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Song-selective auditory circuits in the vocal control system of the zebra finch

(birdsong/brain/auditory feedback/complex auditory neuron/learning)

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ABSTRACT **Birdsong is a learned behavior controlled by a distinct set of brain nuclei. The song nuclei known as area X, the medial nucleus of the dorsolateral thalamus (DLM), and the lateral magnocellular nucleus of the anterior neostriatum (L-MAN) form a pathway that plays an important but unknown role in song learning. One function served by this circuit might be auditory feedback, which is critical to normal song development. We used single unit recordings to demonstrate that all three of these nuclei contain auditory neurons in adult male zebra finches (*Taeniopygia guttata*). These neurons are song selective: they respond more robustly to the bird's own song than to songs of conspecific individuals, and they are sensitive to the temporal structure of song. Auditory neurons so highly specialized for song within a pathway required for song learning may play a role in the auditory feedback essential in song development. Recordings in the robust nucleus of the archistriatum (RA), the nucleus to which L-MAN projects, showed that RA also contains highly song-selective neurons. RA receives a direct projection from the caudal nucleus of the ventral hyperstriatum (HVc) as well as from L-MAN. We investigated the contributions of these two inputs to auditory responses of RA neurons by selectively inactivating one or both inputs. Our results suggest that there is a song-selective pathway directly from HVc to RA in addition to the circuit via L-MAN. Thus the songbird brain contains multiple auditory pathways specialized for song, and these circuits may vary in their functional importance at different stages of learning.**

Birdsong is a complex motor act learned during the course of a young bird's life. Behavioral studies have shown that auditory experience and feedback are crucial to normal song learning. In the first or sensory learning phase, a young bird must hear and memorize a tutor song (1). During the subsequent vocal practice phase, the bird gradually matches its vocalizations to the memorized song model or template, using auditory feedback. Birds deafened before the onset of singing are entirely unable to refine and correct their song (2). Once the song is adult or "crystallized," it is much less dependent on auditory input, although adult birds clearly use auditory information for many purposes, including recognition of conspecific individuals (3).

The songbird brain contains a set of discrete and interconnected nuclei involved in song learning and production (Fig. 1; ref. 4). Because song is learned and corrected with reference to auditory information, there must be a link between the auditory and vocal motor systems. Many basic questions about the auditory response properties and inputs of the song control system remain unanswered, however. Field L, the primary auditory area of the avian forebrain, is known to project to the vicinity or "shelf" of the caudal nucleus of the ventral hyperstriatum (HVc) and of the robust nucleus of the archistriatum (RA) (5); these nuclei form part of the motor pathway for song (4, 6). The connections between Field L, the shelf, and HVc and RA are not well understood, but HVc and RA contain auditory neurons, and those in HVc have been well characterized (7–11). Evoked potential and multiunit studies point to auditory responsiveness in or near a number of other forebrain and thalamic song nuclei as well (12, 13), but the exact location and physiological response properties of these cells remain to be elucidated.

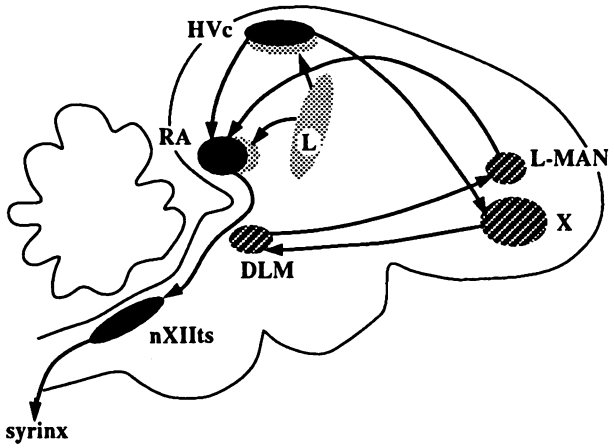

FIG. 1. Simplified schematic of the current view of the song control system. The nuclei shown in solid black, the caudal nucleus of the ventral hyperstriatum (HVc), the robust nucleus of the archistriatum (RA), and the tracheosyringeal portion of the hypoglossal nucleus (nXIIts), form part of the descending motor pathway for song. The avian primary auditory area field L (stippled) projects to the vicinity of HVc and RA. Nucleus X, the medial nucleus of the dorsolateral thalamus (DLM), and the lateral magnocellular nucleus of the anterior neostriatum (L-MAN) (hatched) form a pathway connecting HVc to RA.

