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Compromised Neural Selectivity for Song in Birds with Impaired Sensorimotor Learning

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Summary

Anterior forebrain (AF) neurons become selective for song as songbirds learn to produce a copy of a memorized tutor song. We report that development of selectivity is compromised when birds are prevented from matching their output to the tutor song. Finches with denervated vocal organs developed stable song, but it usually did not resemble the tutor song. In those birds, numerous neurons in Area X responded selectively to both tutor and bird's own song (BOS), indicating the importance of both in shaping AF responses. The degree of selectivity for BOS was less, however, than that of normal adults. In contrast, neurons in denervated birds that successfully mimicked tutor song exhibited normal adult selectivity for BOS. Thus, during sensorimotor learning, selectivity for complex stimuli may be influenced by how well motor output matches internal sensory models.

Introduction

To learn to sing, songbirds depend on two types of auditory experience (Figure 1A) (Konishi, 1965; Price, 1979). First, during the sensory phase of learning, a young bird listens to and memorizes the song of its tutor; this memory is often called the template. Then, in the sensorimotor phase of learning, the juvenile uses auditory feedback from its own vocalizations to compare them to the tutor song template; it gradually refines its immature sounds until it produces a song that, although it has distinctive and individual features, resembles the tutor song. Thus, neural circuits mediating song learning must process auditory experiences both of the bird's own song (BOS) and of the tutor song to which BOS will be matched.

Neurons of the songbird anterior forebrain (AF) might process BOS and tutor song experiences during learning. The AF belongs to a system of nuclei dedicated to song learning and production (Figure 1B) and is essential for normal song learning (Bottjer et al., 1984; Sohrabji et al., 1990; Scharff and Nottebohm, 1991; Basham et al., 1996). Furthermore, the AF seems specialized for processing song. In adult birds, the AF contains auditory neurons that respond selectively to BOS, strongly preferring it to songs of other zebra finches (conspecific song) or to BOS played in reverse (Doupe and Konishi, 1991; Hessler and Doupe, 1999). This song selectivity is not present early in the bird's life: although AF neurons in 30-day-old birds are auditory, they are not selective, responding equally well to any song stimulus (Doupe, 1997). By the end of sensory learning and partway through sensorimotor learning (60 days), however, song selectivity is apparent in two nuclei of the AF, Area X (X) and the lateral portion of the magnocellular nucleus of the neostriatum (LMAN) (Solis and Doupe, 1997). Thus, selectivity emerges rapidly in juvenile birds and could function during vocal learning.

Song selectivity in adult AF neurons was originally identified using BOS, conspecific song, and reversed versions of BOS (Doupe, 1997), whereas the tutor's song was not used as a stimulus and often was not even known. In the studies of 60-day-old zebra finches, however, tutor songs were systematically used as test stimuli. This revealed that at this age, song selectivity is shaped by experience of both BOS and tutor song: many individual AF neurons respond equivalently to both of these stimuli, yet also show selectivity for BOS and tutor song relative to other song stimuli (Solis and Doupe, 1997). This "dual selectivity" is not simply due to the acoustic similarity between BOS and tutor, since such neurons are found even in juvenile birds that have been experimentally manipulated so that their songs do not resemble the tutor song (Figure 1C) (Solis and Doupe, 1999).

Selectivity of individual neurons for both BOS and tutor song could be useful during song learning. Selective neurons could provide feedback about the current state of BOS, store tutor song information, or compare BOS to tutor song during learning. Once song learning is complete, however, tutor song information may lose its importance. This could be indicated by a loss of tutor song responses in AF neurons from adult birds, leaving selectivity only for BOS. Thus, the dual selectivity for BOS and tutor song prevalent in 60-day-old birds might be lost as these birds mature to adulthood. Alternatively, tutor song responses may persist throughout life, perhaps providing a song reference for the bird. To investigate whether dual selectivity for BOS and tutor song in the AF is maintained into adulthood (>90 days of age), we examined the responses of AF neurons in adult birds that had been manipulated as young birds so that their songs did not resemble the tutor song. Furthermore, these adult birds persistently experience a state that normally is found only in juvenile birds and is presumably transient: their vocal output does not match their internal tutor song model. Thus, we were also able to ask whether neural properties such as song selectivity would be affected by this experimental disruption in song learning.

We have previously shown that denervating the syrinx, the avian vocal organ, in young birds prior to song onset (<30 days of age) reliably prevents birds from producing a copy of their tutor song at 60 days (Solis and Doupe, 1999). For the present study, we again performed bilateral transections of the tracheosyringeal portion of the hypoglossal nerve (NXIIts or ts; Figure 1) before song onset and found that, even in adulthood, most but not

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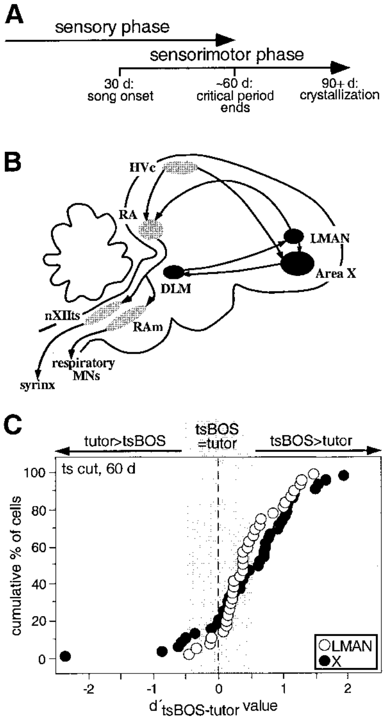

Figure 1. Song Development and Anatomy

(A) The two phases of song learning. For zebra finches, the sensory phase, in which the bird memorizes the tutor song, ends at ∼60 days, and the sensorimotor phase begins at ∼30 days, when juveniles begin to sing and match their vocalizations to the memorized tutor song. Song learning is complete at 90+ days. Note the overlap between the two song phases.

(B) Anatomy of the song system: motor pathway nuclei are gray, and AF nuclei are black. Area X is a specialized basal ganglia nucleus, and LMAN is its cortex-like outflow after a connection through the thalamus (DLM). The musculature of the avian vocal organ, the syrinx, was deafferented by transecting the neural outflow of the tracheosyringeal portion of the hypoglossal nucleus (nXIIts).

(C) Distribution of the preference of individual AF neurons for BOS versus tutor in 60-day-old birds that had been manipulated so that their song did not resemble the tutor song ("ts cut"): the discriminability between BOS and tutor is quantified with a $d'_{\text{tsBOS-tutor}}$ value for each neuron (Experimental Procedures). The cumulative percentile plot shows that the population of AF neurons has a range of tsBOS versus tutor song preferences, and many neurons responded equally well to both BOS and tutor song ($-0.5 < d'_{\text{tsBOS-tutor}} < 0.5$). LMAN cells are represented with open circles, and X cells with closed circles.

all "ts cut" birds produced songs that were unlike the tutor song. Both ts cut BOS ("tsBOS") and tutor song stimuli were then used to characterize AF neurons recorded extracellularly in adult, anesthetized ts cut zebra finches.

Results

Many Songs of ts Cut Adults Remained Acoustically Different from Tutor Song

The goal of the ts transections was to generate birds with songs very different from their tutors' songs, by deafferenting the musculature of their vocal organ, the syrinx. Since these transections do not interfere with the respiratory pathways involved in producing sound, ts cut birds were able to sing throughout song development. Due to the loss of control over their syringeal musculature, however, these ts cut adults had songs that were often extremely abnormal. For many birds, ts transections at or before 30 days of age resulted in adult song that consisted of simple syllables, which were either noisy, broadband signals or harmonically related notes. These ts cut adult songs did not obviously resemble their tutor song, either spectrally or temporally. For example, Figure 2A shows the tutor song of two birds that received ts transections as young birds. The resulting ts cut adult songs for each bird are shown in Figures 2B and 2C: neither resembled its tutor song. To judge the similarity between the ts cut songs and tutor song, human observers tried to match each ts cut song to its tutor song, which was present among six potential tutor songs; the number of correct matches indicates the degree of similarity between the two songs (see Experimental Procedures). The songs in Figures 2B and 2C were matched to their correct tutor song by 0/9 and 3/9 observers, respectively. Thus, despite the long time after nerve transection (258 and 255 days, respectively), these ts cut songs remained acoustically different from the tutor song; 12/17 birds were similarly affected.

