Published in final edited form as:

J Neurophysiol. 2007 January ; 97(1): 806–816.

Developmental Modulation of the Temporal Relationship Between Brain and Behavior

Shane R. Crandall, Naoya Aoki, and Teresa A. Nick

Department of Neuroscience and Center for Neurobehavioral Development, The University of Minnesota, Minneapolis, Minnesota

Abstract

Humans and songbirds shape learned vocalizations during a sensorimotor sensitive period or “babbling” phase. The brain mechanisms that underlie the shaping of vocalizations by sensory feedback are not known. We examined song behavior and brain activity in zebra finches during singing as they actively shaped their song toward a tutor model. We now show that the temporal relationship of behavior and activity in the premotor area HVC changes with the development of song behavior. During sensorimotor learning, HVC bursting activity both preceded and followed learned vocalizations by hundreds of milliseconds. Correspondingly, the duration of bursts that occurred during ongoing song motif behavior was prolonged in juveniles, as compared with adults, and was inversely correlated with song maturation. Multielectrode single-unit recording in juveniles revealed that single fast-spiking neurons were active both before and after vocalization. These same neurons responded to auditory stimuli. Collectively, these data indicate that a key aspect of sensory critical periods—prolonged bursting—also applies to sensorimotor development. In addition, prolonged motor discharge and sensory input coincide in single neurons of the developing song system, providing the necessary cellular elements for sensorimotor shaping through activity-dependent mechanisms.

INTRODUCTION

The brain mechanisms of sensorimotor integration and motor skill learning are not well understood. However, the bird-song model has begun to illuminate fundamental principles of forebrain control of behavior (Perkel 2004). The birdsong system has the necessary characteristics to enable a systematic investigation of the neural bases of sensorimotor learning. Song production requires precise coordination of muscles in the vocal organ and respiratory system (Goller and Cooper 2004; Suthers and Zollinger 2004) and these coordinated patterns are learned using sensory feedback (Konishi 1965). In addition, the song behavior depends on a series of anatomically and functionally distinct brain areas (Nottebohm et al. 1976) and, importantly for plasticity studies, matures over several months (Immelmann 1969), enabling day-by-day assessment and comparison of neural and behavioral changes.

The pallial (cortical) song area HVC resides at the interface of sensory and motor pathways and projects directly to a cortico-basal-ganglia-thalamocortical loop (the anterior fore-brain pathway) that enables song plasticity (Bottjer et al. 1984; Brainard and Doupe 2000; Williams and Mehta 1999). Thus HVC is anatomically well positioned to have a role in sensorimotor integration and learning. HVC both controls learned song (Ashmore et al. 2005; Nottebohm 2004; Vu et al. 1994) and receives auditory information (Katz and Gurney 1981; Margoliash 1983; McCasland and Konishi 1981; Mooney 2000; Theunissen and Doupe 1998). Moreover,

HVC exhibits patterned activity that is locked to song production (Fee et al. 2004; McCasland 1987), implicating this area in the control of song timing. Recent studies indicate that HVC contains a pattern generator (Mooney and Prather 2005; Solis and Perkel 2005), suggesting that temporal aspects of song are stored in the HVC circuitry. Although these and other data identify HVC as a crucial player in song production, its role in song plasticity and learning is not clear. No recordings of HVC activity in singing finches in the process of song learning have ever been published.

As with human speech, song learning is divided into two phases: the sensory phase during which the songbird memorizes a tutor song (explicit memory) and the sensorimotor phase during which he then uses comparison of auditory feedback and the memorized tutor song template to shape his song (implicit memory) (Bolhuis and Gahr 2006; Konishi 1965). During the sensorimotor phase, HVC serially recalibrates its response to playback of the bird's own song. During sleep (Nick and Konishi 2005a) and anesthesia (Volman 1993), HVC always responds most to the current version of the bird's own song, even when it is little more than repeated begging calls. During waking in the sensorimotor phase, HVC responds preferentially to the memorized song of an adult male tutor (Nick and Konishi 2005b). Collectively, these data show that HVC contains or receives signals that code for both the current version of the bird's own song and the tutor song memory. Further, they indicate that HVC activity has a functional and dynamic role in song learning. However, changes in HVC activity during song learning have not been investigated.