Three song nuclei, area X, the medial nucleus of the dorsolateral thalamus (DLM), and the lateral magnocellular nucleus of the anterior neostriatum (L-MAN), form an accessory circuit linking HVc and RA (refs. 12 and 14; Fig. 1). This accessory loop plays an important but unknown role in song learning. Bilateral lesions of either L-MAN or X in young birds disrupt song markedly (15–17). In contrast, lesions of these areas in adult birds have no immediate effect on the production of crystallized song (4, 15). The requirement for auditory feedback in song development has similar timing. Auditory information is essential first for formation of the song template and then for matching of the bird's motor output to this template. After song crystallization, auditory

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Abbreviations: DLM, medial nucleus of the dorsolateral thalamus; HVc, caudal nucleus of the ventral hyperstriatum; L-MAN, lateral magnocellular nucleus of the anterior neostriatum; RA, robust nucleus of the archistriatum.

feedback is much less important for song production. Thus, the crucial role of the three nuclei of the accessory loop during song learning might be to provide auditory feedback. Nothing is known, however, about the type of information carried by this circuit. We therefore characterized the stimulus selectivity of neurons in these areas. We report here that in adult male zebra finches all three of these nuclei contain highly selective auditory neurons, which respond best to each bird's own song. Furthermore, we found similar song-selective neurons in RA, a premotor nucleus that receives the major output of the accessory pathway.

MATERIALS AND METHODS

Experiments were conducted with adult (older than 90 days) male zebra finches (*Taeniopygia guttata*) obtained from local breeders or raised in our colony. Prior to each experiment the bird's own song was recorded on analog tape and digitized at 20 kHz with 12-bit resolution with the aid of either a PDP-11/40 (Digital Equipment) or a Masscomp 5600 (Concurrent, Westford, MA) computer (with software written by Daniel Margoliash and by Larry Proctor, California Institute of Technology). The song was then stored on computer disk along with a library of songs of other zebra finch individuals, to be used for playback during the physiology experiments. Songs were also reversed and edited on the computer.

Two days prior to the experiment, birds were anesthetized with Equithesin (2 ml/kg i.m.; 0.85 g of chloral hydrate/0.21 g of pentobarbital/0.42 g of $MgSO_4$/2.2 ml of 100% ethanol/8.6 ml of propylene glycol to a total volume of 20 ml with water) or with a mixture of ketamine (40 mg/kg i.m.) and xylazine (50 mg/kg i.m.) and placed in a stereotaxic head holder. A stainless steel post was then cemented to the skull in a fixed location centered on the midsagittal sinus. This stereotaxic post served to immobilize the head during the recording sessions and to provide a fixed point from which to measure the location of various song nuclei. In some experiments, a Formvar-insulated stainless steel electrode was lowered into L-MAN at the time of pin implantation, and electrolytic lesions were made by passing 100 μA of anodal current for 60 sec. On the day of the experiment, birds were anesthetized with 20% urethane (Sigma; 5 ml/kg i.m.). Glass-coated platinum/iridium microelectrodes were used to make stable single unit extracellular recordings of neuronal responses to a variety of acoustic stimuli. These stimuli were presented in freefield conditions by a small calibrated speaker (JBL, Northridge, CA) 1.7 m in front of the bird inside a sound-attenuating chamber. The sound stimuli, whose peak amplitude was 70 decibels, included broad-band noise and pure tone bursts, the song of the experimental subject (including reversed and edited versions), and the songs of other zebra finches. In some experiments 4% aqueous lidocaine was injected into a brain area, either with a Hamilton syringe or with a glass electrode attached to a pneumatic Picopump (WPI Instruments, New Haven, CT), while the activity of the target nucleus of that area was simultaneously recorded with a platinum/iridium electrode.