In contrast, some songs from ts cut adults were highly similar to the tutor song. Of all birds receiving ts cuts as fledglings, 5/17 of them ultimately produced songs that were correctly matched to the tutor song with the same frequency as normal adult songs (7/9 or more observers correctly matched the two songs [≥78% correct]). For example, the ts cut adult song in Figure 2E shared many of the syllables found in its tutor song (Figure 2D), with the exception of a high, tonal note (circled). The absence of this note could reflect the weakened syringeal musculature, because notes with high fundamentals require strong contraction of these muscles (Vicario, 1991; Goller and Suthers, 1996a). For comparison, the song of a normal adult that had the same tutor is also shown in Figure 2F. The ts cut adult song in Figure 2E was correctly matched to its tutor song by 8/9 observers, whereas the normal adult song in Figure 2F was correctly matched by 9/9 observers.

The similarity between ts cut adult song and tutor song was compared to the similarity that normally arises between adult song and tutor song in a variety of ways, which confirmed that in 12/17 cases we had greatly decreased the usual similarity to tutor (Figure 3A). In contrast, the 5 ts cut birds with a high incidence of matching to the correct tutor song resembled tutor song to a degree similar to that found in normal adults (Figure 3A). The songs of normal adult birds are also highly stereotyped from rendition to rendition and are therefore called "crystallized" song. All ts cut adults had attained normal adult levels of stereotypy, even those birds with low similarity to tutor song (Figure 3B).

The ability of some ts cut birds to produce a song resembling their tutor song may have been due to partial reinnervation of the syrinx over time: there was a weak positive correlation between each ts cut bird's syrinx weight (which includes muscle mass and thus reflects the extent of reinnervation) and similarity to tutor song, as measured by the percentage of correct matches ($r^2 =$

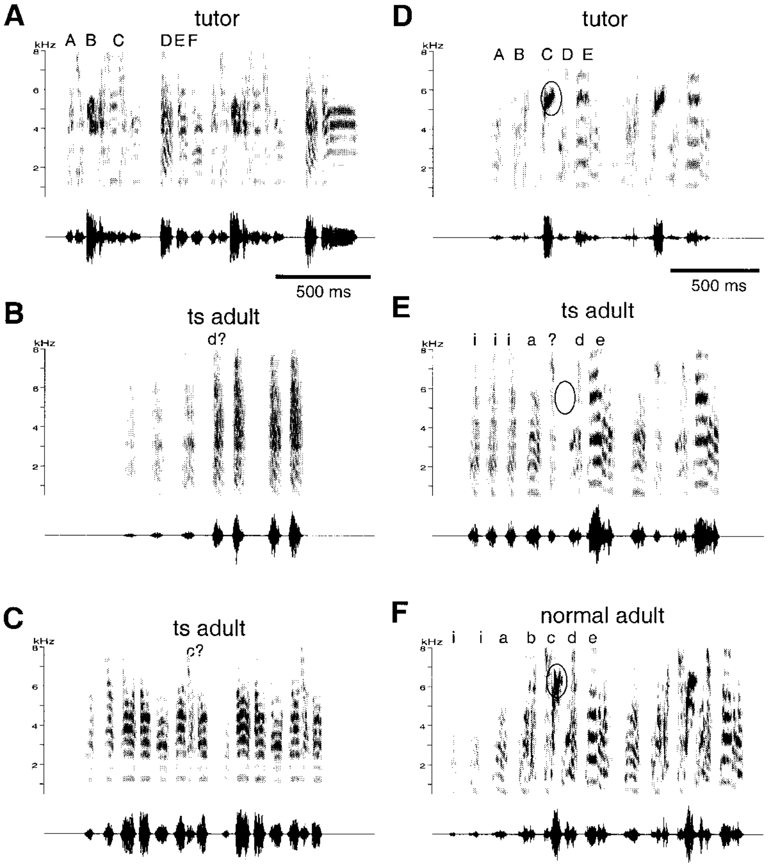

Figure 2. Long-Term Effects of Juvenile ts Transections on Adult Song

(A) The tutor song of two sibling ts cut adults is illustrated with a sonogram and oscillogram. Sonograms plot frequency versus time, and the intensity of each frequency band is indicated by its darkness; oscillograms show the sound waveform. Syllables are labeled with capital letters. This bird was the tutor for the birds whose songs are shown in (B) and (C).

(B) Adult song of a bird that received ts transections at ~30 days; this bird was exposed to the tutor in (A) during song learning. The labeled syllable ("d") bears a slight resemblance to syllable D in the tutor song, but overall the song was neither spectrally nor temporally similar to the tutor song.

(C) Song of another ts cut adult that had little similarity to its tutor song, shown in (A). One syllable ("c") was slightly similar to syllable (C) in the tutor song.

(D) The tutor song of the two birds whose songs are shown in (E) and (F). Syllables are labeled with capital letters; note the high tonal note in syllable C (circled).

(E) The song of a ts cut adult that developed high similarity with its tutor song (in [D]). Syllables resembling those found in the tutor song are labeled with lowercase letters; the high tonal note is missing (circle). Introductory notes are labeled with "i."

(F) The degree of similarity that normally develops between adult and tutor song is illustrated with the song of a normal adult bird that also had the tutor shown in (D). Syllables resembling those found in the tutor song are labeled with lowercase letters; the high tonal note is present (circle).

0.24, $p < 0.032$). Moreover, the average syrinx weight of ts cut adults with a high incidence of matching to tutor song was slightly greater than that of birds with a low incidence of matching, although this difference was not statistically significant. Overall, however, as expected from successful ts transections, the syrinx weights of both ts cut adults groups were significantly less than those of normal adults (Figure 3C).

AF Responses to tsBOS and Tutor Song

To reliably assess the separate contributions of tsBOS and tutor song experience to AF selectivity in ts cut adults, we first examined the responses of neurons recorded from those ts cut adults with songs acoustically different from their tutor song ($n = 12$). Extracellular recordings of 51 single units in X, the first nucleus in the AF pathway, revealed a range of tsBOS and tutor song preferences in these birds: whereas some neurons preferred tsBOS over tutor song, and a few preferred tutor song over tsBOS, many responded equivalently to these two songs, despite their acoustic differences (Figure 4A). For each neuron, the relative responses to tsBOS and tutor song were quantified with a measure of discriminability, d′ (see Experimental Procedures). Neurons were considered not to discriminate between the two stimuli if their d′ for BOS relative to tutor song fell in a range close to zero ($-0.5 < d'_{\text{tsBOS-tutor}} < 0.5$). The distribution of these values for all 51 X neurons recorded is shown in Figure 4B (open circles): 53% of these cells (27/51) had equivalent responses to tsBOS and tutor song (gray region in Figure 4B). For comparison, the individual $d'_{\text{tsBOS-tutor}}$ values from X cells recorded in ts cut juveniles at 60 days are also shown in Figure 4B (closed circles) ($n = 41$; data from Solis and Doupe, 1999); the average $d'_{\text{tsBOS-tutor}}$ value for ts cut adults was not significantly different from that for ts cut juveniles. Thus, in these ts cut adults with songs very different from their tutors, a population of individual X neurons that did not discriminate between tsBOS and tutor song was maintained, despite the maturity of the birds and their crystallization of song.

In contrast to X, in LMAN, a nucleus farther along the AF pathway, auditory responses were unusually rare in birds with long-term ts transections. Of the 8 ts cut adults in which LMAN was sampled, auditory data were obtained from only 2 animals (25%), in contrast to 6/9 normal adults (67%). Of the 54 LMAN neurons recorded in ts cut adults, only 3 yielded auditory responses (6%). This frequency of auditory neurons in LMAN was significantly less than that in normal adults, where auditory data were obtained from 15/31 LMAN cells sampled (48%) (χ^2 test, $p < 0.0001$). The nonauditory cells in LMAN appeared to be normal based on their spike shapes and spontaneous firing rates. The 8 birds that