To understand the neural signals that drive song learning during development, we used long-term population and single-unit (multielectrode) recording techniques to examine HVC activity in juvenile finches as they actively shaped their song toward the mature adult form.

Aspects of this study previously appeared in abstract form (Aoki and Nick 2006; Crandall and Nick 2006; Nick 2005).

METHODS

Subjects

For chronic population recording experiments, 35 juvenile (<90 days) and 18 adult (>200 days) male zebra finches (*Taeniopygia guttata*) were surgically implanted. Of these, 14 juveniles had high-quality recordings [root-mean-square (RMS) premotor signal:noise ≤ 2] and five sang while in the sensorimotor phase (earliest ages recorded for each juvenile: 62, 65, 66, 72, 72 days). Of the adults, six had high-quality recordings and three sang. The low song yield is presumably explained by our strict requirement for undirected song (emitted in the absence of a female). All recordings were made in the absence of a female. Undirected song is thought to reflect song practice (Jarvis et al. 1998) and is thus most relevant to learning studies. One finch implanted as a juvenile was not recorded singing until adulthood (Red-87, age 141 days) and was therefore used in the adult group (total adult $n = 4$; age recorded for other three adults: >200 days). All juvenile and all but three adult finches were reared in our facility on a 12:12 light:dark cycle. Three adult birds were obtained from Magnolia Bird Farm (Anaheim, CA). None of the finches used in the population recording experiments was ever exposed to auditory playback.

For the multielectrode (triode) experiments, 88 juvenile (<70 days) finches were implanted. Of these, six juvenile finches (age 53–62 days) vocalized (undirected) before deterioration of the recording. All juveniles used in the triode experiments were reared in an acoustic chamber that also contained their parents and same clutch siblings until day 45 and then isolated in an acoustic chamber thereafter. All procedures were approved by the University of Minnesota Institutional Animal Care and Use Committee.

Chronic physiological recording

For population recordings, finches were implanted with a set of recording electrodes: one or two 50- μ m nichrome-formvar electrodes in HVC (1.1–1.8 M Ω , AM Systems, Carlsborg, WA), a 50- μ m nichrome-formvar electrode adjacent to HVC for use as a reference electrode (1.1–1.8 M Ω), a ground electrode (relative to lambda: 5–6 mm lateral, 6 mm anterior. Depth: 0; inserted between the skull and the dura), and an electroencephalogram electrode (relative to lambda: 2–3 mm lateral, 7–8 mm anterior. Depth: 0; inserted between the skull and the dura). The headstage and recording environment were previously described (Nick and Konishi 2001; Schmidt and Konishi 1998).

For increased stability and recording quality in chronic population recordings, we 1) modified the implant such that the recording electrodes extended rigidly from the connector in the implantable orientation, which decreased wire handling and accidental stripping; 2) scored the juvenile one-layer skull with a scalpel blade and made tiny holes in the top layer of the more mature two-layer skull to increase adherence of the dental cement; 3) did not plate the recording electrode wires and reference, with the aim of increasing tissue adherence; and 4) gave the finch an antiinflammatory (Metacam, Boehringer-Ingelheim, Ingelheim, Germany) and antibiotic (Baytril, Bayer, Leverkusen, Germany) in lactated ringers immediately after surgery and antibiotic in lactated ringers for 3 days after surgery.

For multielectrode single-unit recordings, the electrode configuration consisted of a gold-plated triode in HVC (wire diameter: 12 or 18 μ m, AM Systems or Kanthal, Palm Coast, FL), a gold-plated reference electrode with the same resistance as a single wire of the triode adjacent to HVC (≤ 1 M Ω), and a ground wire placed as above. Two of the triode implants also consisted of a titanium microdrive and bipolar stimulating electrodes placed in or near Area X and the robust nucleus of the arcopallium (RA).

