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Sound-induced monoaminergic turnover in the auditory forebrain depends on endocrine state in a seasonally-breeding songbird

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Abstract

Sensory responses to courtship signals can be altered by reproductive hormones. In seasonally-breeding female songbirds, for example, sound-induced immediate early gene expression in the auditory pathway is selective for male song over behaviourally irrelevant sounds only when plasma estradiol reaches breeding-like levels. This selectivity has been hypothesized to be mediated by release of monoaminergic neuromodulators in the auditory pathway. We previously showed that in oestrogen-primed female white-throated sparrows, exposure to male song induced dopamine and serotonin release in auditory regions. In order to mediate hormone-dependent selectivity, this release must be (1) selective for song and (2) modulated by endocrine state. Therefore, in the current study we addressed both questions by conducting playbacks of song or a control sound to females in a breeding-like or non-breeding endocrine state. We then used high performance liquid chromatography to measure turnover of dopamine, norepinephrine, and serotonin in the auditory midbrain and forebrain. We found that sound-induced turnover of dopamine and serotonin did in fact depend on endocrine state; hearing sound increased turnover in the auditory forebrain only in the birds in a breeding-like endocrine state. Contrary to our expectations, these increases occurred in response to either song or artificial tones; in other words, they were not selective for song. The selectivity of sound-induced monoamine release was thus strikingly different from that of immediate early gene responses described in previous studies. We did, however, find that constitutive monoamine release was altered by endocrine state; whether the birds heard sound or not, turnover of serotonin in the auditory forebrain was higher in a breeding-like state than in a non-breeding endocrine state. Our results suggest that dopaminergic and serotonergic responses to song and other sounds, as well as serotonergic tone in auditory areas, could be seasonally modulated.

Keywords

dopamine; norepinephrine; serotonin; catecholamine; song; white-throated sparrow

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Introduction

Courtship signals command the attention of their intended receivers. [1–4]. Because courtship signals are relevant only to receivers in breeding condition, however, they should command more attention during the breeding season than outside it. In seasonally-breeding female *Zonotrichia* sparrows, breeding-typical plasma levels of estradiol are required for proceptive responses to playback of male song [5–7]. Because behavioural responses to courtship signals depend on endocrine state, we expect the neural mechanisms underlying the salience of those signals to depend on endocrine state as well. In a variety of seasonally breeding songbirds, auditory responses to conspecific song are in fact greater during the breeding season. Such changes have been demonstrated using auditory evoked potentials [8, 9], neurophysiological recording [10, 11], *in vivo* imaging [12–14], and the expression of immediate early genes [5, 15]. There is thus good evidence that the auditory system becomes more attuned to conspecific song when a bird enters a breeding-like endocrine state.

The brain ascribes salience to sensory signals in part *via* the monoaminergic neuromodulators dopamine, norepinephrine, and serotonin [2]. These systems are evolutionarily ancient and can be found in all vertebrate taxa [16]. Fibres carrying these neuromodulators originate in a small number of nuclei but project widely throughout the brain. Each of the monoaminergic systems serves multiple functions, and it is beyond the scope of this study to review them all here; we focus instead on their shared function of modulating auditory responses. Noradrenergic, dopaminergic, and serotonergic fibres terminate at all levels of the auditory system in vertebrates [17] and are well-understood to participate in attending to and learning of auditory stimuli [2].

In birds, monoaminergic cell bodies project from the brainstem to nearly every area of the brain, including auditory areas [18–20]. Lesions of brainstem noradrenergic neurons inhibited behavioural responses to song in female canaries [21, c.f. 22] and eliminated behavioural preferences for more attractive vs. less attractive song in female zebra finches [23]. Noradrenergic receptor antagonism in European starlings disrupted female preferences for conspecific over heterospecific song [24] and treatment with a dopamine agonist disrupted preferences for attractive song [25]. In zebra finches, playback of novel songs induced more immediate early gene expression in noradrenergic cell bodies than playback of familiar song, and this induction was correlated with induction in the auditory forebrain, particularly in cells contacted by catecholaminergic fibres [26]. Together, these studies suggest that hearing song engages monoaminergic systems, which may be necessary for appropriate auditory and behavioural responses.

