Sexual Dimorphism of Auditory Activity in the Zebra Finch Song System

HEATHER WILLIAMS

Rockefeller University Field Research Center for Ecology and Ethology, Tyrrell Road, Millbrook, New York 12545

While the tracheosyringeal motor neurons of anesthetized male zebra finches fire in response to acoustic stimuli, the same motor neurons in females show no such response. Females masculinized by estradiol implants on Days 1 or 2 after hatching may develop auditory responses in their tracheosyringeal motor neurons; the presence of the response is directly related to the degree of masculinization of the estradiol-treated females' telencephalic song centers. In male zebra finches, neurons in HVC (Hyperstriatum Ventrale pars caudalis) respond to sound, and the HVC is necessary for the tracheosyringeal auditory response. Multiunit auditory activity was demonstrated in the HVC of female zebra finches. A single 20-μA pulse delivered to the male HVC elicits a large volley in the tracheosyringeal nerve; microstimulating the female HVC does not evoke a response in the motor nerve. This failure of both auditory and HVC stimulation to elicit a response in the female tracheosyringeal nerve is attributed to the lack of a functional HVC–nucleus Robustus Archistriatals projection in females. If, as has been suggested, the tracheosyringeal auditory response may be important for the processing of song, female zebra finches might not process song in the same manner as do males.

Female zebra finches (as well as most other female songbirds) do not normally sing, while male passerines are well known to be prolific songsters (Nottebohm, 1975). This sexual dimorphism in behavior corresponds to a sexual dimorphism in the neural machinery that controls birdsong (see Fig. 1 for a diagram of the avian song system). In both canaries and zebra finches several forebrain song system nuclei, including HVC (Hyperstriatum Ventrale pars caudalis) and RA (nucleus Robustus Archistriatals), as well as the tracheosyringeal motor nucleus (nXIIIs of the hypoglossus) and the muscles it innervates in the syrinx (the avian vocal tract),

1 I thank Linda Crane for implanting hatchlings, Susan Kasparian for tracing sections, and Dr. Fernando Nottebohm for helpful comments on the experiments and manuscript. Send requests for reprints to the author.
AUDITORY SEXUAL DIMORPHISM IN FINCHES

Fig. 1. Auditory and song system brain centers in male passerine birds. Filled arrows show afferents to HVc (Nottebohm et al., 1981), unfilled arrows show the efferent pathway from HVc to the syrinx (Nottebohm et al., 1976), and stippled pathways show the known auditory projections (Kelley & Nottebohm, 1979). These pathways were originally described in canaries, but the nuclei and connections shown here are consistent for all male oscine songbirds examined to date, including zebra finches. HVc: Hyperstriatum Ventralis pars caudalis; L: field L, the primary telencephalic auditory projection; NIf: Nucleus interfacialis of the neostriatum; nXIIIts: the tracheosyringeal portion of the hypoglossal motor nucleus; NXIIIts: the tracheosyringeal motor nerve; OV: nucleus ovoidalis, the avian homolog of the medial geniculate; RA: nucleus Robustus Archistriatalis; Uva: nucleus uvaeariformis of the thalamus.

organ), are larger in males than in females (Arnold, 1974; Nottebohm & Arnold, 1976; Arnold, 1980; Nottebohm, Kasparin, & Pandazis, 1981). The HVcs and RA's of female zebra finches have fewer and smaller cells than do the same nuclei in males (Gurney, 1981). Additionally, it has been shown that the dendritic arborizations of the neurons in the canary RA are less extensive in females than in males (DeVoogd & Nottebohm, 1981a). The neurons of many song system nuclei, including HVc, RA, and nXIIIts, concentrate steroid hormones; here again there is sexual dimorphism, as proportionately fewer HVc neurons are labeled by tritiated testosterone injected into female zebra finches (Arnold & Saltiel, 1979).