Finches were recorded 24-h per day. Food and water were available without restriction. All data were acquired with custom-written Lab-View software (Datafleet, Minneapolis, MN) at a sampling frequency of 44.1 kHz. During recording, a lightweight operational amplifier was attached to the bird and connected to a mercury commutator by a flexible cable. HVC neural activity was amplified and filtered 300–10,000 Hz. Song behavior was high-pass in-line filtered at 100 Hz (Shure, Niles, IL) and recorded with a microphone (Earthworks, Milford, NH). Localization of electrodes to HVC was confirmed with premotor activity in all cases and cresyl-violet histology in seven of 13 finches.

Sound playback (multielectrode single unit only)

Behaviorally relevant and artificial sounds were played back to four of six birds implanted with multielectrodes. Stimuli consisted of noise (3 or 15 s), silence, the Bird's Own Song (available for only two birds), the tutor song, and two conspecific songs from two different adult males that were not the song tutor. All stimuli were delivered in random order during every trial set. The onset interstimulus interval of stimuli was 20 s. Trials during which movement or vocalization occurred during the playback stimulus were discarded. The method of detection of movement and vocalization was previously described (Nick and Konishi 2005b).

Behavioral analysis

The Sound Analysis Pro (Tchernichovski et al. 2000) program was used to compare 25 randomly selected motifs from each day to 25 randomly selected motifs from the most mature day available to obtain percentage similarity values. Feature calculation was tuned to zebra finch sounds (default). Sound Analysis Pro was also used to cluster syllables based on the following features: syllable duration, mean pitch, mean entropy, mean FM, and mean goodness of pitch (see the Sound Analysis Pro manual for specific details, available at the website

http://ofer.sci.ccny.cuny.edu/html/sound_analysis.html). After clustering, the longest-duration syllable was analyzed for entropy variance across development.

Chronic population analysis

All data were analyzed with custom-written Matlab functions (by TA Nick). Initial analysis consisted of the sorting of sound data. Unlike vocalizations, movement noise tends to be highly variable at time intervals <20 ms, which enabled the detection and elimination of most movement noise. Sound data were further sorted according to temporal properties. Preliminary songs were defined as sounds lasting ≥ 500 ms with time gaps of no more than 20 ms.

To examine prolonged bursting associated with vocalizations, we analyzed HVC activity that occurred within 5 s of vocalization. Ongoing activity was measured during 2-s silent periods that occurred between vocalizations. Twenty randomly selected silent periods were measured per day; 3 days were analyzed per electrode using RMS, which is a measure of the power of the neural population recording. These data were compared using a two-tailed Student's *t*-test.

To specifically study activity during learned behavior, a canonical motif was identified by a skilled observer from the oldest day available from each finch and used to extract motifs and corresponding neural activity throughout the recording period. Multiple motif forms could have been selected depending on the developmental stage. However, we were most interested in how HVC activity changed once the overall rhythm had been established and thus selected the most mature motif available. Because perfusion and histology were performed as soon as the electrode recording declined, the final adult motif may not have been achieved by some subjects.

We focused our analysis on action potentials (instead of, for example, low frequency potentials) and behaviorally relevant vocalizations (instead of, for example, movements) by band-pass filtering our electrode and vocal data before low-pass filtering at 50 Hz to obtain an amplitude envelope. Thus amplitude envelopes of the canonical motif and all preliminary songs (about 4 terabytes of data) were constructed by band-pass filtering the sound recording at 1–8 kHz and then low-pass filtering the rectified waveform at 50 Hz (all digital filters were fixed-impulse response hamming). Amplitude envelopes of the canonical motif and preliminary songs were cross-correlated. Sharp peaks in the cross-correlation revealed the onset of a motif that matched the canonical motif (Supplementary Video 1¹). Behavioral song motifs and corresponding neural activity were then excised and saved for further analysis.