Spike activity was collected and displayed by the PDP-11/40 or Masscomp 5600 computer both as a raster pattern and as a summed peristimulus time histogram of 10–20 stimulus presentations. Electrolytic lesions were placed at the sites of selected units. At the end of an experiment, animals were given a lethal dose of Equithesin and fixed with 4% (vol/vol) formaldehyde via intracardial perfusion. Electrode tracks and electrolytic lesions were located on 30-μm frozen sections stained with cresyl violet. Neuronal data were included in the data analysis only if the recording site could be unambiguously identified histologically. The extent of electrolytic lesions of L-MAN was determined by tracing the area of any remaining portions of the nucleus and expressing it as a percentage of the average total area of L-MAN in adult zebra finches.

The firing rate to a given song stimulus was quantified from recorded data as the average spike rate during the song, normalized to spikes per sec. To compare the evoked response to the baseline firing rate, the strength of a unit's response to a stimulus was calculated as the average spike rate during the song minus the average baseline firing rate for the same trial (determined from 2–4 sec of spontaneous firing prior to each stimulus). A neuron was considered to prefer a stimulus if the strength of its response to that stimulus was greater than the strength of its response to the other stimuli being tested.

RESULTS

We recorded from a total of 44 isolated auditory units in L-MAN in 14 birds. Small clusters of units as well as multiunit recordings showed qualitatively similar auditory responses but were not used in the quantification. L-MAN auditory neurons were in general much more responsive to complex acoustic stimuli than to simple ones, and most strikingly, each bird's own song was the most effective

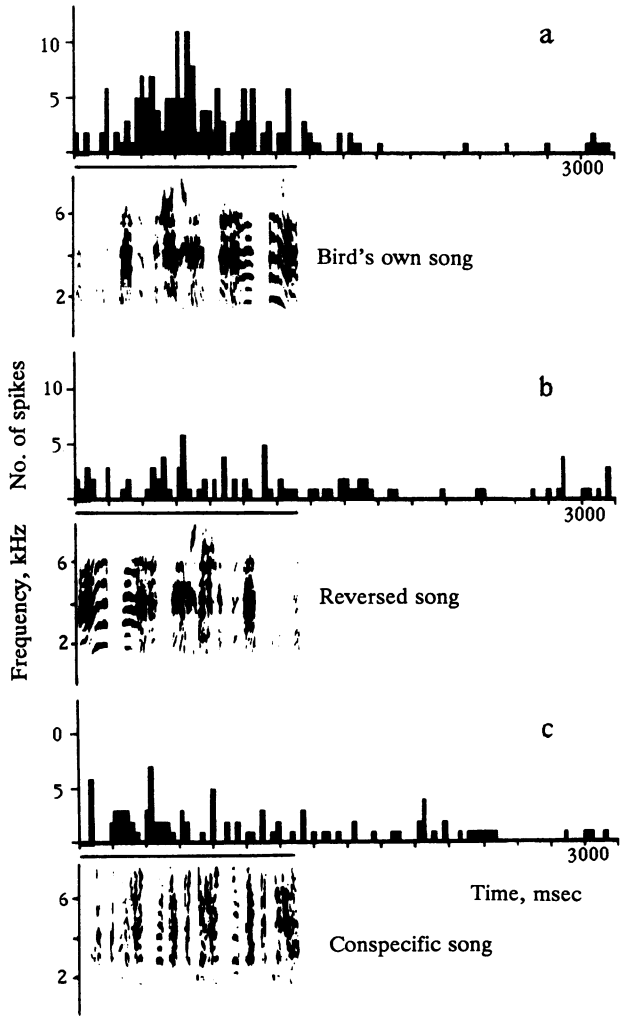

FIG. 2. Peristimulus time histograms of the response of an isolated L-MAN neuron to the bird's own song (*a*), the same song played in reverse (*b*), and the song of another zebra finch individual (*c*). Histograms represent the summed responses to 20 presentations of the stimulus. A sonogram (frequency vs. time plot) of the song stimulus and a line indicating stimulus duration are shown under each histogram.