Because monoaminergic systems are highly sensitive to reproductive hormones, they are prime candidates for seasonal modulation of behavioural responses [2, 27]. The transition to breeding, accompanied by increases in plasma sex steroids, may bring increases in constitutive monoamine release that persists over weeks, priming the auditory pathway to respond to courtship signals [2]. In female white-throated sparrows, treatment with estradiol increased the density of both catecholaminergic and serotonergic fibres at multiple levels of the auditory pathway [19, 20, 28]. Estradiol could, therefore, effectively increase the amount of catecholamine or serotonin available for release in response to song. Alternatively, or in

addition, estradiol may increase the rate of tonic or nonsynaptic release that occurs independently of sensory stimulation. In rats, stimulus-induced and stimulus-independent release of dopamine occur even in the same population of neurons [29]. These actions of monoamines, i.e., induced by stimuli or *via* priming (Fig. 1a), are thus not mutually exclusive.

In a previous study of female white-throated sparrows, playback of male conspecific song induced release of both dopamine and serotonin in the auditory forebrain [20, 30]. This release was detectable, using HPLC of micropunched tissue, after 30 minutes of song exposure. Thus, there is evidence that hearing song can induce monoamine release fairly quickly in an auditory area. Those studies left several unanswered questions, however. Because all of the birds in those studies were treated with estradiol, we do not know whether the song-induced monoamine release depended on endocrine state. In addition, because no control sounds were presented, we do not know whether the monoamine release was caused by exposure to song specifically, or whether it would have occurred in response to any sound. In this study, we manipulated endocrine state in female white-throated sparrows and then exposed them to conspecific male song, a control sound, or silence. Following stimulus presentation, we measured turnover of dopamine, norepinephrine, and serotonin in the auditory midbrain and two regions of the auditory forebrain. We predicted that if neural responses to song are driven or modulated by monoamine release on a rapid timescale, then monoamine turnover in these areas would be higher in birds hearing song than in birds hearing tones or silence. In addition, we predicted that such effects would be apparent only in birds in a breeding-like (estradiol-primed and photostimulated) endocrine state. Finally, we predicted that if constitutive release of monoamines depends on endocrine state, turnover would be higher in birds in breeding-like condition than in birds in non-breeding condition, regardless of the type of stimulus presented. These results would support our hypothesis that the salience of male song, which attracts females only during the breeding season, is mediated by hormone-dependent monoamine release within the auditory system.

Experimental Procedures

Animals

Animals were collected under appropriate local, state, and federal permits. Emory University's Institutional Animal Care and Use Committee approved all the procedures that involved the use of animals. Sixty female white-throated sparrows (*Zonotrichia albicollis*) were trapped using mist-nets around Emory University campus during their fall migration. After capture, the birds were housed in indoor aviaries in the Division of Animal Resources at Emory University. The birds were held on a winter-like photoperiod (9.5L:14.5D) until at least mid-January so that they would regain photosensitivity [31, 32]. Housing on short photoperiod prevents increases in endogenous plasma estradiol in this species [5, 6]. Food and water was provided *ad libitum*.

The sex of the birds was determined by PCR analysis [33] of a blood sample obtained by venipuncture of the brachial vein. Sex was then confirmed at the end of the study by inspection of the gonads during necropsy. Age and plumage morph, which could affect behavioural responses to song playback [34], were balanced across our experimental

treatments (age: $n = 23$ hatch-years, $n = 8$ after hatch-years, $n = 5$ unknown age; morph: $n = 15$ tan-striped, $n = 21$ white-striped). Age was determined by non-invasive inspection of plumage and skulls in live birds [35]. Morph was determined by PCR analysis [36].

Manipulation of endocrine state

Our experimental design is depicted in Fig. 1b. All birds were transferred to individual cages and housed in small rooms, $n=5$ per room. We assigned the birds to one of two treatment groups. For birds in the first group ($n = 30$), the light cycle was changed to a summer-like photoperiod (14L:10D) to facilitate a breeding-like endocrine state. Because photostimulation alone is not sufficient to elevate plasma estradiol to levels typical of the reproductive season [6, 7, 37], after three weeks of photostimulation we treated birds in this group with exogenous estradiol via a subcutaneous implant [19]. Each implant consisted of a silastic capsule (length 12 mm, ID 1.47 mm, OD 1.96 mm, Dow Corning, Midland, MI) filled with 17β -estradiol (Steraloids, Newport, RI) and sealed on both ends with silicon adhesive. This dose has been demonstrated to increase plasma estradiol to breeding-typical levels in captive females of this genus after two days of treatment for up to 80 days [7]. In this species, this dose brings plasma estradiol levels up to 1.3 ± 0.2 ng/ml [5] which is within the physiological range for free-living, breeding females early in the breeding season when they are likely attending to male song [38]. We have previously shown, in multiple studies, that this dose of estradiol increases the selectivity of immediate early gene responses in the auditory forebrain and midbrain [5, 6, 39, 40]. Moreover, hearing song increased concentrations of dopamine and serotonin metabolites in NCM in females treated with this dose [20, 30]; thus we needed to use this dose to replicate that finding in the current study.

