MHPG levels differed between females and males from the high-performance treatment, but not the low-performance treatment.

($F_{1,6.31}$ = 26.35, p = 0.002; Fig. 4a). Levels of norepinephrine did not significantly differ between females and males in the high-performance treatment ($F_{1,7.43}$ = 0.75, p > 0.2; Fig. 4a). For MHPG, females in the high-performance treatment had lower levels of MHPG than males in the high-performance treatment ($F_{1,13}$ = 4.72, p = 0.049; Fig. 4b). Levels of MHPG did not significantly differ between females and males in the low-performance treatment ($F_{1,6.65}$ = 0.20, p > 0.2; Fig. 4b).

We did not find an effect of treatment, sex, or an interactive effect on levels of dopamine or its metabolite (Table 2). When we tested for correlations between each monoamine and its metabolite, we found a correlation between serotonin and 5-HIAA (r_s = 0.61, p < 0.001) and a correlation between dopamine and DOPAC (r_s = 0.56, p = 0.002). We did not detect a significant correlation between norepinephrine and MHPG (r_s = 0.15, p > 0.2).

Discussion

This study tested the hypothesis that the sexes differ in the auditory system's monoaminergic response to variation in social signals. We found detectable differences in monoaminergic responses to song performance in female but not male Lincoln's sparrows. Females exposed to the high-performance songs had lower levels of both norepinephrine and its metabolite MHPG in NCM compared to females exposed to the low-performance songs. Males from the 2 treatments did not reliably differ in the levels of any of the monoamines or metabolites. When comparing the sexes within each treatment, in the low-performance treatment, females had higher levels of norepinephrine than males, while in the high-performance treatment, females had lower levels of MHPG than males. Females and males also differed in the influence of the treatments on serotonin levels in CMM, and there was a trend for females exposed to high-performance songs to have lower levels of the serotonin metabolite 5-HIAA in the NCM than females exposed to low-performance songs. Together, the results from this study suggest that norepinephrine and potentially serotonin in the auditory telencephalon respond to differences in trill performance more strongly in females than in males.

Several studies have detected sex differences in song perception and in brain regions that regulate song perception and production [Williams, 1985; Cynx and Nottebohm, 1992; Del Negro et al., 2000; Del Negro and Edeline, 2001; Gall et al., 2013]. The best known differences are sexual dimorphisms in the size and composition of the song-control nuclei, which regulate song learning and production and are typically larger in males [Nottebohm and Arnold, 1976; Arnold, 1992; Ball and Macdougall-Shackleton, 2001; Ball, 2016]. However, researchers have also detected effects of sex on gene expression and protein levels in the songbird auditory system as well as on auditory perception [Phillmore et al., 2003; Ikebuchi et al., 2003; Pinaud et al., 2006; Krentzel and Remage-Healey, 2015]. Similar to this study in which we found that monoamines in the auditory forebrain discriminated between song performance in females but not males, many of these studies show female-biased sensitivity to songs or calls.

For example, song attractiveness modulates gene expression in the NCM of female, but not male, zebra finches *(Taeniopygia guttata)* [Gobes et al., 2009], and song novelty modulates heart rate in female, but not male, Bengalese finches *(Lonchura striata)* [Ikebuchi et al., 2003]. This study found that for a sexual signal that differs in valence between females and males, females have greater monoaminergic sensitivity to differences in the quality of the sexual signal than males. This finding is consistent with theoretical work predicting that it would be more costly for females to fail to discriminate between songs' attractiveness than it would be for males to fail to discriminate between songs' threat levels [Searcy and Brenowitz, 1988; Searcy, 1992].

