It takes a seasoned bird to be a good listener: communication between the sexes

Eliot A. Brenowitz

Departments of Psychology and Biology, Virginia Merrill Bloedel Hearing Research Center, University of Washington, Box 351525, Seattle, WA 98195, USA

Luke Ramage-Healey

Neuroscience and Behavior Program, Center for Neuroendocrine Studies, Department of Psychological and Brain Sciences, University of Massachusetts, Amherst, Massachusetts 01003, USA

Corresponding author: Eliot Brenowitz, eliotb@uw.edu, 206-543-8534 (phone), 206-685-3157 (FAX)

Department of Psychology, Box 351525, University of Washington, Seattle, WA, USA 98195

Abstract:

Birds commonly use sound for communication between the sexes. In many songbird species, only males sing and there are pronounced sex differences in the neural song control circuits. By contrast, the auditory circuitry is largely similar in males and females. Both sexes learn to recognize vocalizations heard as juveniles and this shapes auditory response selectivity. Mating vocalizations are restricted to the breeding season, when sex steroid levels are elevated. Auditory cells, from the ear to the cortex, are hormone sensitive. Estrogens are synthesized in the brain and can modulate the activity of auditory neurons. In species that breed seasonally, elevated levels of estradiol in females transiently enhance their auditory responses to conspecific vocalizations, resulting in sex differences in audition.

Introduction

The use of sound for communication between the sexes is a prominent trait of birds. Sounds are commonly used for attracting and recognizing mates. Birds present an interesting model for studies of sex differences in vocal communication for several reasons. 1) Among the 9000 species of birds there is extensive diversity in the degree of sex differences in vocal behavior. 2) Species-specific vocalizations in songbirds and parrots are typically developed by juveniles through sensorimotor learning. The auditory selectivity of cortical neurons to those sounds is also shaped by learning. 3) Vocalizations used for mate attraction are usually restricted to the breeding season. Sex steroid hormones are elevated in breeding birds. Auditory cells, from the ear to the cortex, often have steroid receptors and are therefore sensitive to these hormones. Emerging research shows that estrogens can enhance auditory responses to conspecific signals. This raises the hypothesis that pronounced sex differences in neural responses, when they occur, are only present in breeding birds, caused by transient high estrogen levels in the blood and brain of females. Here we review the evidence for sex differences in vocal production and perception, and the contribution of learning and hormones to sex differences in audition. We will focus on songbirds since most of the relevant research has been done on this group.

Sex differences in song learning and production

There is great diversity in sexual patterns of song learning and production among the 4000 species of songbirds, from species in which only males sing to those in which both sexes sing in complex vocal duets [1-3]. Song learning has been studied mostly in species where only males sing. Even females that do not sing may learn to discriminate songs of different males. Different behavioral assays show that females of several species prefer songs heard as juveniles, as do males [reviewed in 4], which demonstrates that early exposure to song is important for song recognition in both sexes, even for those species in which only males learn to produce song [5]. The sexes may differ, however, in what aspects of

perception are learned. Adult male Zebra Finches (*Taeniopygia guttata*) placed in isolation during the juvenile sensitive period for song learning fail to show selective neural responses to conspecific songs or calls, as measured by magnetic resonance imaging or event-related potentials. By contrast, female finches that were similarly isolated as juveniles *do* show selective neural responses to conspecific vocalizations [6]. The behavioral preference of adult females for song features heard as juveniles shows, however, that while they may hatch with a broad filter for conspecific song, experience sharpens their discrimination among the songs of individual males.

In species where females learn to sing there may be sex differences in different aspects of learning. Female Northern Cardinals (*Cardinalis cardinalis*) learn the same number of songs as males. The sensitive phase for memorizing songs starts at the same age in males and females, but ends much earlier in females [7]. This difference allows females to selectively learn the songs in their natal area, whereas males continue to learn after they disperse to new areas. Females raised in acoustic isolation do not learn to sing normally, whereas isolated males can improvise *de novo* normal cardinal song.