stimulus (Fig. 2a). Despite their overall acoustic similarity, songs of other zebra finch individuals were less effective (Fig. 2c). For all 29 neurons tested, the response to conspecific songs was less than that to the bird's own song; on average, the firing rate to conspecific song was 44.3% ± 18.3% (range 1.9–92.2%, n = 71) of the firing to the bird's own song. A comparison of the response strengths (see *Materials and Methods*) of individual L-MAN neurons for the bird's own song to those for two other zebra finch songs shows that for each neuron conspecific song is less effective and in some cases even inhibitory (Fig. 3a). Despite the variability of neuronal firing rates, even the mean response strength for the whole population of neurons studied was much higher in response to the bird's own song than to conspecific songs [4.22 spikes per sec ± 1.86 (SD) vs. 0.22 spikes per sec ± 1.33; P = 0.0001, unpaired t test].

Because bird song has complex temporal structure (see sonograms in Fig. 2, for example), we tested the sensitivity of song-selective L-MAN neurons to temporal features of song. Playing the song in reverse completely alters the temporal order, but does not change any of the stationary spectral properties of the song. In all 29 well-isolated L-MAN neurons tested, this manipulation dramatically reduced the effectiveness of the bird's own song as an acoustic stimulus for L-MAN neurons (Fig. 2 a vs. b). For each neuron tested, the reversed song was much less effective and frequently inhibitory, as shown by a comparison of response strengths to forward and reversed song (Fig. 3b). On average, the firing rate to reversed song was 40.0% ± 13.8% (range 13.4–65.0%, n = 29) of that to forward song. This difference in firing rate to forward and reversed song is also evident in a comparison of mean response strengths for the whole group of neurons studied [3.57 spikes per sec ± 1.53 (SD) to forward song vs. −0.34 ± 0.91 to reversed; P = 0.0001, unpaired t test].