To examine patterns of population spiking activity, amplitude envelopes of rectified neural activity during motifs were created by band-pass filtering 300–6,000 Hz and then low-pass filtering at 50 Hz. The line plots of amplitude envelopes shown in Figs. 2 and 3 were normalized to the maximum. Peaks in activity (bursts) were identified as continuous periods above the mean of neural motif activity from the same day. The findings that we report were not dependent on a specific threshold (mean) because they held if median or mean + SD were used instead (Supplementary Fig. 1). For assessment of differences in activity peak duration and rate between groups, means of the youngest juvenile and adult recordings were compared using a Wilcoxon rank-sum test ($\alpha = 0.05$). Each electrode contributed one datum per comparison. Comparison of activity across age within the same finch was achieved using an ANOVA ($\alpha = 0.05$). All data points are means and all errors are SEs. Although experience and not age is probably the strongest predictor of song system maturity, age is correlated with experience and presents a more quantifiable parameter.

¹The online version of this article contains supplementary data.

Chronic single-unit analysis

Waveforms for clustering were found by filtering the triode data (band-pass 300–5,000 Hz), finding all spikes above the threshold (mean + 2 SD; calculated independently for each channel) that did not occur during a movement artifact and saving the spike time (relative to the entire cluster session), waveform (16-bit; about 0.36 ms), vocalization, and movement data for each data file such that they could be retrieved later, across trials, by a custom loading function. A subset of Matlab functions taken from the MClust suite (by AD Redish) was used to obtain the clustering parameters (Energy, Derivative of Energy, First Principal Component) across all three wire electrodes [giving a total of nine parameters in all cases but one stereotrode (two wires), from which six parameters were available], to feed these parameters to the clustering algorithm KlustaKwik (by K Harris), and to assess the quality of clusters using the L-ratio (a measure of the amount of noise or contamination near a cluster; “good” quality <0.05) and isolation distance (a measure of how far a given cluster is from the noise distribution; “good” quality >16) metrics (Schmitzer-Torbert et al. 2005). For display and spike width analysis, a matrix of 48-bit waveforms was created with the same indices as the 16-bit waveform matrix used for clustering.

Clustering was performed either on a Dell PC with a 64-bit processor running Matlab in 64-bit mode with a Fedora Linux operating system or on a Sun V880 provided by the Supercomputing Institute at the University of Minnesota (Matlab and KlustaKwik codes were parallelized for the workstation by S. Zhang). Clusters that were not well separated (L-ratio <0.05 and isolation distance >16) were discarded. In pilot studies, manual manipulation of clusters after KlustaKwik sorting and selection based on L-ratio and isolation distance did not further increase cluster quality (i.e., decrease L-ratio and increase isolation distance). Thus clusters were not manually manipulated after KlustaKwik sorting, but checked by an observer who was blind to all aspects of the clusters except energy and principle component 1. (Note that timing of spikes within clusters is of primary interest and thus most likely to be subject to experimenter bias.) A given cluster was discarded if it overlapped by more than roughly half of its area with noise (all spikes in clusters that did not have acceptable L-ratios or isolation distances) in a two-dimensional energy plot and in a two-dimensional principal component 1 plot.

Spike width was measured from negative to positive peak and at 25% positive peak amplitude, in accordance with a previous study in adults (Rauske et al. 2003). Baseline spike rate was measured in 500-ms bins that contained no vocalizations or playback stimuli (number of bins: 60–148). Premotor and postmotor spike rates were measured for 50 ms before or after the vocalization, respectively. Responses to an array of auditory stimuli were compared using a one-way ANOVA with post hoc Tukey–Kramer ($\alpha = 0.05$) analysis. An “Auditory” neuron responded significantly above baseline to at least one of the auditory stimuli 250–750 ms after stimulus onset.