The lack of an effect of song exposure on monoamines in males could indicate a lower sensitivity to song's trill performance, as suggested above. However, male songbirds are behaviorally sensitive to trill performance, with free-ranging, territorial males of many species, including Lincoln's sparrows, typically increasing aggressive behavior in response to an intruder song that is high-performance compared to low-performance [Illes et al., 2006; de Kort et al., 2009; Sewall et al., 2010; Moseley et al., 2013; Lyons, 2016]. Therefore, it is possible that males' monoaminergic responses to trill performance occur on a timescale that differs from the one we measured. Future studies that track the change in monoamine levels and the corresponding change in female discrimination and male competitive singing over the course of exposure to songs of different trill performance will further elucidate how exposure to song modulates monoamine-induced neuroplasticity. Moreover, although this study focused on sensory regions of the brain, male behavioral responses to variation in sexual signals may principally manifest in other regions, such as motivational or motor regions, or they may be regulated principally through non-monoaminergic systems [Sewall et al., 2010; Maney and Goodson, 2011; Rosvall et al., 2012].

Monoamines may act in the auditory telencephalon to integrate information about the value of song with information about the state of the individual to ultimately affect behavior [Sockman, 2007; Salvante et al., 2009; Sewall et al., 2013]. Several studies have found that extended exposure to songs of differing value influence neural activity and behavior in female and male songbirds. One week of experience with more attractive compared to less attractive songs increased neural discrimination for male song in the female European starling *(Sturnus vulgaris)* [Sockman et al., 2002; Sockman et al., 2005] and decreased behavioral responsiveness to male song in the female Lincoln's sparrow [Lyons et al., 2014], suggesting that long-term experience with attractive songs increases female discrimination of song attractiveness [Bateson and Healy, 2005; Sockman, 2007; Lyons et al., 2014]. At the same time, male songbirds that experienced 1 week of exposure to high-quality songs increased singing effort more than males that experienced low-quality songs, indicating that males increase competitive behavior in response to experience with competitive signals [Salvante et al., 2009; Sewall et al., 2013].

In support of the hypothesis that monoamines in the auditory system mediate the influence of song exposure on neural and behavioral plasticity, previous studies in both European starling females and males and in Lincoln's sparrow males found that monoaminergic activity in CMM and NCM was higher following extended exposure to high-quality songs compared to low-quality songs [Sockman et al., 2002; Sockman and Salvante, 2008; Salvante et al., 2009; Sewall et al., 2013]. However, these studies measured both monoamine levels and behavior in birds on the morning following 1 week of exposure to songs. In this study, we measured monoaminergic activity in the auditory telencephalon of females and males after one morning of exposure to songs, and thus the different experiments captured the responses to different stimuli. In the previous experiments, changes in monoamines may have reflected long-term changes in the brain (i.e., neuroplasticity) due to the weeklong exposure to stimuli, whereas in the current study, variation in monoamines may have reflected short-term, real-time responses to the stimuli as they were occurring, even if those short-term responses might form an underlying basis to long-term plasticity.

Several studies have collected tissue punches and used HPLC-ECD to measure monoamines and their metabolite levels following exposure to auditory stimuli (e.g., birds [Sockman and Salvante, 2008; Salvante et al., 2009; Matragrano et al., 2012a; Sewall et al., 2013], amphibians [Rodriguez Moncalvo et al., 2013]). These studies found that auditory stimuli affected levels of monoamines and metabolites after stimulus exposure that lasted for as short as 15 min [Matragrano et al., 2012a] to as long as 1 week [Sockman and Salvante, 2008; Salvante et al., 2009; Sewall et al., 2013]. The methodology used in these studies allows for a direct measurement of monoamine and metabolite levels in the sampled tissue at the time of collection. However, this methodology does not provide direct insight into patterns of monoaminergic synthesis or secretion that occurred during stimulus exposure prior to collection.