Sex differences in behavioral auditory perception

Song discrimination has been measured with various behavioral assays. Male red-winged blackbirds (*Agelaius phoeniceus*) respond aggressively to an imitation of redwing song by a Northern Mockingbird (*Mimus polyglottos*). Female redwings, however, do not perform a copulation solicitation display in response to this imitation [8,9]. Male and female Zebra Finches both make "long calls" (learned in males but not females) preferentially in response to female long calls, but discriminate using different acoustic components of these calls [10-12]. Females rely on call duration, whereas males make a categorical discrimination based on the absence of frequency modulation [10]. There is a strong motivational aspect to these behavioral measures, which makes it challenging to determine perceptual limits.

Operant training procedures isolate sensory ability from motivational factors and have been used to examine psychoacoustics in a broad range of birds (e.g., canaries, pigeons, owls, chickens, parakeets, hawks) [reviewed in 13]. These methods are generally used either to measure thresholds for detection or discrimination, or to measure the discrimination among two or more sounds [14,15]. Few psychoacoustic studies have directly compared hearing in conspecific male and female birds. Dooling et al. [16] showed that male and female parakeets (*Melopsittacus undulatus*) differed in how they categorized the same set of conspecific vocalizations. Female Zebra Finches required more operant trials to learn to discriminate between two conspecific songs than did males [17].

Changes in heart rate have also been used to measure perception. Ikebuchi et al. [14] found that male Bengalese finches (*Lonchura striata*) did not differentiate between songs of other individual males, but females did distinguish among them. Male and female Chiffchaffs (*Phylloscopus collybita*) did not differ in their heart rate response to conspecific songs [18]. Both sexes of European Robins (*Turdus merula*) increased heart rate more to conspecific than heterospecific song, and this increase lasted longer in males [19].

The overall impression from these operant and heart rate studies is that there are not substantial sex differences in behavioral song discrimination (R. Dooling, G. Klump, personal communication). A caveat to this conclusion, however, is that birds used in captive studies are likely not to be in breeding condition, and females therefore would not experience enhanced auditory responses due to high levels of estrogenic hormones in the plasma and brain (see below). Consistent with this suggestion, Zebra Finches required more operant trials to reach criterion in the winter than summer [17]. Future research should explicitly compare hearing in breeding and nonbreeding conspecific males and females, especially in species in which females sing [e.g., 20].

Sex differences in brainstem auditory responses

Peripheral auditory function can be evaluated using the auditory brainstem response (ABR), a short-latency evoked potential typically emitted 10–15 ms after the presentation of an auditory stimulus. Sex differences in brainstem auditory function were observed using ABRs in the Brown-headed Cowbird (*Molothrus ater*) [21]. Female cowbirds are brood parasites and lay their eggs in the nests of other species. They may detect their specific host species by eavesdropping on their songs, which typically have peak energy at 2 – 4 kHz. Male cowbird songs, by contrast, have energy above 4 kHz. Females are much more sensitive (by ca. 15 dB), and have better frequency discrimination, at 2 – 4 kHz than males, which may improve their ability to detect and localize the species they parasitize from a distance. In Zebra Finches, tones and clicks evoked higher amplitude and shorter latency peaks for the early ABR waves in females, suggesting more synchronous activity [22]. A study of White-crowned Sparrows (*Zonotrichia leucophrys*), however, found that males and females did not differ in ABR thresholds, peak latencies, or frequency tuning [23]. Thresholds were elevated and latencies delayed in both sexes by exposure to breeding-typical levels of steroid sex hormones (testosterone in males, 17 β -estradiol (E2) in females).