RESULTS

Vocal-associated activity in HVC changes with development

We investigated the development of the brain–behavior relationship during the sensorimotor phase, when auditory feedback and a memory of the tutor song are used to sculpt song production (Konishi 1965). Unlike activity in the adult HVC, which is tightly correlated with song behavior (Fig. 1B), juvenile HVC activity both preceded and followed vocalizations by up to several seconds (Fig. 1A; Supplementary Video 2). To quantify the apparent prolonged bursting before and after vocalizations, we analyzed the RMS of 2 s of ongoing activity during silent periods that occurred within 5 s of vocalization. We found that HVC near-vocalization activity in juveniles was significantly greater than that of adults [RMS μ V; Juvenile ($n = 18$

days): 26.31 ± 2.25 ; Adult ($n = 18$): 15.38 ± 1.72 ; $P < 0.0005$, t -test]. These data suggest that a substantial amount of ongoing activity in the juvenile HVC is not directly involved in vocal production and may thus have a role in vocal learning.

Activity bursts during learned motifs are longer in juveniles

To investigate the role of developmental modulation of HVC activity patterns in song plasticity, we examined HVC activity during ongoing learned vocal behavior (singing). We were able to maintain stable population recordings over several weeks, which allowed us to obtain detailed maps of the changes in neural activity and vocalizations during song development. We precisely excised behavioral and neural song motifs (highly stereotyped sequences of learned sound that are concatenated to produce songs) from all vocalizations that each finch produced during the recording period. Motifs were excised from multiple days for each animal by cross-correlating the amplitude envelope of a “canonical” (Williams 2004) behavioral motif with all available song data across development (see Supplementary Video 1). For each finch, an experienced observer selected a single relatively mature behavioral motif. This canonical motif was used to extract motifs from all recording days, enabling the precise alignment of song motifs and corresponding neural activity by an unbiased computer program. The canonical motifs of all of our adults were longer in duration than all of the canonical motifs of our juveniles (note the duration of motifs in Fig. 2, *A* and *B*). This is consistent with previous studies that demonstrated the emergence of longer sequences of different syllables with song learning (Arnold 1975; Tchernichovski et al. 2001).

We found that HVC neural activity during the act of singing was different in juveniles compared with adults. In Fig. 2*A* (juvenile) and Fig. 2*B* (adult) each show, from the top: 1) a sonogram for the selected canonical motif to show the spectral properties of the song behavior; 2) amplitude envelopes of the canonical motif and a subset of extracted motifs to emphasize that the motif extraction relied on the matching of amplitude envelopes of the two sounds; 3) a three-dimensional plot of the behavioral motif vocalization amplitudes produced on the same day (plotted from top to bottom in the order in which they were produced) to illustrate the precision of the behavioral alignment; 4) amplitude envelopes for the neural activity accompanying the motifs shown in 2 above, to show the neural activity associated with well-aligned motifs and to illustrate the threshold (mean, yellow line) above which we considered continuous neural activity a “burst”; and 5) a three-dimensional plot of the associated neural activity that occurred during the behavioral motifs shown above in 3. The columns of activity that can be seen in the *bottom panels* of both Fig. 2, *A* and *B* emphasize that 1) our motif identification technique is temporally precise and 2) the electrodes were stable.

For illustration purposes only, two bursts [peaks above the mean (yellow line in the HVC activity amplitude line drawing)] are indicated by green bars in Fig. 2, *A* and *B* above the three-dimensional HVC activity amplitude plots. The HVC activity bursts during juvenile motifs (Fig. 2*A*) were longer in duration than those of adults (Fig. 2*B*). In addition, the rate of bursts was higher in adults. Statistics on group data revealed that both burst duration and rate were significantly different between adults and juveniles (Fig. 2, *C* and *D*; $P < 0.02$).