These song-related behavioral and neural deficits in female songbirds can be reduced by treatment with steroid hormones. Testosterone propionate (T) administered to adult female canaries induces singing (Shoemaker, 1939; Baldwin, Goldin, & Metfessel, 1940), an increase in the volumes of HVc and RA (Nottebohm, 1980), and the lengthening of RA neuron dendrites (DeVoogd & Nottebohm, 1981b). Zebra finch females do not respond to T treatment as adults (Arnold, 1974); however, the females' HVc and RA volumes, the size and number of neurons in RA, and singing behavior can be masculinized by estradiol (E) implants administered immediately after hatching and followed by T treatment in adulthood (Gurney, 1982; Pohl-Apel & Sossinka, 1984).

The marked sexual dimorphism in many areas and aspects of the songbird brain might merely reflect the complex circuitry required for learning and producing the intricate series of vocal motor acts that is
the male's song. However, the number and magnitude of sexually dimorphic brain areas in the song system suggests that functions other than the purely vocal-motor might also differ between males and females.

HVC, the forebrain nucleus which is necessary for normal song production (Nottebohm, Stokes, & Leonard, 1976) and is markedly sexually dimorphic (Nottebohm & Arnold, 1976; Gurney, 1981), has also been shown to respond to auditory stimuli (Katz & Gurney, 1981; McCasland & Konishi, 1981). A small proportion of the auditory units in the HVC of white-crowned sparrows respond specifically to elements of the bird's own song (Margoliash, 1983). In light of the recent discovery of auditory activity in the tracheosyringeal portion of the hypoglossal motor nerve (NXIIIts) of male song birds, and the finding that this auditory activity in the motor nucleus and nerve does not occur when HVC or RA is lesioned (Williams & Nottebohm, 1985), it seems possible that the sexual dimorphism in the forebrain song system might also reflect a sexual dimorphism in auditory processing.

METHODS

Adult zebra finches for use as breeders and experimental subjects were obtained commercially. Ten pairs were placed in a large communal cage kept on a constant 14-h day length at the Rockefeller University Field Research Center, where they bred readily and continuously. Hatchlings were implanted with E pellets containing 100 μg of hormone and prepared according to the protocol developed by Gurney (1981). This treatment masculinizes females; some masculinized females produce song of male-like quality, while some sing poorly and others do not sing at all (Pohl-Apel & Sossinka, 1984). A total of 91 zebra finches was tested for auditory responses in the tracheosyringeal motor nerve (NXIIIts): 68 intact males (which were used in other studies as well), 12 intact females, and 11 E-treated females.

Birds were isolated without food for 1 h prior to surgery and anesthetized with either Chloropent, urethane, or a 1:1 mixture of ketamine and xylazine. The feathers on top of the head and the front of the neck were removed and the bird's head was placed in a Kopf small animal stereotaxic located in a soundproof chamber. Especially designed perforated and hollow earbars were used to allow transmission of sound to the ear. The bill was clamped at 45° below horizontal, and the bird's body rotated to expose the front of the neck. The tracheosyringeal nerves were exposed, dissected free, and mounted on hook electrodes in an oil pool. The skull was exposed and then cut away over the stereotaxic coordinate locations for nuclei of interest. Acoustic stimuli (pure tones in the audibility range, 300 Hz to 7 kHz) were generated (Krohn-Hite 5300 function generator), attenuated (Hewlett-Packard 350B attenuator set), and delivered to the
bird by a Nagra speaker mounted 1 m above the bird's head. The system was calibrated with a Bruel and Kjaer 2206 precision-sound-level meter.

Glass-insulated tungsten microelectrodes with 10- to 25-μm tips (Asanuma, 1981) were placed stereotaxically and used for multiunit recording and microstimulation. Stimuli were monopolar or biphasic pulses: 5–100 μA, 0.1–0.8 ms, initially anodal or cathodal; parameters were varied within these bounds for each experiment, in order to minimize the stimulus artifact and maximize the evoked response. These pulses were delivered singly or in trains. Data were recorded as photographs of oscilloscope tracings or as computer-averaged plots on thermal paper.

Some birds received injections of HRP (Sigma type VI) delivered iontophoretically through a glass micropipet with a tip diameter of 30–40 μm (20% HRP, 3 μA for 5–10 min); others received small electrolytic lesions to confirm electrode placement. After a 24- to 48-h survival time, these zebra finches were killed with an overdose of Chloropent and perfused intracardially with 10% formol saline (lesioned birds) or 1% paraformaldehyde, 1.5% glutaraldehyde, and 4% sucrose in pH 7.4 phosphate buffer (HRP-injected birds). Sections 50 μm thick were cut on a vibratome, and alternate sections were reacted with TMB to show transport of HRP (Mesulam, 1978) or mounted and stained with cresyl violet.