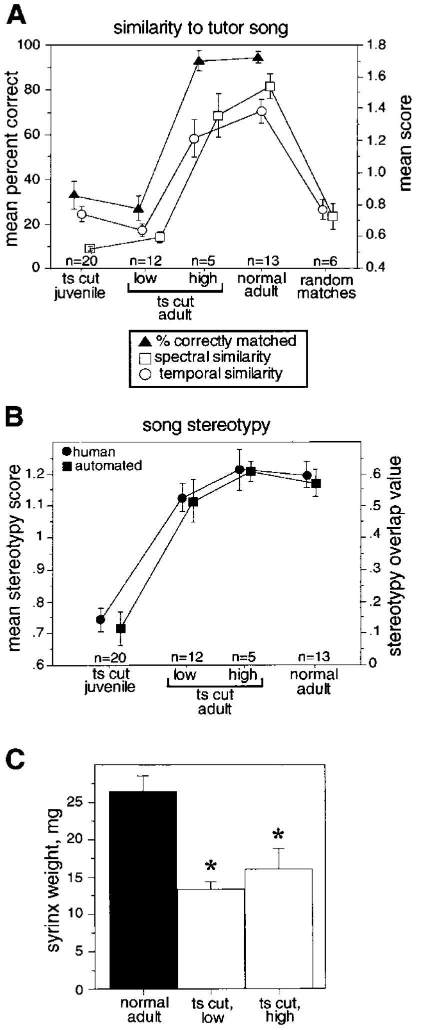

Figure 3. Song Analysis

(A) Measures of similarity to tutor song are shown for different bird groups. Black triangles show the mean percentage of observers that matched a song to the correct tutor song (left-hand ordinate). This value averages the frequency of matching across all songs in each song type. There is no percentage for random matches because for these songs, the correct tutor was not present among the choices. Adult ts cut songs were subdivided according to the incidence of correct matching to the tutor song ("low" or "high" similarity). For low similarity ts cut adult songs, the percentage of correct matches to tutor song was significantly less than that between normal adults and their tutor songs (unpaired t test, $p < 0.0001$). In addition, there was no significant increase in frequency of correct matches to tutor song relative to that found in songs from 60-day-old juveniles that had also received transections at 30 days (data from Solis and Doupe, 1999). The mean spectral (open squares) and temporal (open circles) similarity scores for each song pair are plotted along the right-hand ordinate. For ts cut adult songs with low incidence of matching, mean similarity scores were significantly lower than those for normal adult songs (unpaired t tests, $p < 0.0001$ for both spectral and temporal similarity scores). Relative to ts cut juveniles, the spectral similarity to tutor song in these ts cut adults was slightly increased (unpaired t test, $p < 0.031$); however, there was no significant difference in temporal similarity scores. Furthermore, these ts cut adult songs had similarity scores similar to those given to randomly matched songs, indicating the lack of resemblance to tutor song. The 5 ts cut birds with a high incidence of matching to tutor song showed no significant differences in the percentage of correct matches, spectral similarity, or temporal similarity between their ts cut adult songs and normal adult songs. Also, their similarity to tutor song was significantly greater than that of ts cut adults with low incidence of matching (unpaired t tests, $p < 0.0001$ for all three measures of similarity). Error bars are SEM.

(B) Measures of song stereotypy are shown for different bird groups. Closed circles show the human scores of stereotypy; closed squares show the automated overlap values of stereotypy (see Experimental Procedures); error bars are SEM. For both human and automated measures of stereotypy, ts cut adult song with low incidence of matching was not significantly different from normal adult song. In addition, these ts cut adult songs were significantly more stereotyped than ts cut 60-day juvenile song (unpaired t test, $p < 0.0001$ for both human scores and automated measures). The ts cut birds with a high incidence of matching also had normal adult stereotypy.

(C) A histogram compares the average syrinx weights from different bird groups. Asterisks indicate significant differences from the average syrinx weight obtained from normal adults (unpaired t tests, $p < 0.0001$ for ts cut adults with low similarity and $p < 0.019$ for ts cut adults with high similarity).

yielded only 3 auditory LMAN neurons were not completely devoid of auditory responses, however, as indicated by the 34 auditory X neurons also recorded in these animals. The difference in the frequency of auditory LMAN cells relative to X cells was significant between ts cut and normal adults (15 LMAN and 13 X cells obtained in normal adults) (χ^2 test, $p < 0.0001$). In contrast to ts cut adults, auditory LMAN neurons were readily recorded in ts cut juveniles (Figure 4C). Thus, the decreased auditory responsiveness in LMAN of ts cut adults might be a long-term effect of ts transections.

The three LMAN cells obtained responded more to tsBOS than to tutor song (Figure 4C, open circles). The mean $d'_{\text{tsBOS-tutor}}$ value calculated from the three LMAN neurons from ts cut adults was also significantly greater than that from ts cut juveniles (compare to closed circles, Figure 4C; unpaired t test, $p < 0.029$). Although this shift toward tsBOS tuning supports the idea that in LMAN, tsBOS responses overwrite tutor song responses in adulthood, the small sample of LMAN neurons precludes any clear interpretation.

Neurons with Similar Responses to tsBOS and Tutor Song Were Selective

X neurons with equivalent responses to tsBOS and tutor song clearly exhibited selectivity, as assayed by their responses to other song stimuli. Unlike AF neurons in 30-day-old birds, which respond equally to all song stimuli (Doupe, 1997), the responses of neurons in these ts cut adult birds discriminated between stimuli. For example, the cell shown in Figure 5A responded strongly to both tsBOS and tutor song, despite the marked acoustic differences between these stimuli. Yet, it did not respond well to either reverse tsBOS or reverse tutor song, in which both the temporal structure of notes within a syllable as well as the sequence of syllable order within the song have been reversed. Furthermore, this X neuron did not respond to two different conspecific songs. Thus, the ability of this cell to discriminate tsBOS and tutor song from other song stimuli indicates that the cell was indeed selective. Such selectivity was a common feature of X neurons with equal responses to tsBOS and tutor song. Neurons were categorized as selective if they had a d' value that was ≥ 0.5 for at least one of

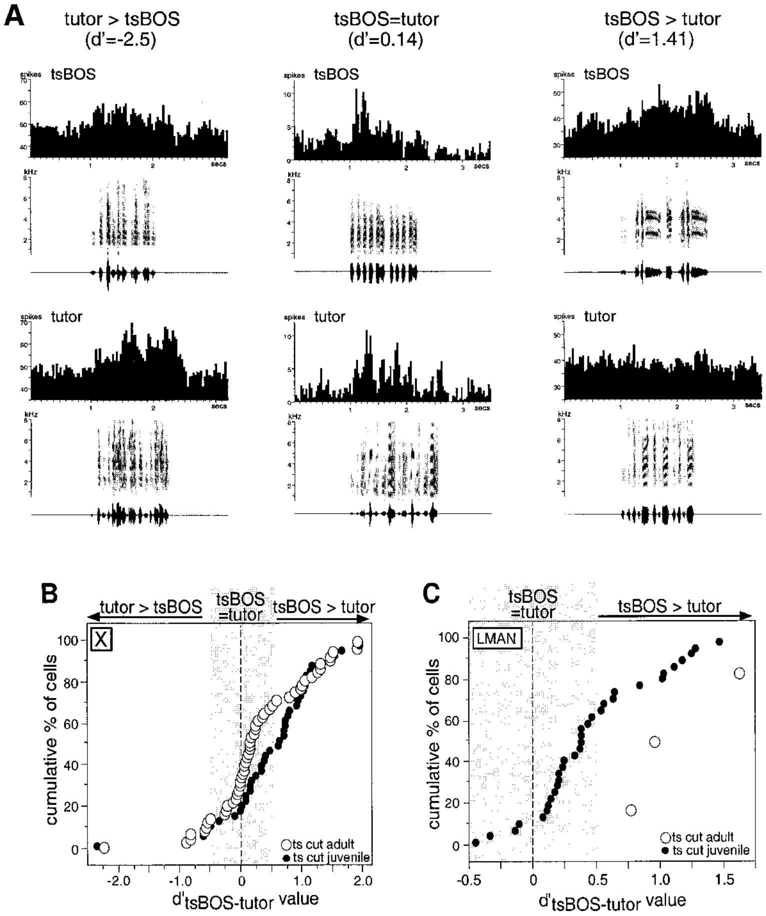

Figure 4. Preferences for tsBOS versus Tutor Song Recorded in X and LMAN Neurons from ts Cut Adults

(A) Peristimulus time histograms (PSTHs) show the responses to tsBOS and tutor song of three different X neurons. The left-hand pair shows the cumulative responses of a neuron that preferred tutor song over tsBOS; 20 trials of each song were presented, and the ordinate of the PSTH begins at 35 spikes/s. The middle pair shows the responses of a neuron that responded equally well to tsBOS and tutor song; 15 trials of each song are shown. The right-hand pair shows the responses of a neuron that preferred tsBOS over tutor song; 15 trials of each stimulus are shown, and the ordinate of the PSTH begins at 25 spikes/s.

(B) The cumulative distribution of tsBOS versus tutor song preferences for all X neurons recorded, as quantified with $d'_{tsBOS\text{-}tutor}$ values, is shown with open circles. For comparison, the distribution of $d'_{tsBOS\text{-}tutor}$ values from ts cut juvenile birds (60 days) is shown with closed circles. Gray shading highlights those cells considered to respond equally well to tsBOS and tutor song.