We were initially concerned that the observed differences between adults and juveniles might have arisen because of time since surgical implantation and not a real developmental effect. To address this issue, we compared adults and juveniles that had been implanted the same number of days. Controlling for implant days, we found that burst duration and rate were significantly different between adults and juveniles [Days implanted: 6, Adult (bird Blk161): Duration: 23.1 ± 0.4 ms, Rate: 22.2 ± 0.2 Hz, $n = 116$ motifs; Juvenile (Bu70): Duration: 24.7 ± 0.3 ms, Rate: 20.3 ± 0.2 Hz, $n = 216$ motifs; Days implanted: 7, Adult (Blk161): Duration: 20.7 ± 0.4 ms, Rate: 24.1 ± 0.4 Hz, $n = 103$; Juvenile (Bu81): Duration: 28.2 ± 1.2 ms, Rate: 17.3 ± 0.7 Hz, $n = 41$; for all $P < 0.0001$, t -test].

HVC vocal activity is sculpted daily during song development

The preceding experiments revealed that HVC bursting activity changes between the sensorimotor phase and adulthood. To determine the time course of these changes, we made longitudinal recordings of both brain activity and behavior in the same animals throughout the sensorimotor phase. We found that, similar to song itself, HVC activity matures over many days (Fig. 3). An abrupt crystallization of HVC activity was not observed, but instead motif burst duration and rate were shaped daily and plateaued at adult values at the approximate time of song crystallization (about 90 days). For timing comparisons relative to behavior, Fig. 3A shows the sonogram of the canonical motif used to extract song motifs over both behavioral and neural activity columns below. The behavior extracted on each day and corresponding neural activity are shown side by side in Fig. 3, *B* and *C* (and Supplementary Fig. 2). Consistent with adult–juvenile comparisons, longitudinal recordings from the same finch reveal that the duration of activity bursts decreased and their rate increased as song learning proceeded (Fig. 3, *D* and *E*). Comparison across all days revealed a transition from long bursts at a low rate to short bursts at a higher rate at about 80–85 days posthatching in this animal. The interactions between age and burst duration and between age and burst rate were significant [ANOVA: Duration: $F(24,267353) = 63.8$, $P < 0.0001$; Rate: $F(24,15825) = 92.2$, $P < 0.0001$]. By using a canonical motif to extract the data, we sampled only the most mature vocalizations. The fact that developmental changes in bursting activity are clear in spite of this bias indicates that examination of less-mature vocalizations may reveal an even stronger effect. In addition, the finding that initially long continuous bursts appear to split into narrower bursts during development may provide a neural correlate of behavioral chunking (Williams 2004).

Transitions in HVC activity correlate with behavioral changes

Both HVC bursting activity and singing behavior change during development (see above). To determine the relationship between brain activity and behavior, we compared song behavior and HVC burst duration on a day-by-day basis. Individual song syllables were tracked during development using the computer program Sound Analysis Pro (Tchernichovski et al. 2000), which enables characterization and clustering of individual syllables. For each day, syllables were extracted from 50 randomly selected motifs. The entropy variance feature of a single syllable decreased during vocal learning (Fig. 4A, red line, bird Blue-70), as previously reported (Deregnacourt et al. 2005). This change in the vocalization was paralleled by a decrease in HVC burst duration during learned motifs (Fig. 4A, gray line). The correlation between the changes in HVC bursting activity and song behavior was significant and fairly strong (Fig. 4B). Depending on the syllable, entropy variance may increase or decrease with development (Deregnacourt et al. 2005). Analysis of entropy variance data from another juvenile finch (shown in Supplementary Fig. 3) indicated that HVC burst duration correlated with entropy variance regardless of the direction of developmental change of this syllable feature. Entropy variance only measures a single syllable, whereas our burst data are from the entire motif. Thus we also analyzed motif maturation by computing the percentage similarity of 25 randomly selected motifs from each day to 25 randomly selected motifs from the most mature day recorded. As with syllable entropy variance, song maturity and HVC burst duration were correlated (Supplementary Fig. 4). Collectively, these data indicate that the time course of the shortening of HVC bursts parallels the maturation of song behavior.