Sections containing HVc, RA, and SpM (Spiriformis medialis, a midbrain nucleus that is not sexually dimorphic and not part of the song system) were placed in a microprojector and the outlines of the nuclei traced by a technician unaware of the experimental treatment or sex of the bird. The area of each tracing was determined using a polar planimeter. The areas were then multiplied by the interval between sections and totaled to obtain volumes. To control for the differences in brain size and shrinkage due to use of the two fixatives described above, birds' HVc and RA volumes were expressed as a ratio of their SpM volumes; this method has been used routinely to control for gross differences in brain size and in tissue shrinkage (Nottebohm et al., 1981; Nottebohm, 1981).

RESULTS

Figure 2 shows examples of the tracheosyringeal nerve (NXIIts) auditory response to pure tones in an intact male and an intact female zebra finch. Spontaneous activity in the tracheosyringeal nerve increases dramatically during expiration (Manogue and Paton, 1982; Fig. 2a). Because this activity might mask a weak auditory response in NXIIts, all female zebra finches were also tested with sound stimuli delivered during inspiration, when spontaneous activity levels are low and any auditory response would be readily apparent. As the NXIIts auditory response can be obliterated by deep anesthesia, birds were examined for a NXIIts response to sound over a period of at least 3½ h before being classified as nonresponders. All zebra finches tested were classified as NXIIts auditory responders.
Fig. 2. Auditory responses in NXIIIs. (a) "On" and "off" responses to a pure tone in the NXIIIs of an intact adult male zebra finch. Expiratory activity in NXIIIs can also be seen in the first 2/3 of the oscilloscope trace. (b) The NXIIIs auditory response cannot be seen in an adult female zebra finch, although a strong auditory response in HVC is evoked by the pure tone stimulus. Although the amplification is identical to (a) and (c), this trace shows the low level of spontaneous firing in HVC which is seen during inspiration; the relatively low firing level in NXIIIs would allow even a small auditory response to be perceptible. (c) An NXIIIs auditory response in an adult female zebra finch which had been implanted with estradiol as a hatchling. Expiratory activity can also be seen in this recording.
AUDITORY SEXUAL DIMORPHISM IN FINCHES

or nonresponders, and the results are presented in Table 1. None of the 12 intact females showed an auditory response in their NXIIIts, while 53 of the 68 intact males were NXIIIts auditory responders (why some males are nonresponders has not yet been explained, although some failures of the response can be attributed to damage to the nerve caused by poor surgical techniques during the early phases of the study). This difference between males and females in the occurrence of the NXIIIts auditory response is statistically significant ($\chi^2(3) = 28.2, p < .001$). Among the 11 early E-treated females, 7 showed responses to sound in NXIIIts; this distribution of responders closely approximates that in intact males ($\chi^2(3) = 1.465, p > .5$) while differing significantly from intact females ($\chi^2(3) = 10.96, p < .02$). Clearly, normal zebra finch females lack the NXIIIts auditory response found in males, while females masculinized by early estradiol treatment have male-like NXIIIts responses to sound.

The volumes of HVc and RA (as a proportion of SpM) were determined for the early E-treated females that survived surgery (six responders, three nonresponders) and for matched intact males and females (Table 2). The normalized HVc and RA volumes of the NXIIIts auditory responding and nonresponding E-treated females are significantly different. The size of the song control nuclei of E-treated auditory-responding females is masculinized; although not as large as those of intact males, their HVcs and RAs are significantly larger than the same nuclei in E-treated nonresponders (Student’s $t$ test, $p < .05$). The HVc and RA volumes of the E-treated nonresponders did not differ from the nucleus volumes of intact females. Thus, the presence or absence of the NXIIIts auditory response in the E-treated females is directly correlated to the degree of masculinization as measured by the size of song control nuclei.