The birds in the second group ($n = 30$) were kept in a non-breeding endocrine state. These birds continued to be held on a winter-like photoperiod (9.5L:14.5D) and received empty silastic capsules. Under these conditions, ovaries remain regressed and plasma estradiol remains very low [5, 6]. After completion of the study, ovaries were inspected to confirm a regressed state.

Playback experiment

Four weeks following the onset of the above treatment (and one week following implant administration), we exposed each bird to one of five auditory stimuli: silence, conspecific male song for 15 or 30 min, or artificial tones for 15 or 30 min. Six birds in each of the two endocrine states heard each type of stimulus. Type of stimulus was balanced across birds that were housed in the same room during the manipulation of breeding state, so that each of the five birds in a room was exposed to a different stimulus during the playback experiment (Fig. 1b). The sound presentations have been described elsewhere [5, 20, 30, 41]. Briefly, songs from several male white-throated sparrows were downloaded from the Borror Bioacoustic Library. Using Audacity, we constructed sound presentations in which a song from a unique male was repeated every 15 sec and the identity of the singer changed every 3 min. Thus, each 15-min presentation contained five different male songs, each one repeated 12 times, and each 30-min presentation contained ten male songs each repeated 12 times. Six versions of each presentation were constructed, one for every bird in each treatment

group assigned to hear song. The versions differed in the order in which the songs were presented, following a Latin-square design.

For each song, a sequence of artificial tones was generated to match the dominant frequencies in the song [5]. The order of the tones within each sequence was randomized and their durations equalized, while keeping the overall duration of the tone sequence equal to that of the song. The tone sequences were automatically generated with a script (available upon request) written in R (R Core Team, 2013), using functions from the software packages *seewave* [42] and *tuneR* [43]. The tone sequences were arranged into stimulus presentations exactly as is described above for the songs, so that for each of the six unique song presentations, there was a matching tone presentation. As was the case for the birds hearing song, each of the six birds per experimental group heard a unique tone presentation.

On the afternoon before the playback, each bird was isolated in a sound-attenuating booth (Industrial Acoustics, Bronx, NY). The booth was equipped with a microphone, a speaker (AudioSource LS-300), and a video camera (Panasonic WV-CP240) that recorded video into a Digital Video Recorder (Dahua DVR 1604 HF-U). The bird remained isolated in the booth overnight, and presentation of conspecific song, tones, or silence (no sound stimulus) started between 30 and 75 minutes after lights-on (between 08:00 and 08:45 AM). The birds' behaviour was recorded on video throughout the stimulus presentation. Birds assigned to the silence group remained in the booth for the same amount of time as did the birds hearing song or tones.

Tissue collection

For birds that heard song or tones, brains were collected immediately after playback. For birds that heard silence, brains were collected within 60–100 minutes after lights-on such that they were collected at the same time of day as were the other brains. Brains were rapidly frozen in powdered dry ice within 10 min after rapid decapitation. Sagittal frozen sections (300µm) were mounted on microscope slides by rapid thawing and refreezing. We used the Palkovits punching technique [44] to extract brain tissue from three auditory regions of interest (ROIs) as depicted in Fig 1c: the caudomedial mesopallium (CMM) and caudomedial nidopallium (NCM), which are regions of the auditory forebrain homologous to secondary auditory regions of mammals [45, 46], and the inferior colliculus (IC; in birds also called the dorsal lateral nucleus of the mesencephalon). The diameter of the punches was 0.5 mm for CMM and IC, and 1.0 mm for NCM (Fig. 1c). Two punches were collected for each ROI. Tissue from one hemisphere was used from each bird; whether it was left or right was balanced across groups.