(C) The cumulative distribution of $d'_{tsBOS\text{-}tutor}$ values for the three LMAN neurons recorded in ts cut adults is shown with open circles. For comparison, the same distribution is shown for ts cut juveniles with closed circles.

four selectivity categories: tsBOS-reverse, tutor-reverse, tsBOS-conspecific, and tutor-conspecific. Classified in this way, 72% of neurons with equivalent responses to tsBOS and tutor song were selective. This percentage was similar to that found in normal and ts cut 60-day-old birds (68% and 66%, respectively; data from Solis and Doupe, 1997, and Solis and Doupe, 1999). Thus, when based on d′ measures of selectivity, a majority of X neurons with similar responses to tsBOS and tutor song were selective.

This selectivity was also confirmed in a more rigorous analysis based only on selectivity for tutor song. Because 30-day-old birds have not yet developed their own song, the selectivity of their neurons was characterized relative to their responses to tutor song only (Doupe, 1997). Thus, to compare neural selectivity from ts cut adults to that of 30-day-old birds, the X neurons from ts cut adults were reclassified as selective based only on their $d'_{tutor\text{-}con}$ or $d'_{tutor\text{-}reverse}$ values. Assessed this way, the prevalence of selective neurons in ts cut adults was still significantly greater than that in 30-day-old birds (data from Doupe, 1997; compare 58% in ts cut adults to 21% in 30-day-old juveniles, χ^2 test, $p < 0.002$). Furthermore, population measures of neural responses also indicated that X neurons from ts cut adults with similar responses to tsBOS and tutor song were selective: on average, they responded significantly more to tutor song than to reverse tutor or conspecific song (Figure 5B). This selectivity distinguishes X neurons with similar responses to tsBOS and tutor song in ts cut adults from the unselective neurons found in 30-day-old juveniles.

Neural Responses to tsBOS and Tutor Song Were Not Related to Song Properties

Although the neurons examined here came from birds whose songs were unlike their tutor song, it was theoretically possible that residual acoustic similarities between these songs produced the equivalent neural responses to tsBOS and tutor song. If this was true, then neurons with equivalent responses to tsBOS and tutor song should have come from birds with tsBOS acoustically similar to tutor song. Furthermore, neurons with strong preferences for either tsBOS or tutor song ought to have come from birds with tsBOS having little similarity to tutor song. This "similarity hypothesis" predicts a negative correlation between the absolute value of $d'_{tsBOS\text{-}tutor}$ for each bird and the acoustic similarity between tsBOS and tutor song (dotted line, Figure 6A). Such a correlation was not evident in our data, however, when, for each ts cut adult, we compared the mean $|d'_{tsBOS\text{-}tutor}|$ value of the X neurons to the percent of correct matches between its song and tutor song (Figure 6A) ($r^2 = 0.02$). Correlations were similarly low when other measures of similarity with tutor song were used (data not shown) ($r^2 = 0.03$ for spectral similarity scores, and $r^2 = 0.01$ for temporal similarity scores). Thus, the neural responses

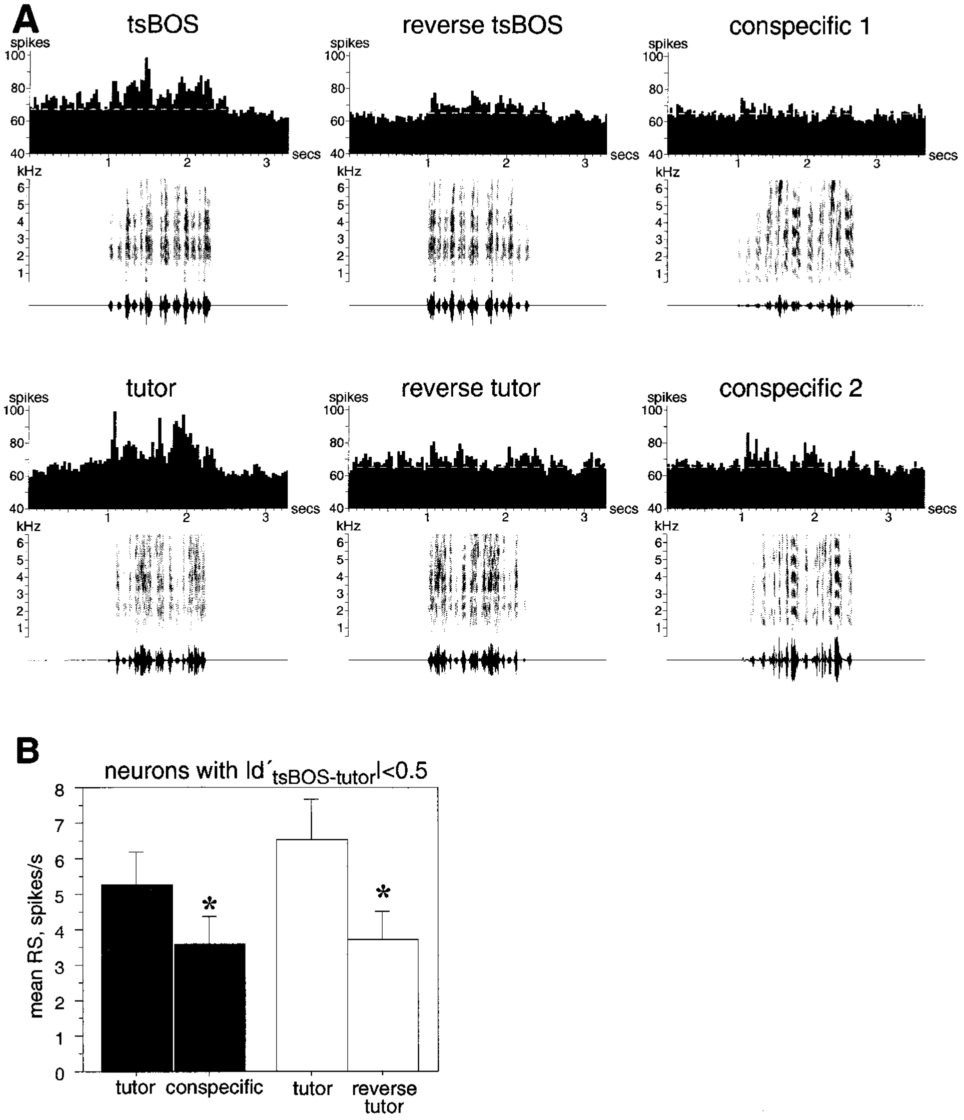

Figure 5. Neurons with Equivalent Responses to tsBOS and Tutor Song Are Also Selective

(A) PSTHs show the responses of a single X neuron to 20 presentations of each song. While this neuron responded equally well to tsBOS and tutor song ($d'_{\text{tsBOS-tutor}} = -0.05$), it did not respond well to either reverse stimulus ($d'_{\text{tsBOS-reverse}} = 1.14$ and $d'_{\text{tutor-reverse}} = 1.64$) or to two different conspecific songs ($d'_{\text{tsBOS-con1}} = 1.69$, $d'_{\text{tsBOS-con2}} = 1.45$, $d'_{\text{tutor-con}}1 = 2.52$, $d'_{\text{tutor-con}}2 = 2.00$). The white dashed line indicates the neuron's average spontaneous firing rate. Note that the ordinate of the PSTH begins at 40 spikes/s. This particular tsBOS was correctly matched to the tutor by only 2/9 observers.

(B) For those neurons considered to respond equally well to tsBOS and tutor song ($-0.5 < d'_{\text{tsBOS-tutor}} < 0.5$), histograms show paired comparisons of the mean RS to tutor song and conspecific (closed bars) or to tutor song and reverse tutor song (open bars). Error bars are SEM, and asterisks indicate significant differences (paired t tests: $p < 0.010$ for tutor–reverse comparisons, $n = 16$; $p < 0.050$ for tutor–conspecific comparisons, $n = 23$).

to tsBOS and tutor song were not accounted for by acoustic similarity between these two song stimuli.

The relative responses to tsBOS and tutor song of individual X cells also did not depend on the song maturity of a bird, as estimated by age and song stereotypy. The age of each ts cut adult did not correlate with the mean $d'_{\text{tsBOS-tutor}}$ value obtained from X cells in each bird ($r^2 = 0.01$; data not shown). Furthermore, there was no strong correlation between mean $d'_{\text{tsBOS-tutor}}$ values obtained from each bird and its song stereotypy, as measured by human scoring (Figure 6B; $r^2 = 0.02$) or by an automated measure (data not shown; $r^2 = 0.03$) (see Experimental Procedures). Thus, the song maturity of a bird did not predict its X neuron preferences for tsBOS or tutor song.