The correlation between the size of the song control nuclei and the presence of the NXIIIts auditory response suggests that the absence of an NXIIIts auditory response in normal females might be attributable to either (a) a lack of auditory inputs to the smaller nuclei in the forebrain song system of females or (b) differences in connectivity within the females’ song system. The known auditory response in the male HVc, coupled with the gross disparity of HVc size in males and females,

| TABLE 1 |
| Occurrence of the NXIIIts Auditory Response in Male, Female, and E-Treated Female Zebra Finches |

<table>
<thead>
<tr>
<th></th>
<th>Responders</th>
<th>Nonresponders</th>
<th>Percentage responders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>53</td>
<td>15</td>
<td>77.9</td>
</tr>
<tr>
<td>Females</td>
<td>0</td>
<td>12</td>
<td>0.0</td>
</tr>
<tr>
<td>E-treated females</td>
<td>7</td>
<td>4</td>
<td>63.6</td>
</tr>
</tbody>
</table>
### TABLE 2

Volumes of the Song System Nuclei HVC and RA in Intact Male Zebra Finches, NXIIIIs Auditory Responding and Nonresponding Females, and Intact Adult Females

<table>
<thead>
<tr>
<th></th>
<th>HVC (mm$^2$)</th>
<th>RA (mm$^2$)</th>
<th>SpM (mm$^2$)</th>
<th>HVC:SpM</th>
<th>RA:SpM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Intact males (n = 6)</td>
<td>0.194</td>
<td>0.0616</td>
<td>0.146</td>
<td>0.0608</td>
<td>0.034</td>
</tr>
<tr>
<td>Females + E, + NXIIIIs auditory response (n = 6)</td>
<td>0.165</td>
<td>0.0852</td>
<td>0.100</td>
<td>0.0224</td>
<td>0.0426</td>
</tr>
<tr>
<td>Females + E, - NXIIIIs auditory response (n = 3)</td>
<td>0.0328</td>
<td>0.0109</td>
<td>0.0215</td>
<td>0.0032</td>
<td>0.0398</td>
</tr>
<tr>
<td>Intact females (n = 3)</td>
<td>0.0372</td>
<td>0.0184</td>
<td>0.0191</td>
<td>0.0063</td>
<td>0.0502</td>
</tr>
</tbody>
</table>

*Note.* Spiriformis Medialis is a thalamic nucleus that is not sexually dimorphic and is not part of the song system. The volumes of HVC and RA were expressed as a proportion of the volume of SpM in order to control for the variability introduced by differential tissue shrinkage. Statistical comparisons were performed on the normalized data.
suggested that an absence of auditory activity in the female HVC might account for the lack of a female NXIIIts auditory response. As the female HVC is small and difficult to locate using stereotaxic coordinates, auditory responses in the lateral hemispheres near the putative location of HVC were investigated in two normal females and two NXIIIts nonresponding
E-treated females. Multiunit responses were found on 26 penetrations and mapped (Fig. 3a). The depths at which auditory activity was seen for each penetration and representative computer-averaged auditory responses are also shown (Fig. 3b,c). The 26 penetrations can be separated into three groups by their anterior–posterior location, depth, and latency. The posterior-most, shallowest, and longest-latency responses were attributed to HVc neurons; the anterior-most deep area may be nucleus interfacialis (NIf); and the central set of penetrations, covering the most brain area, have auditory responses typical of field L, the primary telencephalic auditory projection (Karten, 1968). The posterior auditory area tentatively labeled HVc is thicker and extends to a greater depth than the HVc normally seen in Nissl-stained female zebra finch brains. The added thickness may be due to auditory potentials recorded in the “auditory shelf,” an area which underlies HVc and contains cells receiving inputs from field L (Kelley and Nottebohm, 1979; Fig. 1). Two additional pieces of evidence, the timing of the auditory response and the pattern of HRP backfills, served to confirm that this posterior auditory area in females included HVc. Nucleus NIf projects to HVc (Nottebohm, Kelley, & Paton, 1981), as does field L (Kelley and Nottebohm, 1979); the latency of the auditory response in the putative female HVc lags behind the responses in both field L and NIf (Fig. 3c), as would be expected from the connectivity. HRP was injected into the center of the posterior auditory area identified as HVc in two of the NXIIIts nonresponding females. The resulting pattern of labeled cells is consistent with the identification of the posterior auditory area as HVc: cells were backfilled in NIf and in the thalamic nucleus uveiformis (Uva), both of which are known to project to HVc in males (Nottebohm et al., 1981; see Fig. 1). Extensive labelling in nucleus ovoidalis (the avian medial geniculate) would be expected if the injection site were in field L; however, a maximum of only seven cells were backfilled. Thus, the inputs to the female HVc and the auditory responses recorded there suggest that any anatomical basis for the sexual dimorphism of the NXIIIts auditory response must lie farther downstream in the song motor system.