Sex differences in central auditory processing

Studies of neural activity in telencephalic auditory nuclei of songbirds (see Figure 1) show that males and females are largely similar, with only subtle differences [reviewed in 24]. In the caudomedial nidopallium (NCM, analogous to mammalian A2) and other auditory areas, neurons in female and male Zebra Finches respond similarly to familiar stimuli (e.g., tutor songs). By contrast, NCM in females shows diminished response magnitude and shallower rates of stimulus adaptation to novel song stimuli [25]. In male finches, neurons in the adjacent caudomesopallium (CM, also analogous to mammalian A2) respond selectively to conspecific songs over white noise [26], whereas female CM lacks this response

selectivity [27]. These observations suggest that higher-order song processing regions may encode stimulus novelty and perhaps social relevance differently in the sexes, though the differences are subtle. These neuronal response differences in finches may reflect the divergence in behavioral contexts of auditory processing between males, who use songs for mate attraction and inter-male aggression, and females, who use songs to choose a mate.

Studies examining immediate-early gene (IEG) expression profiles in developing songbirds show some subtle but quantifiable early sex differences. As early as 30 days post-hatch, IEG expression in both NCM and CM differ between male and female Zebra Finches. Conspecific song upregulates the IEG *FOS* in both of these auditory regions in females but not in males, whereas conspecific song-induced *EGR1* upregulation occurs in both regions in males but not in females [28]. These sex differences are no longer present at 45 days post-hatch [29] or in adulthood (> 100 days) [30]. This brief developmental period of sex difference aligns with the time of intensive sensorimotor song learning in males (up to ca.35 days post-hatch), and indicates that there are early sex-specific genetic mechanisms operating in the auditory forebrain. In adult finches, in general there are no sex differences in song-induced IEG responses in the auditory forebrain [31,32]. The one exception is greater *EGR1* expression in CM in response to conspecific male vocalizations in adult female finches [10,33]. The functional consequences of these sex differences in IEG expression are not yet clear, but are consistent with the general subtlety of sex-specific response patterns in the auditory pathway.

Hormone effects on auditory responses

Steroid hormones influence activity in auditory regions and contribute to sex differences in response patterns in songbirds. Neurons within the auditory forebrain, including NCM, CM, and also the thalamic region IC (central nucleus of the inferior colliculus, see [34]; Figure 1) exhibit elevated *EGR1* induction in response to song following treatment with estradiol implants [35,36]. In NCM and CM this is due to

decreased response to simple stimuli like tones, compared to complex stimuli like song, and this increases the selectivity for biologically relevant sounds. The degree of sex-specificity for hormonal influences on neural activity in auditory regions is an active topic of investigation. The hormone-dependent IEG selectivity for song stimuli over tone stimuli appears to occur only in the female auditory forebrain, since hormone treatment of males did not alter stimulus selectivity in *EGR-1* responsiveness [37]. Similarly, Caras et al. [38,39] showed that estrogen increased the sensitivity and response strength of spike-timing neurons in the primary telencephalic auditory region field L (Figure 1) to tones and conspecific songs in breeding female, but not male, White-crowned Sparrows. These findings support the general idea that prolonged increases in circulating hormone levels that bring female birds into breeding condition also produce sex-specific changes in auditory responsiveness.

Sex differences in audition have also been examined by measurement and manipulation of local steroid hormone levels in songbird brain. In vivo microdialysis measurement in Zebra Finches has shown that steroid levels in the brain can rapidly fluctuate, analogous to neuromodulators. In cases where baseline neuroestradiol levels in NCM were directly compared between male and female finches, however, no difference was detected [40]. The auditory NCM therefore appears to have a similar neuroestrogen 'set point' for baseline functions in adulthood, and in each sex the neuroestrogen levels are elevated in NCM compared to nearby brain regions that exhibit lower expression of the estrogen synthesis enzyme, aromatase [24].

Similarly, the elevation in neuroestradiol levels within NCM in response to auditory stimulation is similar in magnitude in male and female finches [40,41]. Furthermore, no qualitative difference exists between sexes in the acute actions of E2 on enhancing the neuronal encoding of songs in NCM [40]. This is generally consistent with a lack of sex differences in the NCM of finches in the expression of any of the major classical estrogen receptors [42,43], as well as non-classical estrogen receptors [44]. The acute

estrogen signaling that regulates auditory function in the Zebra Finch NCM thus does not appear to differ qualitatively or quantitatively between males and females.