Degree of Selectivity in ts Cut Adults Was Less than that for Normal Adults

Although X neurons in ts cut adults with low similarity to the tutor song were selective, they did not exhibit the high levels of selectivity characteristic of normal adults. This was apparent when the average selectivity of all X neurons ($n = 51$), regardless of their tsBOS versus tutor song preference, was compared to that obtained from

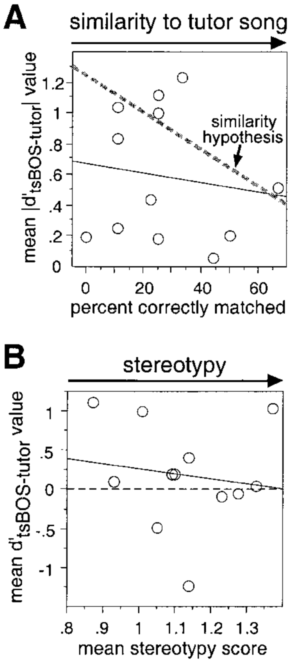

Figure 6. Comparisons between Song Quality and Neural Preferences for tsBOS over Tutor Song

(A) If residual acoustic similarity between tsBOS and tutor song accounts for the neural responses to tsBOS and tutor song, then the trend shown with the gray dashed line is expected ("similarity hypothesis"): equivalent responses to tsBOS and tutor song would occur when tsBOS and tutor song are similar, and strong preferences for one song or the other (quantified by the absolute value of $d'_{\text{tsBOS-tutor}}$) would result when tsBOS and tutor song are dissimilar. When the mean $|d'_{\text{tsBOS-tutor}}|$ values obtained for each bird are plotted against the percent correctly matched, the resulting linear least-squares fit of the data (black line) does not approximate the trend predicted by the similarity hypothesis.

(B) The human score of song stereotypy for each ts cut adult bird is compared to its mean $d'_{\text{tsBOS-tutor}}$ value obtained from the responses of X neurons in each bird. The black line is the linear least-squares fit of the data.

34 X neurons recorded from 13 normal adults (Figure 7A). The ts cut adult selectivity was significantly less than that from normal birds for all comparisons except for BOS responsiveness relative to tutor song. Furthermore, X neurons from these ts cut adults had not significantly increased their selectivity relative to that measured for 61 neurons from ts cut juveniles (open circles in Figure 7A; data from Solis and Doupe, 1999). The lower selectivity relative to normal adults was due at least in part to significantly greater than normal adult responses to nonpreferred stimuli, such as conspecific and reverse songs, as well as a trend for decreased responses to tsBOS and tutor song (Figure 7C). Thus, although these ts cut adults had crystallized song, the selectivity of their X neurons was compromised.

Although the degree of their selectivity for tsBOS and tutor song was lower than normal, the entire population of X neurons in these ts cut adults clearly exhibited selectivity, like the subset of neurons with equivalent responses to tsBOS and tutor song. The average $d'_{\text{tutor-reverse}}$ and $d'_{\text{tutor-con}}$ values from all X neurons were significantly greater in ts cut adults than in 30-day-old birds ($n = 51$;

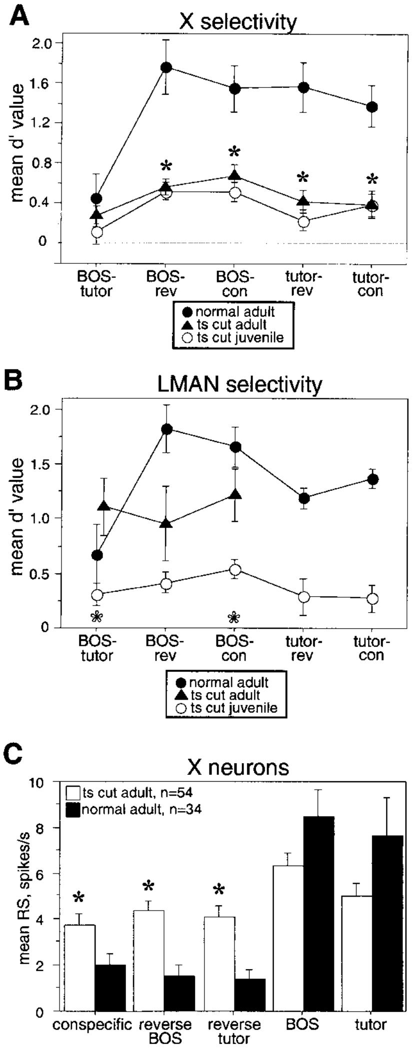

Figure 7. AF Selectivity of ts Cut Adults Relative to that Found in Normal Adults

(A) The degree of selectivity of X neurons from ts cut adults (closed triangles) is compared to that from ts cut juveniles (open circles) and normal adults (closed circles). The mean d′ values for each selectivity category (listed on the abscissa) are plotted along the ordinate; error bars are SEM. Lines connect the mean d′ values obtained for each bird group. Asterisks indicate significant differences in selectivity between ts cut adult and normal adult X neurons (unpaired t tests, for $d'_{\text{BOS-tutor}}$ $p = 0.456$, for $d'_{\text{BOS-reverse}}$ $p < 0.0001$, for $d'_{\text{BOS-con}}$ $p < 0.001$, for $d'_{\text{tutor-reverse}}$ $p < 0.0001$, for $d'_{\text{tutor-con}}$ $p < 0.0002$).

(B) The average selectivity obtained from the three LMAN neurons recorded in ts cut adults (closed triangles) is compared to average selectivity measured for ts cut juveniles (open circles) and normal adults (closed circles); lines connect the d′ values obtained from each neuron in ts cut adults. Error bars are SEM, and asterisks denote the selectivity categories for which there were significant differences between the mean d′ values of ts cut adult and ts cut juveniles (data from Solis and Doupe, 1999; $n = 52$ neurons; unpaired t tests, $p < 0.028$ for $d'_{\text{tsBOS-tutor}}$ and $p < 0.036$ for $d'_{\text{tsBOS-con}}$).

(C) A histogram compares the mean RS to different stimuli measured from X neurons in ts cut adults (open bars) and normal adults (closed bars); error bars are SEM. X neurons from ts cut adults had significantly greater response strengths to these stimuli than normal adults; asterisks indicate significant differences between ts cut and normal responses (unpaired t tests, $p < 0.010$ for conspecific, $p < 0.0001$ for reverse BOS, and $p < 0.0003$ for reverse tutor).

30-day data from Doupe, 1997) (unpaired t tests, $p < 0.0004$ for $d'_{\text{tutor-reverse}}$ and $p < 0.022$ for $d'_{\text{tutor-con}}$). Furthermore, although ts cut songs tend to be simpler than normal songs (Figure 2), X neurons from ts cut adults could make the finer discrimination between their own ts cut song and the songs of other ts cut conspecifics, responding significantly more to tsBOS than to ts cut conspecific songs (paired t test, $p < 0.0001$; $n = 20$). Thus, the unusually low selectivity of X neurons from ts cut adults did not indicate that these neurons were insensitive to differences between song stimuli. Rather, these neurons had an intermediate level of selectivity, similar to that observed for 60-day-old juveniles.

The lower selectivity of ts cut adults relative to normal adults could not be accounted for by age or trophic effects of ts transections. If selectivity increases with age, even in adulthood, this could contribute to the difference in X selectivity found between ts cut and normal adult birds. Although these ts cut adults were slightly younger than the normal adults that we tested (unpaired t test, $p < 0.004$), no positive correlation resulted between age and any measure of selectivity within ts cut adult or normal adult data. Thus, we did not find evidence for selectivity increasing with age in adulthood. Alternatively, trophic effects on upstream neurons could have been induced by ts transections, potentially leading to lower selectivity in ts cut adults. X cells from ts cut birds appeared to be normal, however, as their spontaneous firing rates were not significantly different from those in normal adults.

Given the low prevalence of auditory LMAN neurons from ts cut adults ($n = 3$), it was difficult to make conclusive comparisons of their selectivity relative to other bird groups. The mean d' values obtained from the three LMAN neurons are shown in Figure 7B. On average, LMAN selectivity in ts cut adults was not significantly different from that found in normal adults (Figure 7B; $n = 15$ LMAN neurons; no ts cut adult data were obtained for tutor song categories of selectivity). Furthermore, LMAN neurons from ts cut adults were more selective for BOS relative to tutor and conspecific song than those in ts cut juveniles, although not for comparisons of BOS to reversed song. Thus, the average selectivity obtained from 3 LMAN neurons in ts cut adults approximated normal adult levels; however, the low sample size undermines the significance of this greater selectivity relative to that found in ts cut juveniles.