After HRP is injected into the HVc of female zebra finches, anterogradely labeled fibers can be seen leaving the ventro-caudal borders of the nucleus; however, this projection does not terminate in RA as do the fibers defined by HRP injections into the male HVc (Nottebohm et al., 1981). Using tritiated amino acids as tracers, Konishi and Akutagawa (1985) have also found that the axons of HVc neurons terminate before penetrating RA in adult female zebra finches. Attempts were made to record multiunit auditory responses in the RAs of three intact adult females. A total of seven penetrations that passed through RA failed to record responses to pure tones of 0.8 to 3.0 kHz. In two of the females electrodes were also placed in HVc and multiunit auditory responses to pure tones recorded;
the lack of response in RA cannot be attributed to anesthesia. In males, such pure tone stimuli elicit large responses in both RA and NXIIIts, which makes it unlikely that the lack of response was due to inadequate stimulus parameters. These results suggest that the absence of the NXIIIts auditory response in intact female zebra finches may reflect a failure of the auditory units in the HVC of females to excite neurons in RA.

The functional connectivity of HVC and RA was investigated by microstimulating the nuclei while recording from the tracheosyringeal nerve; electrode locations were subsequently verified by examining electrode tracks (RA) or by stimulating at a large number of sites in the area of the posterior-most auditory responses (HVC). In males, and in early E-treated females that show a NXIIIts auditory response, a single bipolar pulse of 30 μA or less elicits a large volley in the ipsilateral NXIIIts (Fig. 4a). A large number of different stimulus parameters were used in an attempt to elicit a response in NXIIIts by stimulating the HVC of female zebra finches: pulse lengths of 0.1 to 0.8 ms, monopolar and bipolar pulses, initially anodal or cathodal, trains of up to 100 stimuli delivered at intervals of 0.5 to 3 ms, currents of up to 100 μA; for each site, intensity of stimulation was gradually increased in order to preclude tissue damage at early stages of testing. None of these stimuli delivered to the HVCs of 12 intact adult females and 4 nonresponding E-treated females succeeded in evoking any activity in NXIIIts (Fig. 4b). However, short trains delivered to RA were effective in eliciting a volley in NXIIIts (Fig. 4c). These results fail to confirm Arnold’s (1980) report of activity in the syringeal muscles evoked by microstimulating the HVC of a female zebra finch, but coincide with Konishi and Akutagawa’s (1985) observation that HVC axons terminate outside RA in adult female zebra finches. Thus, HVC neurons in adult female zebra finches do not appear to make any anatomical or functional connection with the cells in RA which project to the tracheosyringeal motor nucleus. When the connection between HVC and RA is masculinized by early E treatment of females, the NXIIIts auditory response appears, presumably because auditory units in HVC now synapse upon and excite the RA cells which project to tracheosyringeal motor neurons.