Measurement of monoaminergic activity

We measured the concentrations of the monoamines dopamine, norepinephrine, and serotonin, and their respective metabolites dihydroxyphenylacetic acid (DOPAC), 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), and 5-hydroxyindolacetic acid (5HIAA) using high liquid performance chromatography (HPLC) with electrochemical detection [47]. Preparation of the samples and the mobile phase for HPLC followed protocols published elsewhere [48]. HPLC was carried out using an HTEC 500 HPLC-ECD

detector system (Eicom). Analytes were automatically identified using the software PowerChrome (edaq). The software matched the retention times of peaks in the HPLC output trace to the retention times of peaks corresponding to 1 pg/ul and 10 pg/ul external standards. A 5% error margin was allowed for matching retention times. When the software failed to recognize a peak, we classified that peak manually. The peak heights of the samples were normalized to that of an internal 10 pg/ul isoproterenol standard. The concentration of the analytes was estimated from a linear interpolation between the concentrations and normalized peak heights of the external standards.

Because we were interested in quantifying monoaminergic activity rather than absolute concentration, we estimated turnover. Turnover is thought to be related to the rate of release [47] and has been used to estimate such in songbirds [49–51]. To be consistent with this literature, we defined turnover of each monoamine as the ratio of each metabolite to the corresponding monoamine. In other words, dopamine turnover was calculated by dividing the DOPAC value by the dopamine value, and so on.

Statistical analysis

Our primary goal was to replicate and extend the findings of Matragrano et al. [20, 30]; we therefore took a similar statistical approach. We performed a separate analysis for each of the three monoamine turnover rates (dopamine, norepinephrine, and serotonin) in each of the three auditory regions (NCM, CMM, and IC). Because Matragrano et al. [20, 30] found effects of playback at 30 min, we were primarily interested in that time point and analysed those data first. To ask whether the response to hearing song depends on endocrine state (Fig. 1a), we were interested in whether the difference between silence and song depended on treatment (Fig 1a, Question 1); in other words, we were interested in the *interactions* with endocrine state. To test for these interactions we used mixed effects linear models in which monoamine turnover was the response. Each of these models included the following five predictors: treatment (breeding-like or non-breeding), the contrast between silence and song (to test whether hearing song induced turnover), the contrast between song and tones (to test for selectivity of the response), and each two-way interaction (to test whether either effect depended on endocrine state). To account for the non-independence of each group of individuals that were housed together in the same room (one hearing silence, one hearing song for 30 min, and one hearing tones for 30 min; see Fig. 1b), observation was nested within group as a random intercept and as a random coefficient for all predictors except treatment. This random effects structure followed Schielzeth and Forstmeier [52].

To get an idea of how rapidly effects could be seen, we then analysed the data from the 15 min time point as described above; for that time point the data from the birds hearing silence were re-used. As described above, non-independence of the individuals housed together (one hearing silence, one hearing 15 min of song, one hearing 15 min of tones; see Fig. 1b) was accounted for.

When the contrast between song and silence interacted with treatment, in other words when the effect of hearing song depended on endocrine state, we followed up with *post-hoc* comparisons to test whether the response to song differed from the response to tones within

each endocrine state, and whether endocrine state affected responses to each type of stimulus.

The statistical approach described above allowed us to test both parts of Question 1 (Fig. 1a). We next wanted to test Question 2, in other words whether endocrine state had a main effect on constitutive (stimulus-independent) monoamine turnover. In this test, we wanted to include all 60 of our experimental animals in the same model in order to maximize our power to detect a main effect of treatment. At the same time, we needed to control for the effects of hearing a sound on monoamine turnover. To accomplish these goals, we fitted an additional set of mixed effects linear models, this time including all 60 birds in each model. Treatment was included as a predictor. In addition, to control for sound playback, we also included the independent contrasts between silence and each duration of song or tones. Interactions were not included in this model. For these analyses, we assessed only the effects of treatment.

To rule out copulation solicitation displays as a source of variation in monoaminergic activity, we tested for a linear correlation between the number of these displays and monoamine turnover. Displays were scored from the videos recorded during playback. Only data from estradiol-treated birds were used, because non-breeding birds of this species are not known to perform courtship displays and none of the blank-treated birds did so in this study. We included data from estradiol-treated birds exposed to song, tones, or silence because estradiol-treated birds in our lab occasionally solicit spontaneously, regardless of any sound stimulus.

Results

Sound-induced monoamine turnover

The effects of hearing sound on monoamine turnover are plotted in Fig. 2. The means and standard errors are also shown in Supplemental Table 1. The statistical models, including z and p values for all contrasts and posthoc tests, are shown in Supplemental Table 2.