A subset of single HVC neurons are both premotor and postmotor

The findings that HVC activity outlasts vocalizations and that burst duration during learned motif production is longer in juveniles than in adults suggest the potential for temporal overlap of motor command and sensory feedback. However, the data are from a neural population. It is possible that different subtypes of HVC neuron are active during premotor and postmotor time periods. Were this the case, cellular integration of sensory and motor activities would not

occur in these neurons. To resolve this issue, we recorded stably from single units within the HVC of vocalizing juveniles using multielectrode techniques (triode; stereotrode in one case).

All HVC neuronal subtypes respond to auditory stimuli under anesthesia (Mooney 2000). However, auditory feedback during singing can occur only during the wake state (because singing can occur only in the wake state) and most HVC neurons do not respond to auditory stimuli in the wake state (Rauske et al. 2003). The auditory responsiveness of HVC neurons during actual singing behavior is completely unknown. In addition, postmotor activity that follows vocalizations, such as that described above, has not been investigated. Further, auditory responses and motor-associated activity have not been systematically compared in any song area. To understand sensorimotor integration in the song system, we compared premotor, postmotor, and auditory activity in stably recorded and rigorously identified HVC single units in juveniles that were actively vocalizing in the sensorimotor phase of song learning.

Using established spike-sorting techniques and software (Harris et al. 2000), we found that single HVC neurons fired both before and after vocalization (Figs. 5 and 6; Table 1). Figure 5 shows a sample of the simultaneous sonogram (Fig. 5A), raw triode recording (Fig. 5B), and reference recording (Fig. 5C; deflections of the reference recording reveal movement). The activity of a single unit (Fig. 5D, red) was identified using nine clustering parameters, three of which are shown (Fig. 5E, red). Single-wire, single-unit recording traditionally uses one clustering parameter (amplitude). For added rigor, we subjected all multielectrode data to strict criteria previously determined to characterize a reliable or “good” spike cluster (Schmitzer-Torbert et al. 2005) (i.e., the cluster is more likely to contain the activity of only a single neuron and all of the activity of that single neuron). The mean waveforms for each channel and 50 randomly selected clustered spikes are shown (Fig. 5F). We found that single neurons within HVC were active both before and after the vocalization (Fig. 5D).

Pre/postmotor neurons respond to auditory stimuli and belong to a putative subclass of interneurons

If the neurons that fired both before and after vocalizations (pre/postmotor neurons) described above integrate sensory and motor information, then they must also respond to sensory stimuli during waking. To test this hypothesis, we played back a variety of auditory stimuli to juvenile finches and recorded responses of single HVC neurons. Based on spike width, we found the three extracellularly characterized physiological subtypes of HVC neurons (Rauske et al. 2003): projection neurons, fast-spiking interneurons, and slower-spiking interneurons (examples shown in Fig. 6; Table 1).

All putative interneurons were active before vocalizations (premotor) (Table 1, Fig. 6, red and green cells). A subset of putative interneurons also fired after vocalization. The spike widths of these neurons that fired after vocalizations were not different from those of premotor-only neurons. Neurons with longer-duration action potentials (putative projection neurons) (Rauske et al. 2003) did not increase their firing rate immediately before vocalization. One of these neurons was inhibited by auditory stimuli (Table 1; Fig. 6, blue cell). Antidromic stimulation identified this cell as Area X-projecting (D Cygnar and TA Nick, unpublished results). Previous work showed that some RA-projecting neurons fire immediately before calls (Hahnloser et al. 2002). However, it did not appear from the Hahnloser study that all RA-projecting neurons fired immediately before the beginning of vocalizations. Thus except for the identified Area X-projecting neuron, the putative projection neurons that we recorded may have been either Area X- or RA-projecting (Table 1, “Other”).