Previous studies that have measured both monoamines and synthesizing enzymes following exposure to song stimuli have often found that groups with higher levels of monoamines or metabolites following song exposure also have higher levels of synthesizing enzyme expression (e.g., [Sockman and Salvante, 2008; Matragrano et al., 2012a]). This pattern supports the interpretation that, at least in some situations, a higher level of monoamine or metabolite following exposure to a stimulus corresponds to an increase in synthesis. In addition, a difference in metabolite levels between groups is often interpreted as reflecting a difference in monoamine secretion. However, the metabolite measurements likely do not capture the total amount of monoaminergic secretion due to reuptake of monoamines following secretion, and due to intraneuronal metabolism of monoamines [Moore, 1986; Eisenhofer et al., 2004; Meiser et al., 2013]. Therefore, although we tentatively interpret the monoamine and metabolite measurements in the current study to reflect synthesis and secretion activity preceding collection, this interpretation should be taken with caution. Future studies that use repeated measurements of monoamines and metabolites during song exposure could provide clearer insight into the dynamics of synthesis and secretion during song exposure.

Females from the high-performance treatment had lower levels of both norepinephrine and MHPG in NCM compared to females from the low-performance treatment. The difference in both compounds suggests that the treatment affected both synthesis and secretion of norepinephrine [Moore, 1986; Eisenhofer et al., 2004]. Norepinephrine regulates arousal, attention, and goal-directed behavior by enhancing responses to salient stimuli and suppressing responses to nonsalient stimuli [Berridge and Waterhouse, 2003; Aston-Jones and Cohen, 2005; Sara, 2009]. However, noradrenergic secretion follows 2 patterns of release. Tonic release likely regulates overall levels of arousal, while phasic release likely corresponds to stimulus-specific responses and goal-directed behavior [Berridge and Waterhouse, 2003; Aston-Jones and Cohen, 2005; Valentino and Van Bockstaele, 2008]. In this study, differences between the females in levels of norepinephrine and MHPG could occur through phasic release of norepinephrine in response to hearing songs, or through tonic release that corresponds with different levels of arousal.

We also examined sex differences in levels of norepinephrine and MHPG. We found that for the low-performance treatment, norepinephrine levels were higher in females than males, whereas for the high-performance treatment norepinephrine levels did not significantly differ between females and males. We detected a different pattern for MHPG; for the low-performance treatment, MHPG levels did not significantly differ between females and males, whereas for the high-performance treatment, MHPG levels were lower in females than males. Without measuring norepinephrine and MHPG prior to song exposure, it is not possible to determine how levels of the compounds changed in response to the songs. However, future studies that include a "no-song" treatment could help elucidate the direction of the song-induced change in noradrenergic activity for females and males [Sockman and Salvante, 2008].

In female songbirds, norepinephrine has been shown to affect aspects of mate choice, potentially by modulating attention and goal directed behavior [Castelino and Schmidt, 2010]. Disruption of the noradrenergic system decreases female behavioral [Appeltants et al., 2002; Vyas et al., 2008; Pawlisch et al., 2011] and neural [Lynch and Ball, 2008] preference for sexually stimulating, socially salient songs and increases overall sexual receptivity [Riters and Pawlisch, 2007]. Similar to its effects in other sensory systems and other taxa [e.g., Foote et al., 1975], the addition of norepinephrine to auditory neurons in the songbird auditory system decreases spontaneous firing but maintains the stimulus-evoked response. This sharpens the response to and detection of auditory signals [Cardin and Schmidt, 2004; Ikeda et al., 2015]. In addition, norepinephrine increases encoding accuracy of NCM neurons, likely through its suppression of spontaneous firing [Ikeda et al., 2015]. Although previous research found that norepinephrine's enhancement of song-induced firing was similar across different types of song stimuli [Ikeda et al., 2015], an additional study found that exposure to more attractive, potentially more salient songs compared to less attractive songs increased levels of norepinephrine in the NCM in European starlings [Sockman and Salvante, 2008]. However, it is important to note that norepinephrine also responds to salient stimuli that are aversive [Feenstra et al., 2001]. Therefore, regardless of its context, the salience of auditory input could modulate the amount of norepinephrine released from the locus coeruleus to the NCM [Lynch et al., 2012], which could lead to differential responses to songs based on their salience. In this study, noradrenergic activity in the NCM of the low-performance group was higher than that of the high-performance group, giving rise to the hypothesis that the low-performance songs were more salient (although likely less attractive) than the high-performance songs.