To test the hypothesis that song-induced monoamine release depends on endocrine state, we were interested in the interaction between the effects of song (compared with silence) and endocrine state. We found such interactions after 30 min of song playback for dopamine turnover in NCM ($Z = 2.03$; $p = 0.043$) as well as for serotonin turnover in NCM ($Z = 2.24$; $p = 0.025$) and CMM ($Z = 2.30$; $p = 0.021$). In none of these cases, however, did endocrine state interact with the contrast between song and tones (all $p > 0.2$). In other words, we found no evidence that the selectivity of these responses depended on endocrine state. We found no interactions between the silence-song contrast and treatment, or any main effects of song, in IC.

Post-hoc tests showed that in birds in breeding-like condition, although hearing song induced turnover of both dopamine and serotonin, the turnover was not different from that after exposure to tones (Fig. 2). For example, dopamine turnover in NCM was higher in birds hearing 30 min of song than in birds hearing silence ($z = 4.88$, $p < 0.001$) but not higher than in birds hearing 30 min of tones ($z = 0.71$, $p = 0.48$). Dopamine turnover in

NCM was higher in the breeding-like birds than in the non-breeding birds after presentation of either 30 min of song ($z = 1.65$, $p = 0.099$, note trend) or 30 min of tones ($z = 5.38$, $p < 0.001$). The effect of hearing song on dopamine turnover in NCM was significant after hearing only 15 min of song ($z = 2.52$, $p = 0.012$) but again, this response was not different from the response to tones ($z = 0.32$, $p = 0.747$). Overall, for birds in a breeding-like endocrine state, hearing sound did elicit dopamine release but it did not matter whether song or tones was played.

The results of *post-hoc* tests on serotonin turnover in NCM were similar to those for dopamine. Compared with silence, exposure to 30 min of song increased serotonin turnover in the birds in a breeding-like condition ($z = 2.58$, $p = 0.01$) but that turnover was not higher than after exposure to 30 min of tones ($z = 0.58$, $p = 0.563$). As was the case for dopamine, serotonin turnover was higher in the breeding-like than in the non-breeding birds after exposure to either song ($z = 2.92$, $p = 0.004$) or tones ($z = 2.76$, $p = 0.006$). Unlike the song-induced dopamine turnover, however, serotonin turnover did not change by 15 min after onset of song playback ($z = 0.74$, $p = 0.457$).

Post hoc tests on serotonin turnover in CMM revealed results identical to those in NCM. Within the birds in breeding-like condition, exposure to song for 30 min induced serotonin turnover in CMM ($z = 2.20$, $p = 0.028$). This response to song was not, however, different from the response to tones ($z = 0.94$, $p = 0.349$). As was the case for NCM, serotonin turnover in CMM was higher in the breeding-like than in the non-breeding birds after exposure to either song ($z = 3.11$, $p = 0.002$) or tones ($z = 2.72$, $p = 0.007$). Serotonin turnover did not change after only 15 min of song playback ($z = 0.74$, $p = 0.457$). For both NCM and CMM, although exposure to 30 min of song increased serotonin turnover only in the birds in a breeding-like endocrine state, we found no evidence that this turnover was stimulated by song specifically instead of by sound in general.

Effects of endocrine state on constitutive monoamine turnover

Main effects of endocrine state are shown in the insets in Fig. 2. We found significant main effects of endocrine state on serotonin turnover in NCM ($z = 3.61$; $p < 0.001$) and CMM ($z = 3.28$; $p < 0.001$). No other significant main effects of endocrine state were detected. Overall, these results are consistent with the view that serotonergic tone in the auditory system is modulated seasonally in this species.

Correlations with behaviour

To test whether proceptive behaviour could have contributed to increases in monoaminergic activity, we tested for correlations between the number of solicitation displays and monoaminergic activity in birds in estradiol-treated condition. We found no evidence of such a correlation. Spearman rho estimates were low overall, between -0.240 and 0.194 for all tests. Our ability to detect such correlations was limited by sample size, because only the estradiol-treated birds were expected to solicit [7] and of those, only those exposed to song (as opposed to tones or silence) were expected to perform appreciable numbers of displays. In this study, two birds exhibited 95% of all CSD's that were recorded. Because a relatively

low number of birds showed proceptive behaviour, it is unlikely that such behaviour contributed to the significant effects we report here for monoaminergic activity.

Confirmation of ovarian regression

None of the birds with blank implants showed any sign of ovarian development (very small ovary, largest ovarian follicle <1 mm). The birds in the photostimulated group showed signs of mild ovarian development; their ovaries and largest ovarian follicle were on average larger than in the blank-implanted group (Fisher's exact test, $p < 0.0001$).