Potential Role for Matching the Tutor Song in Development of Selectivity

In manipulating birds so that their songs did not resemble the tutor song, we actually interfered with the normal matching process inherent to song learning. The inability of a ts cut bird to match its song to the tutor song template might have influenced neural properties such as selectivity or auditory responsiveness. To investigate this possibility, we examined the selectivity of AF neurons recorded from the 5 ts cut adults that ultimately produced fairly accurate copies of tutor song. Although these birds showed high similarity to tutor song, they had clearly experienced effects of ts transections, as indicated by their low syrinx weight (Figure 3). In contrast to birds with low similarity to the tutor, however, X neurons recorded from these ts cut adults ($n = 14$ neurons) exhibited normal levels of adult selectivity for some selectivity categories (Figure 8A). For BOS comparisons of selectivity, average d' values from ts cut adults with high similarity to tutor song were not significantly different from those obtained from normal adults. Furthermore, these X neurons were significantly more selective for BOS relative to reverse or conspecific song than were ts cut birds with low similarity to tutor song (unpaired t tests, $p < 0.0001$ for $d'_{\text{tsBOS-reverse}}$, and $p < 0.001$ for $d'_{\text{tsBOS-con}}$). In contrast, selectivity for tutor song remained lower than that found in normal adults (unpaired t tests, $p < 0.007$ for $d'_{\text{tutor-reverse}}$ and $p < 0.004$ for $d'_{\text{tutor-con}}$ comparisons) and not significantly different from the levels found in the ts cut birds with low similarity to the tutor song. Thus, the normal adult level of BOS selectivity apparent for X neurons from those ts cut adults with high similarity to tutor song is consistent with the idea that some selectivity development depends on matching between BOS and the tutor song template.

If a mismatch between BOS and tutor song somehow compromises selectivity, then neural selectivity might increase as similarity to tutor song increases. We examined this by comparing each ts cut adult's degree of similarity to tutor song to the mean d' value obtained from its X neurons for each selectivity category (ts cut adults with both low and high incidence of matching to tutor song were included). The strongest correlations occurred between $d'_{\text{tsBOS-reverse}}$ values and song similarity (Figure 8B) ($p < 0.047$ for percent correctly matched, $p < 0.029$ for spectral similarity, $p < 0.007$ for temporal similarity). This trend is consistent with the hypothesis that selectivity increases with accuracy of matching, although the correlations were considerably weaker for other measures of selectivity (for $d'_{\text{tsBOS-con}}$, $d'_{\text{tsBOS-tutor}}$, $d'_{\text{tutor-con}}$, and $d'_{\text{tutor-rev}}$, r^2 values ranged from 0 to 0.10).

Auditory responses of LMAN neurons were also more readily recorded from ts cut birds with high similarity to their tutor song than from birds with low tutor similarity. Three out of these 5 ts cut adults that mimicked tutor song yielded auditory data from LMAN; this percentage of birds (60%) was similar to that in normal adults (67%) and much greater than that obtained for ts cut birds with low similarity to tutor song (25%). In these ts cut adults with high similarity, auditory responses were obtained from 5/19 LMAN neurons sampled. This frequency of auditory neurons was not significantly different from that in normal adults and was significantly greater than for ts cut birds with low similarity to tutor song (χ^2 test, $p < 0.013$). On average, LMAN neurons from these ts cut adult birds exhibited selectivity that was not significantly different from that in normal adult birds (Figure 8C). This selectivity was also not significantly increased relative to ts cut adults with low similarity to tutor song; however, the small sample sizes in both groups limit this comparison. Nonetheless, the greater prevalence of auditory responses in LMAN of these ts cut birds may have been related to their ability to eventually match their vocalizations to the tutor song template.

Discussion

This study addresses the separate contributions of BOS and tutor song experience to AF selectivity in ts cut

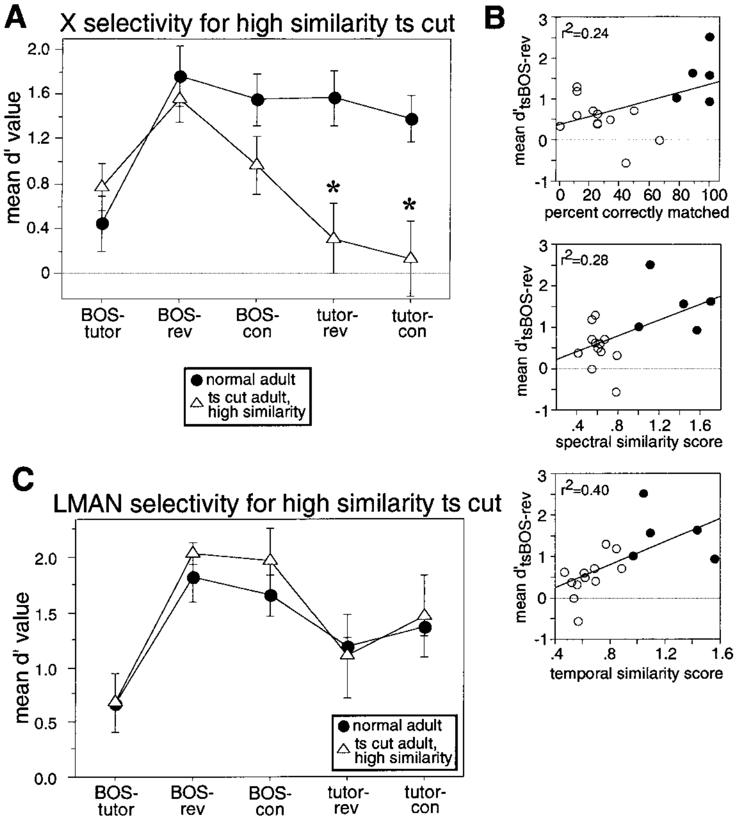

Figure 8. Selectivity of ts Cut Adults with High Similarity to the Tutor Song

(A) The degree of selectivity of X neurons from ts cut adults with high similarity to the tutor song (open triangles) is compared to that measured for normal adults (closed circles). The mean d′ values for each selectivity category (listed on the abscissa) are plotted along the ordinate; lines connect mean d′ values obtained for each bird group. Error bars are SEM, and asterisks denote significant differences between these ts cut adults and normal adults.

(B) Scatter plots compare the mean $d'_{\mathrm{tsBOS\text{-}reverse}}$ values for X neurons from each ts cut bird to the acoustic similarity measured between its tsBOS and tutor song, using percent correctly matched, mean spectral similarity scores, and mean temporal similarity scores. Open and closed circles mark ts cut adults with low and high similarity to tutor song, respectively. Lines are the linear least-squares fit through the data, and r^2 is the coefficient of determination.

(C) The degree of selectivity of LMAN neurons from ts cut adults with high similarity to the tutor song (open triangles) is compared to that measured for LMAN neurons from normal adults (closed circles). Conventions are as in (A).

adults, and the possible consequences of an inability to match BOS to tutor song. In ts cut adults with songs acoustically different from the tutor song, many X neurons continued to respond equally well to tsBOS and tutor song. Thus, the dual selectivity apparent in ts cut juveniles persisted in these ts cut adults. The degree of their X selectivity for BOS and tutor song over other stimuli was considerably less than that in normal adults, however. Furthermore, auditory responses in LMAN of these ts cut adults were unusually rare. In contrast, in ts cut birds with songs resembling the tutor, X neurons developed the normal high selectivity for BOS relative to other stimuli, and LMAN was normally responsive. These results suggest that long-term ts transections can lead to altered neural properties in the AF, perhaps through the chronic mismatch that this manipulation induces between BOS and the tutor song template.

Stereotypy and Similarity to Tutor Song of ts Cut Adult Songs

Adults receiving ts transections as juveniles attained adult levels of song stereotypy, despite the abnormality of their songs. This result indicates that, although stereotypy and song similarity to the tutor song covary during song development in normal birds (Scharff and Nottebohm, 1991; Solis and Doupe, 1997), development of a stable, stereotyped song does not depend on matching to the tutor song. This has also been shown for isolate birds, which develop stereotyped, though abnormal, song in the absence of a tutor song model (Price, 1979; Morrison and Nottebohm, 1993).

Despite ts transections as young birds, some birds produced songs that clearly resembled the tutor song. Although these birds tended to have more reinnervation of the syrinx than the other ts cut birds, their overall syrinx weight was substantially less than that of normal adults. Thus, the ability of some of these ts cut birds to mimic tutor song serves as a reminder that song acquisition does not rely exclusively on an intact syrinx. Temporal similarity can also be accomplished through patterned contractions of respiratory muscles, whose innervation is not altered in the ts cut manipulation (Hartley and Suthers, 1989; Vicario, 1991; Goller and Suthers, 1996b; Wild, 1997). Furthermore, it is remarkable that some birds were ultimately able to produce song similar to the tutor despite their loss of syrinx control early in sensorimotor learning. It would be informative to know whether these birds experienced relatively quick reinnervation, such that they mimicked tutor song with a normal time course, or whether they produced a copy of the tutor song later than normal. Detailed studies of ts cut song development could address whether there are critical periods for sensorimotor learning, as has been previously suggested (Pytte and Suthers, 1996, Soc. Neurosci., abstract).