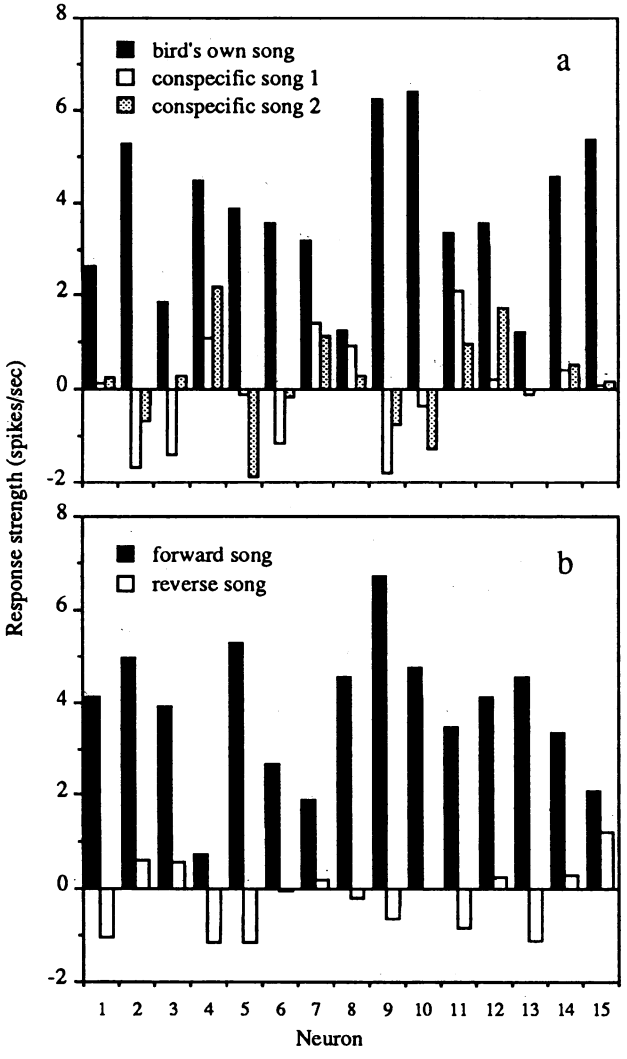

FIG. 3. (a) Strength of the response of 15 L-MAN neurons to the bird's own song and to two other conspecific songs. The conspecific songs tested are not necessarily the same for all neurons. (b) Strength of the response of 15 L-MAN neurons to the forward and reversed versions of the bird's own song. To conserve space the entire set of L-MAN neurons is not shown, but these 2 samples of 15 neurons are entirely representative.

These highly selective L-MAN neurons might integrate inputs from a number of other, possibly simpler, auditory neurons. We therefore investigated the auditory response properties of neurons in nuclei that project to L-MAN (see Fig. 1). We recorded from 15 single auditory units in nine birds in the only known input nucleus to L-MAN, the thalamic nucleus DLM. Like those in L-MAN, the majority of these units had complex properties and a preference for the bird's own song (Table 1). Similarly, the only known input to DLM, the forebrain song nucleus X, contains auditory neurons. The bird's own song was a highly effective stimulus for all 20 single auditory units in nine birds that we recorded in X (Table 1).

The surprising effectiveness of the bird's own song as a stimulus in all three of these interconnected nuclei raised the question of whether there were any differences in response properties among these neurons. We compared the responses of auditory neurons in the different nuclei to simpler stimuli, including broad-band noise and pure tone bursts, 100–500 msec in duration. More than half of the X and DLM units tested responded to broad-band noise bursts, although song was usually a more effective stimulus. Only 1 of 22 L-MAN single units tested was excited by broad-band noise bursts (Table 1), and in some cases the spontaneous activity was inhibited by this stimulus. The difference between these populations was highly significant (P = 0.0001, χ^2 = 17.188, df = 1 for L-MAN vs. X; P = 0.0016, χ^2 = 9.928, df = 1 for L-MAN vs. DLM). In all three nuclei many units responded to pure tone bursts, although X neurons tended to respond to a broader range of frequencies. In addition, all L-MAN neurons tested preferred the forward version of the bird's own song to all other song stimuli, whereas several neurons in X and DLM responded equally well to forward and reverse songs (Table 1). This difference between L-MAN and the two other nuclei was significant (P = 0.0009, χ^2 = 11.053, df = 1).

Although RA is a premotor nucleus essential for song production, it also receives a direct projection from L-MAN. The presence of auditory neurons in L-MAN raised the possibility that RA also contains song-selective neurons, so we tested for auditory responsiveness in RA. We recorded from 32 single auditory units in RA in 10 birds. Twenty-five

Table 1. Response properties of auditory units in four song nuclei

	Responsiveness				
Nucleus	Own song	Forward	Own > conspecific	Noise	Tone
L-MAN	44/44	29/29	31/31	1/22	25/31
DLM	15/15	8/12	6/7	3/5	4/4
X	20/20	6/8	8/11	8/11	11/12
RA	25/30	22/22	14/14	16/23	13/26

Columns show the number of neurons in each area with the described property as well as the total number tested: Own song, responsiveness to the bird's own song; Forward, preference for forward song; Own > conspecific, preference for bird's own song vs. that of conspecific individuals; Noise, responsiveness to broad-band noise bursts; and Tone, responsiveness to tone bursts.

out of 30 auditory neurons tested responded strongly to the bird's own song. All song-responsive neurons tested preferred the bird's own song to the reversed song or songs of conspecific individuals (Table 1). Sixteen of 23 single RA units also responded to broad-band noise bursts.