Discussion

In previous studies, we showed that hearing 30 min of male song caused an increase in dopamine and serotonin metabolites in NCM in oestrogen-primed female white-throated sparrows [20, 30]. Here, we tested whether sound-induced increases in monoaminergic activity are higher in response to song than to a control sound, and whether they depend on endocrine state (Fig. 1a, Question 1). On the basis of popular models, in which monoamines modulate auditory responses in accordance with the behavioural relevance of the stimulus [2, 27, 53–56], we predicted that hearing song, but not a behaviourally irrelevant tone sequence, would increase monoaminergic turnover in the auditory pathway. Our data suggest that although dopamine and serotonin were released in NCM in response to hearing song (Fig. 2); this release did not differ from the response to tones. Thus, the increases in dopaminergic and serotonergic activity described by Matragrano et al. [20, 30] may not have been driven specifically by song; our current results suggest that they would have occurred in response to other sounds.

This result was surprising, because songs are presumably more behaviourally relevant than our tone sequences. It is possible that for some reason, this control stimulus was perceived as particularly behaviourally relevant. Novel sounds induce greater immediate early gene expression in NCM than do familiar sounds [57–59], and our birds were naïve to the tone sequences. We think that this explanation is unlikely, however. We have previously shown in multiple experiments in the same species that these tones drive significantly lower Egr-1 (ZENK) responses in NCM, CMM, the auditory thalamus, and IC than do conspecific songs [5, 39, 40, 60]. The Egr-1 response in the auditory forebrain is so closely related to the behavioural relevance of the stimulus that it has been used as a proxy for such [57, 61]. Egr-1 induction is also closely tied to neuromodulatory activity [2]. In zebra finches, application of norepinephrine directly to NCM induced Egr-1 expression [62], and in European starlings, depletion of norepinephrine significantly attenuated the Egr-1 response to song [63; c.f. 22]. We therefore expected that sound-induced monoamine activity would mirror the previously described Egr-1 response, which is known to be selective for song over tones in all three auditory areas in this study [39]. This prediction, however, was not borne out; the monoamine responses we detected were not any higher to song than they were to tones. Thus, differential monoamine release is perhaps not a good candidate mechanism to explain the selectivity of Egr-1 responses to song.

Other playback studies have produced little evidence that monoaminergic neurons in the brainstem respond selectively to song. In the same birds that showed selective Egr-1

responses in auditory areas, we could not detect selective Egr-1 responses in dopaminergic, noradrenergic, or serotonergic cell bodies [28, 54]. Lynch et al. [63] found that in female canaries, hearing song induced Egr-1 expression in noradrenergic cells, but no control sounds were presented in that study. Chen et al. [64] found that in juvenile male zebra finches, Fos was induced in noradrenergic and dopaminergic cells, but only when song was presented by a live tutor, not by passive playback [c.f. 65; see also 66]. In all of the above studies, monoaminergic cell activation was determined by labelling immediate early gene expression, which may not reflect the relevant activity. Immediate early genes are not always induced by neuronal firing [67] and can usually be sampled at only one time point following the presentation of a stimulus in each animal. Using electrophysiological recording, Gale and Perkel [68] found that dopaminergic cells of the ventral tegmental area in zebra finches fired selectively in response to the bird's own song. Thus, it may be possible to detect song-selective activity of monoaminergic cell groups using more time-sensitive techniques.

Although monoamine release in this study did not depend on the behavioural relevance of the sound playbacks, it did depend on endocrine state. In NCM, sound-induced release of dopamine and serotonin was detected only in birds that had been photostimulated and treated with estradiol. Thus, we replicated our previous finding of song-induced dopamine and serotonin release in estradiol-treated birds [20, 30], and showed further that it does not occur in non-breeding birds. Similarly, artificial tones induced release only in birds in breeding condition, suggesting that the reproductive endocrine state facilitates changes in the neurochemistry of the auditory forebrain that are relevant generally, not just to conspecific vocalizations.

Increased monoamine release during the breeding season may be mediated by an effect of reproductive hormones on releasable stores [2]. In previous studies of female white-throated sparrows, estradiol treatment increased the density of catecholaminergic and serotonergic innervation of CMM and NCM [19, 20]. It is therefore possible that the enhanced sound-induced release we detected in the birds in breeding-like condition can be explained simply by an increase in the availability of monoamines to be released. On a functional level, the result is harder to explain because it is not clear why both song and tone sequences would be more behaviourally relevant in spring than fall. It will be necessary to get a fuller picture of monoaminergic responses by investigating release in regions outside the auditory system. Behavioural responses to relevant sounds are not determined or orchestrated within any one system, particularly not the auditory system alone [27, 54]. Other systems, for example the reward pathway and hypothalamus, are involved and must also be examined [22, 56, 69, 70, 71].