The telencephalic song control circuits are sexually dimorphic in morphology in Zebra Finches, and the masculinization of the song system and singing behavior itself is partially dependent on estrogens [45-48]. However, when directly examining neuroestradiol levels within the auditory forebrain, no differences were detected between males and females during the critical period for masculinization [49]. Therefore, endogenous estrogens cannot solely account for masculinization, and the role of other brain-derived steroids, as well as genetic factors, in regulating sexually differentiated behaviors in songbirds should be considered.

Conclusions:

The neural circuit that regulates the motor production of learned species-specific vocalizations in songbirds provides one of the most pronounced examples of sexual dimorphism in the vertebrate brain. In species where only males sing, such as the Zebra Finch, there are male-biased sex differences in morphological, genetic, neurochemical, and neurophysiological traits. When we look at the auditory system of birds, however, the impression is one of overall similarity between the sexes, especially in nonbreeding birds. What sex differences there are in auditory function tend to be more subtle than are seen in the song production circuitry. The greatest sex differences in auditory responses are seen in seasonally-breeding birds, and seem to result from the elevated levels of estradiol present in the brain of females. The contrast between pronounced sex differences in the song production circuits and similarity between the sexes in the auditory system likely reflects that fact that song detection and discrimination are important for both males and females, even if only males sing in a given species. Male song has its greatest behavioral salience for females when they are breeding, and estradiol is a seasonal internal cue that provides an effective way of enhancing auditory function at that critical time

of year. Future research should address the cellular and molecular mechanisms of the hormonal effects on auditory responses.

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Figure caption:

Figure 1. The neural circuits for auditory processing, song production, and sensorimotor learning in songbirds.

The ascending pathway that processes auditory information including song is shown in blue. Auditory output from the inner ear is conveyed by the VIIIth nerve to the cochlear nuclei in the brain stem (not shown), and then to inferior colliculus (IC) in the midbrain [34]. IC projects to the nucleus ovoidalis (Ov) complex in the thalamus. Ov projects to the telencephalic field L complex (LI, L2, L3) (analogous to mammalian A1) and the caudomedial nidopallium (NCM, analogous to mammalian A2). The field L complex connects reciprocally to the caudomesopallium (CM, analogous to mammalian A2). NCM and CM are also reciprocally connected. There are sex differences in gene expression in NCM of Zebra Finches. Females show higher levels of calbindin expression, and males have more fibers in NCM that label for the estrogen synthesizing enzyme aromatase [50].

The nuclei in green represent the descending motor pathway for song production, which receives premotor information from the nuclei in purple. It originates in nucleus uvaefomis (Uva) in the thalamus and includes projections from HVC (proper name) to the robust nucleus of the arcopallium (RA) in the telencephalon, and from RA to medullary nXIIts, which innervates the muscles of the sound-producing organ, the syrinx, and the brainstem respiratory pre-motor nuclei.

An anterior forebrain pathway (AFP) important for song learning and vocal variability is shown in red. The AFP includes HVC, area X in the striatum, the dorso-lateral division of the medial thalamus (DLM, not shown), lateral magnocellular nucleus of the anterior nidopallium (LMAN), and RA; it is analogous to the mammalian basal ganglia-thalamocortical circuit [51]. The AFP is essential for song learning, for the motor production of song in juveniles [52], and for adult song variability [reviewed in 53].

The song control system receives auditory input at multiple levels. CM projects to ventral HVC, and to the area surrounding RA (not shown). CM also projects to the interfacial nucleus of the nidopallium (NIf), which appears to provide the major auditory input to the song control circuits (not shown) [reviewed in 54,55]. Neurons within HVC, RA, IMAN, area X, and NIf respond to acoustic stimuli. Some neurons within each of these nuclei respond maximally, although not exclusively, to presentation of the bird's own song (BOS), consistent with their primary role in sensorimotor integration.

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Figure 1