Both L-MAN and HVc are known to innervate RA. We assessed the relative contribution of these two inputs to auditory responses in RA by selectively silencing one or both inputs with anesthesia or lesions. In two birds, we injected 0.1–0.4 μl of 4% lidocaine into L-MAN while recording from RA auditory neurons. There was no apparent effect of lidocaine injections into L-MAN on the auditory properties of 4 different RA neurons. In contrast, a similar injection of lidocaine into HVc in one of these birds reversibly eliminated RA song responses. In another approach, we made lesions of 60–100% of the left L-MAN in three birds two days before recording from RA on the same side. We recorded 13 RA units with highly song-selective auditory properties in these birds. In two of these birds with L-MAN lesions we then injected lidocaine into HVc. The auditory responsiveness of the RA units being recorded disappeared upon lidocaine injection and then recovered over the course of 10–25 min. An example of one such experiment is shown in Fig. 4.

DISCUSSION

These results demonstrate that there are auditory neurons in each of the three nuclei in the accessory loop of the song system of adult male finches. These neurons respond best and with a high degree of selectivity to the bird's own song and are sensitive to its temporal structure. In this respect the song units in L-MAN, DLM, and X are similar to the well-described song-selective neurons in HVc of male white-crowned sparrows and zebra finches (7, 10, 11, 18). Intracellular filling of HVc neurons with auditory properties showed that many of them project to X (8). HVc auditory neurons are thus the likely source of auditory input to the accessory loop.

Auditory neurons in all three accessory song nuclei have complex song-responsive properties similar to those of HVc neurons. Thus in this circuit basic song selectivity must arise within or prior to the HVc. This raises questions about the purpose of the sequential loop from HVc via X and DLM to L-MAN. Some differences between auditory neurons in the different nuclei point to a possible function of this circuit. Although they are song selective, many HVc auditory neurons also respond to broad-band noise and tone bursts (10, 11). In this study, many X and DLM neurons also responded to simpler acoustic stimuli, while L-MAN neurons did not. Furthermore, more neurons were strictly order selective in L-MAN than in the other two nuclei. This suggests that the tuning of L-MAN neurons is even narrower than that of X and DLM. Thus one function of this series of interconnected nuclei may indeed be to impart an increase in song selectivity. In many sensory systems complex stimulus selectivity arises gradually in hierarchical circuits like the one described here (19).

In young sparrows that have not yet sung, HVc auditory neurons prefer complex auditory stimuli but are not song selective (20). One possibility is that during development, when this accessory circuit is essential, its proposed function of increasing song selectivity has a more dramatic effect. Thus it might transform nonselective auditory inputs from HVc into highly selective outputs in L-MAN. Clearly, testing of this hypothesis awaits single unit recordings from young birds.

The function of these unusual and complex auditory neurons is unclear. Such neurons are well suited to provide information, in the form of the strength of their firing rate, about how well certain vocalizations match a particular