DISCUSSION

The sexual dimorphism in the zebra finch song motor system occurs both as differences within nuclei (as reflected by the differences in volume) and as a difference in the connectivity between nuclei. Although these differences between the sexes are clearly related to the fact that females do not sing, the lack of a functional connection between the HVC and RA of females also excludes any possibility of auditory processing by neurons in either RA or the tracheosyringeal motor nucleus; males do have auditory responses in these more distal song centers.
In male zebra finches, neurons within different portions of the tracheoesyringeal motor nucleus respond selectively to different natural song syllables (Williams & Nottebohm, 1985). Information derived from this analysis of sound in the motor system is also transmitted back to the forebrain (Williams & Nottebohm, 1985). These findings suggest that male zebra finches might use the song motor system to process certain auditory signals in a manner analogous to that proposed by Liberman.
and his co-workers (Liberman, Cooper, Shankweiler, & Studdert-Kennedy, 1967; Liberman, 1982) as a mechanism for human speech processing. In the motor theory of speech (or song) perception, a listener processes an acoustic signal by running it through the neural system that produces vocalizations and thereby determining which motor commands would be necessary to produce the same sound. This theory implies that the encoding and decoding of species-specific sounds share at least some elements of neural circuitry. Such an overlap in brain areas for the production and perception of human speech has been described (Ojemann, 1983). In songbirds, HVC is known to have auditory responses (Katz & Gurney, 1981; McCasland & Konishi, 1981; Margoliash, 1983; and above) as well as to be important for the production of learned song (Nottebohm et al., 1976); recent results show that, in male zebra finches and canaries, auditory responses can be found in all the descending stations of the song motor system, including the syringeal motor neurons (Williams & Nottebohm, 1985).

Other species that use sound for communication may also use shared neural circuitry to encode and decode their auditory signals. When two Teleogryllus cricket species with dissimilar male calling songs are crossed, the hybrid males produce a hybrid song. In behavioral tests, hybrid females prefer the hybrid song to either parent species' male calling song, suggesting that the mechanisms for production and perception of cricket calling song may also be genetically linked (Hoy, 1974).

Teleogryllus oceaniaus males and females attend to different portions of the males' calling song: males respond to the "chirp" portion of the song, while females respond to the "trill" (Pollack, 1982). Evidence also exists to suggest that males and females of songbird species with sexually dimorphic singing behavior attend to different components of the males' song. Swamp sparrow males respond aggressively to synthetic songs made up of naturally occurring swamp sparrow syllables, although these may be presented in un-swamp-sparrow-like temporal patterns (Peters, Searcy, & Marler, 1980; Searcy, Balaban, Canaday, Clark, Runfeldt, & Williams, 1981). Female swamp sparrows, however, preferentially respond with a soliciting display to songs that are made up of swamp sparrow syllables arranged in the species-specific temporal pattern (Searcy, Marler, & Peters, 1981). Cowbirds also show sexual dimorphism in their responses to conspecific song. Female cowbirds drawn from one of the two eastern cowbird populations mate with males that sing the familiar dialect (King & West, 1983a); males, however, perceive both dialects as cowbird song, and they will learn to sing either variant, presumably in response to the females' preferences (King & West, 1983b).

Female zebra finches can discriminate among the songs of individual conspecific males (Miller, 1979a; 1979b); however, there have not yet been any studies of sexual differences in zebra finch song perception.
In the light of the sexual dimorphism of the auditory response in the thalamic motor neurons, such studies would be of great interest. In the two species for which the perception of song has been systematically studied in both sexes, females respond to a more rigidly defined set of song parameters. If the motor theory of song perception obtains, the sexual dimorphism in perception could be explained as a reflection of the sexual dimorphism of the auditory responses in the syringeal motor neurons: females, without the additional sound analysis provided by the motor neurons, would respond only to song stimuli defined by a very narrow set of rules—while males could perform a finer analysis on a wider range of stimuli by using the motor neurons as an aid in categorizing and discriminating sounds. If, as the most conservative explanation for the NXIIIs auditory responses would have it, the auditory responses within the song motor system are correlated only to the learning of song, then the lack of an NXIIIs auditory response should not affect song perception in females. However, in zebra finches, the song learning process is complete at 90 days (Immelman, 1969), but the NXIIIs auditory response persists in adult males over 2 years old (Williams and Nottebohm, 1985); these older males still hear and extract information from the songs of other male zebra finches. This fact argues against a role for the NXIIIs auditory responses that is limited solely to song learning. If the sexual dimorphism in the zebra finch NXIIIs auditory response reflects a difference in the circuitry used for auditory processing, a corresponding sexual dimorphism in the perception of the song signal may exist—as is suggested for crickets, swamp sparrows and cowbirds in the studies cited above. Female zebra finches, lacking the neural circuitry necessary for song learning, may also have given up the ability to extract some of the information that can potentially be encoded in conspecific male song.

REFERENCES