There was an interaction between the effects of treatment and sex on serotonin levels in the CMM, with females differing more strongly (though not significantly) in their serotonergic response to the treatments than males. Serotonin may be an important modulator of auditory sensitivity [Hurley et al., 2004; Shepard et al., 2013]. Researchers have made great strides in understanding the modulatory effects of serotonin in the mammalian auditory system [Hurley and Hall, 2011]. In bats, adding serotonin to the auditory midbrain decreases responses to conspecific vocalizations in most neurons but enhances the response in a select number of neurons. This results in a population level increase in selectivity of response to conspecific vocalizations [Hurley and Pollak, 2005]. This finding fits within a larger body of evidence that serotonin influences frequency tuning of auditory neurons by selectively enhancing or depressing responses to auditory signals [Hurley and Pollak, 1999, 2001; Hurley et al., 2002]. Less research has focused on the role of serotonin in songbird audition and perception. However, in female white-throated sparrows *(Zonotrichia albicollis)*, serotonergic activity and innervation patterns in the auditory telencephalon are hormone dependent [Matragrano et al., 2012b]. In addition, exposure to salient stimuli such as song (compared to tones) in female white-throated sparrows or exposure to high-quality song (compared to low quality) in male European starlings increases serotonergic activity in the auditory telencephalon [Salvante et al., 2009; Matragrano et al., 2012b]. Therefore, serotonin in the auditory telencephalon could potentially modulate perceptual and behavioral responsiveness to high-performance compared to low-performance songs.

There is evidence that NCM and CMM are both responsible for processing socially relevant stimuli in songbirds [Theunissen et al., 2004; Salvante et al., 2009; Matragrano et al., 2012a]. Studies support that these regions are responsible for recognizing conspecific song [Grace et al., 2003], and for processing female and male responses to variation in conspecific song [Gentner et al., 2001; Gentner et al., 2004; Sockman, 2007]. However, there is also some evidence for functional differences between NCM and CMM. The NCM appears important specifically in processing aspects of song novelty and learning [Gentner et al., 2004; Velho et al., 2012], whereas the CMM appears to be important in processing song familiarity [Gentner and Margoliash, 2003; Gentner et al., 2004]. At the start of playback, all of our songs were novel but may have become familiar by the end of playback. We have only a single monoamine sampling time, which is 5.25 h following the onset of playback. It could be the case that most of the monoaminergic response occurred immediately following the onset of song, that most of the response occurred near the end of playback, or that the response is an integration of many dynamic changes in monoamines over the entire course of playback. Thus, despite evidence for functional differences between NCM and CMM, it is not clear why the effects of the song treatments on monoaminergic activity differed between NCM and CMM in the current study.

Central monoamines are powerful neuromodulators that integrate information about the external environment with information about the internal environment and modulate synaptic connections [Gu, 2002; Briand et al., 2007]. In songbirds, the function of songs in the external environment and the state of the internal environment differ between females and males that are reproductively ready [Searcy and Brenowitz, 1988; Nowicki and Searcy, 2004]. For females, high-performance songs are attractive [Caro et al., 2010; Lyons et al., 2014], whereas for males, high-performance songs are threatening [Illes et al., 2006]. The finding of an interactive effect of song performance and sex on monoaminergic activity in the auditory telencephalon gives rise to the hypothesis that the evolution of sex differences in behavioral responses to sexual signals is mediated, at least in part, by monoamines in perceptual regions of the brain. Future studies that measure sex differences in discrimination of song after manipulating the serotonergic system in CMM or the noradrenergic system in NCM of females and males will further elucidate the role that these systems play in mediating auditory neuroplasticity in response to sexual signals.

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