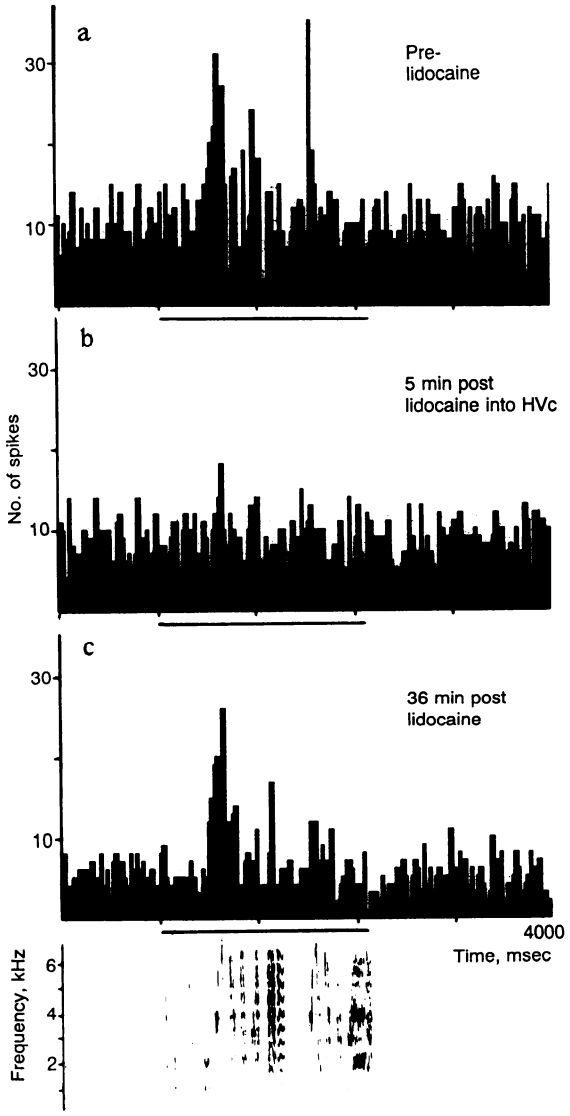

FIG. 4. Peristimulus time histograms of the response of a song-selective RA neuron in a bird with an L-MAN-lesion, pre-lidocaine infusion (*a*), 5 min post lidocaine infusion into HVc (*b*), and 36 min post lidocaine (*c*). The song stimulus is shown in sonographic form under the histogram. This neuron also demonstrates the characteristic high spontaneous firing rate that distinguishes RA neurons from auditory neurons in the accessory loop.

model, that is, to act as a template. Furthermore, these neurons are found throughout a pathway that ultimately projects back into the vocal motor system at RA. This accessory pathway is essential early in development, when song learning is occurring (15–17). Thus one critical role of this circuit may be to provide the auditory feedback and auditory-motor matching so essential to song learning.

In adult birds, these same neurons might be useful in the discrimination of songs of conspecific individuals, perhaps by comparison of the differences between the songs of others and the bird's own song. Behavioral tests of song discrimination will be necessary to demonstrate a role for these neurons in song recognition.

RA auditory neurons have song-selective properties similar to those of the L-MAN neurons that project to them. This is consistent with the idea that the majority of auditory neurons in HVc project to X (8) and therefore that L-MAN is the major source of auditory input to RA. One difference between L-MAN and RA, however, is that, like HVc neurons, many more RA units respond to broad-band noise

bursts than do L-MAN neurons. Williams (13) proposed a direct auditory connection from HVc to RA on the basis of multiunit studies showing tone responses in RA with shorter latencies than similar responses in X. This claim does not take into account the possibility that RA may receive simpler auditory inputs from the "cup" area coming from field L (5). We directly investigated the source of RA auditory responses by silencing possible input areas while recording from RA. Surprisingly, we still observed highly selective RA song neurons after anesthesia or lesions of L-MAN in adult zebra finches. Our results suggest that RA must receive song-related auditory input in addition to that from L-MAN. A source of song-selective inputs directly from HVc is supported by the reversible disappearance of RA auditory activity when HVc is silenced with a local anesthetic, both in birds with and without an intact L-MAN. Thus there are at least two circuits in the songbird brain that in adult birds contain auditory neurons highly specialized for song. It is striking that both these pathways include HVc and converge onto RA.

Because HVc is the source of inputs to both L-MAN and RA, we have not directly assessed the independent contribution of L-MAN auditory neurons to the properties of RA neurons. Nonetheless, although a majority of RA neurons receive inputs from L-MAN (21), it is clear that L-MAN inputs are not essential for adult RA song responsiveness. This fact is consistent with the idea that the accessory loop is particularly important in young birds but wanes in influence in adulthood, while the direct pathway from HVc to RA is essential for song throughout life. Furthermore, the two pathways from HVc to RA form at different times in development. L-MAN neurons make functional synapses with RA neurons by posthatch day 15 in the zebra finch, during acquisition of the template (22). HVc terminals, on the other hand, are not present in RA when RA initially receives L-MAN inputs, and these terminals begin to innervate the nucleus only around day 25, coincident with the onset of the vocal practice phase of learning (23). Thus these two circuits may play crucial and separate roles in the different phases of song learning. An analysis of their auditory properties in various stages of song development should help clarify the role of these pathways and the relationship between them.

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