Our current results suggest that auditory forebrain selectivity for behaviourally relevant sounds cannot be explained by event-related monoamine release during sound exposure. Monoamines could tune responses, however, by acting on a longer timescale. Monoaminergic neuromodulators are released from fibres *via* both synaptic and nonsynaptic mechanisms [72]. Norepinephrine applied directly to NCM in zebra finches increased the signal-to-noise ratio and coding accuracy of single neurons [73], suggesting permissive effects on the detection and encoding of complex sounds such as song. In the current study, photostimulation plus estradiol administration did not increase noradrenergic turnover.

Serotonergic tone, on the other hand, dramatically increased in NCM and CMM. Thus, the answer to our Question #2 (Fig. 1a) was yes, serotonergic activity in the auditory pathway does depend on endocrine state. These results are consistent with our previous findings that estradiol administration increased the concentration of a serotonin metabolite in the auditory forebrain in white-throated sparrows [20]. These increases could serve as a mechanism by which estradiol, working indirectly via serotonin, primes the auditory system to respond selectively to song.

Two potential caveats should be considered when interpreting our results. First, we measured monoamine turnover in dissected tissue, which does not afford the best time resolution. Methods to measure release in real time, such as voltammetry [74–76] should be employed in order to better detect event-related release. Second, our definition of monoamine turnover is based on the assumption that an increase in metabolites, relative to the concentration of the monoamines themselves, indicates release. Our results could also indicate increases in synthesis followed by breakdown without release. Regardless of these potential shortcomings we have shown definitive evidence of hormone-dependent changes in the metabolic rate of monoamines in auditory areas.

Summary.

In this study, we tested predictions of a popular model of hormone-dependent salience of courtship signals [2, 23, 27, 54, 56, 77]. According to the model, reproductive hormones act on monoaminergic modulatory inputs to auditory areas, which themselves respond to external events and encode behavioural relevance. In this way, auditory responses to courtship signals are amplified during the breeding season when they are most behaviourally relevant. Our results show partial support for this model. We did not find evidence of event-related monoaminergic responses that encode behavioural relevance. We did, however, find striking evidence that endocrine state modulates both event-related and event-independent monoaminergic activity in auditory areas. The salience and attractiveness of courtship signals is not determined solely by the auditory system, however [27, 54]. Future investigations should focus on how estradiol shapes monoaminergic responses in the reward pathway and social decision-making network [2, 56].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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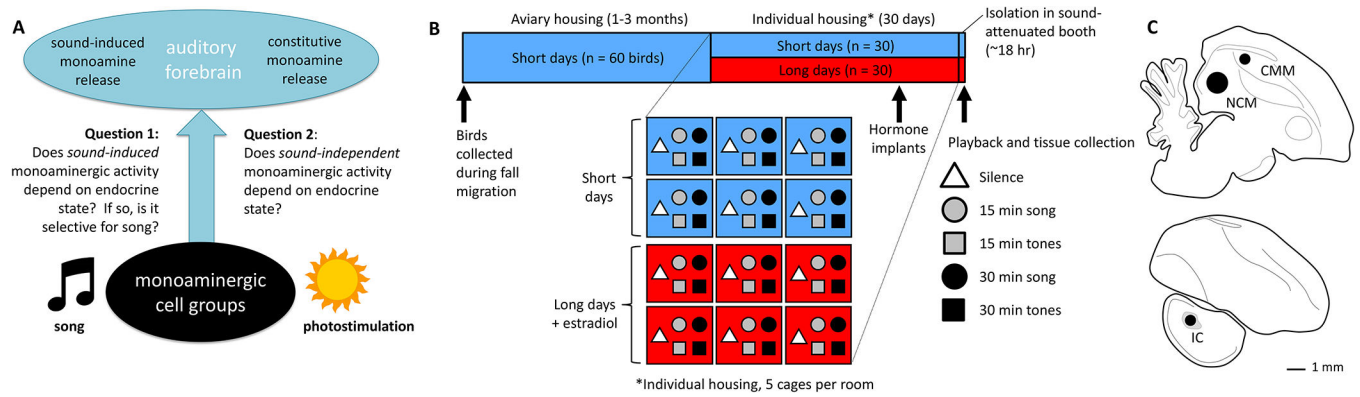


Fig. 1. Experimental design.