The Song Preferences of Neurons Recorded from ts Cut Adults

Adult birds with low similarity of BOS to tutor song allowed us to test whether neurons develop a strong preference for their own song over tutor song once song learning is complete. X neurons from these ts cut adults exhibited a range of tsBOS versus tutor song preferences that was similar to that found in 60-day-old birds:

some neurons preferred tsBOS over tutor song, a few neurons preferred tutor song over tsBOS, and many neurons responded equally well to tsBOS and tutor song. Thus, the dual selectivity for tsBOS and tutor song found at 60 days persisted in ts cut adults, and there was no "overwriting" of tutor song selectivity. This result was unexpected, given the strong selectivity for BOS previously observed for song system neurons in adult birds (Margoliash, 1983; Margoliash and Fortune, 1992; Doupe, 1997) and how rapidly this BOS selectivity emerges once birds begin to sing (Volman, 1993; Solis and Doupe, 1997, 1999). Furthermore, it is remarkable that single neurons are selective for both tsBOS and tutor song, because they are such complex learned vocalizations. Single neurons with receptive fields for two different complex stimuli have rarely been observed. In bat auditory cortex, neurons are tuned to two different interval durations within echolocation or communication calls (Ohlemiller et al., 1996), and some inferotemporal visual cortical neurons respond equally to pairs of intricate fractal patterns (Miyashita, 1988). To further understand dual selectivity for BOS and tutor song, it will be important to determine the cues in each song stimulus that drive neural responses.

Neurons selective for tsBOS and/or tutor song could serve as a reference that the song system of adult birds uses to continuously evaluate BOS. Such a reference has been demonstrated behaviorally: when adult zebra finches receive altered auditory feedback of their song, they will change their songs, and when auditory feedback returns to normal, they will gradually return to their original song (Leonardo and Konishi, 1999); this plasticity may require the AF (Brainard and Doupe, 1997, Soc. Neurosci., abstract). Moreover, individual neurons with dual selectivity for both tsBOS and tutor song could compare the two songs during and after completion of sensorimotor learning. In one potential scheme, auditory feedback of the bird's own vocalizations evokes coincident activity from BOS- and tutor-tuned inputs when the bird produces a good copy of the tutor song, leading to strong activation of dually selective neurons.

Alternatively, the maintenance of neural selectivity for both tsBOS and tutor song may indicate that neurons in ts cut adults remain in a juvenile state, poised to participate further in song learning. This is also supported by the low selectivity of X neurons found in ts cut adults with songs different from the tutor. By this hypothesis, dual selectivity for BOS and tutor song represents a normal transition state as neurons shift their tuning from one song to another during the course of song learning. A similar transition state has been characterized in the barn owl optic tectum: as neurons alter their tuning from one interaural time difference (ITD) to another, they pass through a phase in which they respond equivalently to both ITDs (Brainard and Knudsen, 1995). In ts cut birds in whom successful matching of BOS to tutor was never accomplished, dual selectivity may indicate that such a shift was not completed. In normal adult birds, BOS might come to predominate, but the high acoustic similarity between normal birds' songs and tutor song obscures the individual contributions of each of these stimuli to neural selectivity. Although our ts cut adult birds minimize the acoustic similarity issue, their compromised neural selectivity makes it unclear whether the dual selectivity for tsBOS and tutor song found in ts cut adults would also be true for normal adults.

If the song-selective neurons seen here in anesthetized birds function in song learning, they should be apparent in awake birds. Consistent with this, song-selective neurons have been observed in the AF of awake birds (Hessler and Doupe, 1999), as well as in HVc (McCasland and Konishi, 1981; Dave et al., 1998), the source of inputs to the AF. Recent experiments, however, have also described a state dependence of auditory responses in some nuclei of the song system (Dave et al., 1998; Schmidt and Konishi, 1998), suggesting that the presence of auditory responses may be "gated" by behavioral state. Because the auditory feedback most relevant to song learning occurs when the bird is actually singing, sensory feedback of song may be gated by motor behavior, as it is in many sensorimotor systems (reviewed by Pearson, 1993; Nelson, 1996). The effect of anesthesia may be to remove this gate, allowing observation of auditory responses to song, even without simultaneous motor activity.

Abnormally Low Selectivity in ts Cut Adults

Although songs of ts cut adults had become substantially more stereotyped than songs from ts cut juveniles, there was no parallel increase in selectivity of X neurons from ts cut adults. These neurons were significantly less selective than X neurons in normal adults. These neurons in ts cut adults were not unselective, however: they had selectivity similar to that found in ts cut and normal 60-day-old neurons, which reliably discriminate between different song stimuli.

The chronic mismatch between what a ts cut bird sings and its tutor song template may have compromised AF selectivity development. Consistent with this idea, those ts cut adults with high similarity to the tutor song developed normal adult levels of BOS selectivity, unlike the ts cut adults with low tutor similarity. Also, the selectivity for tsBOS relative to reverse song increased as similarity increased between ts cut adult song and the tutor song. Correlations between selectivity and similarity may not have been stronger, however, because there was not a continuous gradient of similarity among ts cut birds (i.e., their songs were either clearly like the tutor song or not at all like the tutor song); alternatively, it is possible that selectivity itself does not increase gradually but rather in steps. Another study has suggested a relation between song matching and selectivity: LMAN selectivity is not apparent in adult birds raised in isolation that have nonetheless developed stereotyped songs (Maekawa, 1998, Soc. Neurosci., abstract). Isolates are similar to ts cut birds in that they have not been able to match their songs to a tutor song template; however, unlike ts cut birds, this condition was brought about by raising these birds without tutor song experience. The association of song selectivity with the quality of learning rather than with the maturity of a bird suggests that this neural property is related to learning, rather than simply to development, and provides an insight into the potential function of these neurons.

Theoretically, the lower selectivity apparent in these ts cut adults could be the result of tuning to the simpler structures within tsBOS stimuli. For example, a neuron that is sharply tuned to a harmonic stack would likely respond equally well to a reverse version of the stack, which is highly similar to the forward version. We found, however, that X neurons in ts cut adults with low similarity to tutor song could discriminate tsBOS from other ts cut conspecific stimuli; this indicates that these neurons were sensitive to some fine details within song. Although ts cut songs were often composed of simple syllables, they were sung in complex temporal patterns, which provide more cues by which a neuron can distinguish tsBOS from reverse or conspecific stimuli. Neurons in the song system are clearly sensitive to the intervals between syllables and particular syllable combinations (Margoliash, 1983; Margoliash and Fortune, 1992; Lewicki and Arthur, 1996; Doupe, 1997). Thus, the abnormally low selectivity in ts cut adults may represent a specific impairment in sharpness of tuning, rather than resulting from normal tuning to simpler tsBOS stimuli. To assess experimentally what component of this decreased selectivity might be due to simpler structures within ts cut song, the neural selectivity of adult birds raised with ts cut tutors could be examined. These ts cut–tutored birds would learn simpler songs but would not also have a mismatch between their template and their vocal output.

Selectivity for tutor song was markedly lower than normal adult levels for both ts cut adults that achieved good tutor song matches and those that did not. One possible explanation for this is that the level of tutor selectivity in ts cut adults reflects the upper limit of selectivity for tutor song, and that the much higher selectivity found in normal adults is an artifact of the acoustic similarity between normal adult BOS and tutor song. X neurons from ts cut adults with high similarity to the tutor song, however, still had lower tutor song selectivity than normal adults. It could be that, although these songs were easily matched to the tutor song, they were different enough that neurons could discriminate between tsBOS and tutor song (e.g., ts cut song in Figure 2E, which is missing the high tonal note). Neurons in adult HVc, the input nucleus of the AF, are extremely sensitive to the precise spectral and temporal features of song (Theunissen and Doupe, 1998). Alternatively, the emergence of selectivity for tutor song may have been limited by any delay in song development that all ts cut adults experienced.