Our goals in this study were two-fold (A). We previously showed that in estradiol-primed female white-throated sparrows, hearing male song causes release of dopamine and serotonin in the auditory forebrain [20, 30]. Question 1 in the current study is whether that release depends on endocrine state and is selective for song. Question 2 is whether monoamine turnover increases in a breeding-like endocrine state, independent of hearing sound, which may prime the auditory system to respond selectively to song. MA, monoaminergic. We tested these hypotheses using the design depicted in (B). Sixty animals were collected and housed in aviaries on short days before being transferred into individual cages and housed 5 cages per small room. Each of the rooms contained one bird that would hear one of the five types of sound stimulus. Half of the birds remained on a winter-like photoperiod (blue) and half were photostimulated (red). After three weeks of individual housing, birds received subcutaneous silastic capsules containing nothing (blue) or estradiol (red). One week later, each bird was isolated in a sound-attenuated booth and the next morning was presented with a sound stimulus. Brains were collected and frozen immediately following the stimulus presentation. Brains were sectioned sagittally at 300 μ m and regions of interest microdissected as shown by the black circles in (C). CMM, caudomedial mesopallium. IC, inferior colliculus. NCM, caudomedial nidopallium. For further details see *Methods*.

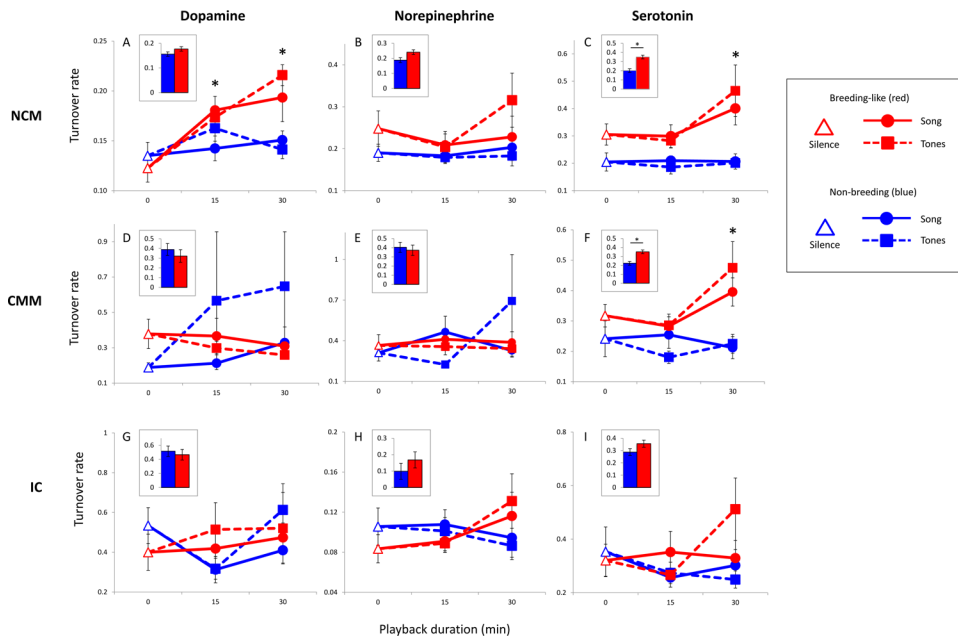


Fig. 2. Effects of sound playback and endocrine state on monoamine turnover in the auditory forebrain.

The effects of song exposure on dopamine and serotonin turnover depended on endocrine state. In birds that were photostimulated and treated with estradiol (“breeding-like”; red lines), hearing song (solid lines) increased turnover of dopamine in NCM (A), serotonin in NCM (C) and serotonin in CMM (F) when compared with silence (open triangles) (* $p < 0.05$). These effects were, however, not different from the effect of exposure to tones (dotted lines). In birds in non-breeding condition (blue lines), monoamine turnover was unaffected by exposure either sound. The line graph in (A) excludes an extreme outlier that did not change the outcome of the statistical tests. Insets in each panel depict the main effects of endocrine state on turnover (* $p < 0.05$), with all animals included in the same analysis and the effects of sound stimulus controlled. Estimated marginal means, i.e., the means adjusted for the effects of sound stimulus, are shown for each endocrine state (blue = non-breeding; red = breeding-like). IC, inferior colliculus. NCM, caudomedial nidopallium. CMM, caudomedial mesopallium.