LMAN seemed differently affected by ts transection: although neurons were readily recorded in ts cut adults with low similarity to tutor song, auditory responses were obtained from these neurons much less often than in normal adults or in ts cut adults with high tutor similarity. Transections of the ts nerve may have independently affected both auditory responses in LMAN and selectivity in X. Alternatively, loss of auditory responses in LMAN alone could have caused the unusually low selectivity in X of these ts cut adults. LMAN has a recurrent projection to X (Vates and Nottebohm, 1995): if this normally sharpens X selectivity, a lack of auditory responsiveness in LMAN could produced the lower selectivity in X.

In other systems, reductions in neural selectivity and responsiveness are also apparent after experience has been altered. In studies of binocular deprivation in the visual system, a lack of visual experience can degrade existing orientation selectivity of cortical neurons (Crair et al., 1998) and result in an increase in visually unresponsive neurons (Wiesel and Hubel, 1965). Similarly, changing the eye through which visual experience is received has been reported to initially reduce both orientation tuning and visual responsiveness in visual cortex (Mioche and Singer, 1989). In general, continued exposure to experiences that are different from what a neuron expects may degrade neural responsiveness and/or selectivity. Our results suggest that deprivation of a very specific type of experience—the successful matching to tutor song that normally accompanies song learning—may be enough to hinder the development of selectivity in X and the responsiveness of LMAN.

This result raises the possibility that the AF participates in the comparison of auditory feedback of BOS to the tutor song template, and is sensitive to how well this match can be accomplished. In this regard, it is intriguing that the AF is comparable to cortical–basal ganglia circuits (Bottjer and Johnson, 1997; Luo and Perkel, 1999), which are generally implicated in motor and reinforcement learning. In primates, striatal neurons appear to have predictive information related to movement and reward and might participate in comparing motor output to an internal model or prediction (Hikosaka et al., 1989; Hollerman et al., 1998; Tremblay et al., 1998). Also, striatal neurons acquire and maintain responses to sounds associated with reward (Aosaki et al., 1994) and show modulation of neuronal sensory responses by expectation of reward (Kawagoe et al., 1998). Whether the comparison of BOS to tutor song and postulated reinforcement of neuronal responses occurs within the AF itself, or whether outcomes of the comparison are stored in the AF, is unknown. In either case, the reinforcement might involve the strong dopaminergic projections to the AF, especially X (Lewis et al., 1981; Bottjer, 1993; Soha et al., 1996). Regardless of mechanism, we found here that during song learning, experience of BOS and tutor song alone did not result in normal adult levels of selectivity. We propose that the quality of that experience, that is, the degree of matching between vocal output and internal models, can also influence the development of neural responsiveness and selectivity in this circuit important for learning.

Experimental Procedures

Experiments used male zebra finches (*Taeniopygia guttata*). The care and treatment of experimental animals was reviewed and approved by a university animal care and use committee. Birds were raised in individual cages, with their parents and siblings, and visually isolated from other conspecfics in the colony in order to restrict their learning to the tutor in their cage (Immelmann, 1969; Eales, 1987, 1989; Williams, 1990).

Surgery

When birds were ~25–30 days old, the tracheosyringeal portion of the hypoglossal nerve (NXIIts) was transected bilaterally as described by Solis and Doupe (1999). The ts cut birds were returned to their home cages where they remained until they were adults. Both ts cut and normal adult birds were used in this study. The mean age for ts cut birds was 200 days (range 92–625) and for normal adults was 241 days (range 105–336). Two days prior to the experiment, we prepared birds for neurophysiological recording by affixing a head post to the skull and marking the location of the song nuclei on the skull (see Solis and Doupe, 1997, for details). On the day of the experiment, the bird was anesthetized with a 20% solution of urethane (5 ml/kg i.m.; Sigma, St. Louis, MO; delivered in three injections at 30 min intervals), placed in the stereotaxic apparatus, and immobilized via its head post. Body temperature was regulated with a temperature controller (FHC, Brunswick, ME). A craniotomy was performed above LMAN and X, the dura was opened, and the electrode was lowered into the brain with a microdrive (Fine Science Tools, Foster City, CA).

Stimuli

The songs of the adult bird and its tutor were recorded as described by Solis and Doupe (1999). Songs were digitized at 32 kHz (Michael Lewicki and Larry Proctor, California Institute of Technology) and had similar peak intensity levels (64–73 db). During the experiment, the stimuli presented included the bird's own song ("BOS" for normal birds and "tsBOS" for ts cut adult birds), tutor song, reversed versions of tsBOS or BOS and tutor song, at least two different songs of other zebra finches (conspecifics), broad-band noise bursts, and tone bursts. Stimuli were presented in a random, interleaved fashion.

Electrophysiology

Extracellular neuronal signals were amplified and filtered between 300 Hz and 10 kHz (A-M Systems, Everett, WA). The electrode was targeted to pass through AF nuclei along the dorsal–ventral axis. The dorsal border of LMAN was generally encountered first, followed by X; for some passes only X was recorded. Neurons were isolated with a window discriminator (UCSF Physiology Shop), and their responses were collected and analyzed using software developed by Mike Lewicki and Larry Proctor (California Institute of Technology) and Frédéric Theunissen (UCSF). Electrolytic lesions were made at selected locations for reconstructing recording sites.

Anatomy

At the end of an experiment, the bird was deeply anesthetized with Metofane (Pitman-Moore, Mundelein, IL) and transcardially perfused with 0.9% saline, followed by 3.7% formalin in 0.025 M phosphate buffer. Brains were postfixed and cut in 40 μm sections with a freezing microtome. Sections were stained with cresyl violet, and electrode tracks and lesions were identified. Only neurons histologically confirmed to be in LMAN or X were used. The syrinx of each ts cut or normal adult bird was also dissected after perfusion. Afterward, each syrinx was cut 1 mm distal and 4 mm proximal of the bifurcation of the bronchi and then weighed to assess relative muscle mass, a marker of denervation success.

Data Analysis

Neural responses to song were measured as described by Solis and Doupe (1997). In brief, we quantified responses to an acoustic stimulus during the period of stimulus presentation, offset by an estimate of the latency. To be considered auditory and included for analysis, a neuron had to have an average firing rate during one of the stimuli that was significantly different from the background rate (two-tailed paired t test, $p < 0.05$). The frequency of auditory neurons in LMAN was compared across different bird groups, all of which had been sampled in the same way.

The response strength (RS) of a neuron to a stimulus was calculated as the difference between the firing rate during the stimulus (offset by the latency) and the background rate. The selectivity of an individual neuron for one stimulus (A) over another (B) was quantified using the $d'_{A\text{-}B}$ measure (Green and Swets, 1966), where

$$d'_{A-B} = \frac{2(\overline{RS_A} - \overline{RS_B})}{\sqrt{\sigma_A^2 + \sigma_B^2}}.$$

In this equation, $\overline{RS_A}$ and $\overline{RS_B}$ are the mean RS to stimulus A and B, respectively, and σ^2 is the variance of each RS. A neuron was considered selective for stimulus A over stimulus B if it had a $d'_{A\text{-}B}$ value ≥ 0.5. Neural selectivity data from previous studies were also used here for comparison (30-day data from Doupe, 1997; normal 60-day data from Solis and Doupe, 1997; ts cut 60-day data from Solis and Doupe, 1999).

Song Analysis

Similarity

The methods of song similarity analysis used here have been described in detail elsewhere (Solis and Doupe, 1999). Briefly, song similarity was judged in a matching task, completed by human observers familiar with zebra finch song but blind to the neural properties of each bird. Human methods of song analysis are standard and are currently the most sensitive (Eales, 1985; Williams, 1990; Nordeen and Nordeen, 1992; Solis and Doupe, 1999). Observers tried to match each experimental song with that of its tutor, which was present among a group of six potential tutors, by listening and looking at sonograms and oscillograms of the songs. Thus, the percentage of observers that correctly matched the experimental song to its tutor song indicated the overall similarity between BOS and tutor song. After selecting a "best match" tutor song, observers also scored the song pair on spectral similarity and on temporal similarity, using a scale from 1 to 5. The normalized scores ranged from 0.41 to 2.07. The final score for each song was the average of each observer's normalized score. For reference, songs of juvenile ts cut birds and randomly matched songs (correct tutor not present) were also analyzed.

Stereotypy

We measured song stereotypy of each bird using human scoring and an automated stereotypy analysis, as described by Solis and Doupe (1999). Briefly, ten song bouts from each bird were randomly selected for analysis. Observers rated on a scale from 1 to 5 how consistently a particular motif was present in each song sample after they listened to and looked at sonograms and oscillograms of each song sample. Normalized stereotypy scores ranged from 0.32 to 1.37. The automated stereotypy analysis measured how consistently the temporal pattern of a motif was repeated in song samples. This measure is the same as the "motif–song overlap" value used in Solis and Doupe (1999).

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