All pre/postmotor neurons were putative interneurons [based on spike width comparisons with a previous study (Rauske et al. 2003)]. Pre/postmotor neurons were also auditory (e.g., Fig. 5, green unit, Supplementary Video 3). Based on spike rate, they were phasically activated by all

auditory stimuli (Supplementary Figs. 5 and 6). These neurons fired phasically to all auditory stimuli for about 250 ms. To assess stimulus preference, we examined spike rate after the phasic burst (250–700 ms after stimulus start). Overall, these neurons were activated most by tutor song (Fig. 6J), although the response to tutor song was not significantly greater than that to other conspecific songs. To assess whether the response was experience dependent, we played back songs from two adult male zebra finches that were not the song tutor. Consistent with biased responding to auditory stimuli resembling the tutor song, response to Conspecific Song 2 (Fig. 6J; 80% similar to tutor song as computed by the Sound Analysis Pro software) (Tchernichovski et al. 2000) tended to be greater than that to Conspecific Song 1 (51% similar).

These data indicate that a putative subclass of HVC inter-neuron 1) fires before vocalization (forms part of the motor command network), 2) fires after vocalization (exhibits the temporal dynamics necessary for sensorimotor integration), and 3) responds to auditory stimulation (receives auditory inputs). These are required characteristics for sensorimotor integration in this system. Interestingly, the identification of premotor neurons that respond phasically to sounds suggests a role for the introductory notes that occur before singing (Williams 2004): they may be used to “boot up” the song system pattern generator by driving it with autogenous sounds.

DISCUSSION

Understanding song development requires the measurement of neural motor activity during singing. However, previous studies of the developing song system have focused either on auditory responses (Nick and Konishi 2005a,b; Solis and Doupe 2000; Volman 1993) or on brain areas that are not necessary for song production (Oliveczky et al. 2005). We used extremely stable recording techniques to assess the development of activity in the song control nucleus HVC. Recording neural population activity over several weeks revealed that activity bursts during learned song behavior decrease in duration and increase in rate with song learning. In addition, multielectrode recording combined with spike-sorting techniques have enabled reliable identification and systematic analysis of the auditory and motor activities of single neurons over several hours. We found that the same neurons are premotor and auditory. Further, these premotor neurons exhibit prolonged bursting that outlasts the vocalization. This “postmotor” bursting was not previously described and may offer a key to the mechanisms underlying song learning.

Prolonged bursting was also previously recorded in developing sensory systems before periods of extreme environmental sensitivity, such as the critical period for ocular dominance plasticity (Fagiolini and Hensch 2000). Our data indicate that a subpopulation of HVC neurons exhibit prolonged bursts that outlast the vocalization in the plastic juvenile. These data suggest that prolonged bursting may have a role in the environmental shaping of neural circuits, whether they are sensory or sensorimotor. Prolonged bursting in HVC occurs before, during, and after learned vocalizations in juveniles. Whether prolonged bursting is also associated with unlearned vocalizations is an important question for future study.

It is possible that the prolonged bursts after vocalizations result from prolonged sensory feedback. Because sensory feedback in the adult takes <100 ms (Sakata and Brainard 2006; Troyer and Doupe 2000) and some observed prolonged bursts were much longer (>1 s), the sensory feedback would have to be considerably delayed. In addition, sensory feedback cannot explain the prolonged activity that occurred before the start of the vocalization that we observed. A more parsimonious hypothesis is that the prolonged bursting reflects the activity of the HVC pattern generator (Mooney and Prather 2005; Solis and Perkel 2005). Early in the sensorimotor phase, the pattern generator or its synaptic targets may not effectively engage or

coordinate the muscles of the respiratory system and vocal organ, resulting in the observed disconnect between HVC activity and song behavior.

Is there a function for the prolonged activity? There are three main hypotheses that are not mutually exclusive. 1) The prolonged activity may be necessary for the development of individual neurons within HVC; for example, HVC neurons may require activity-dependent release of trophic factors from their synaptic targets to develop appropriately (e.g., see Nick and Ribera 2000). 2) The prolonged bursting may be required for the appropriate synaptic connectivity of neural circuits in the song system; for example, prolonged inhibition of Area X-projecting neurons may be necessary to release a downstream nucleus from inhibition and thus enable vocal experimentation (Kao et al. 2005; Olveczky et al. 2005). 3) Sensorimotor integration may not be possible without the prolonged bursting (Fig. 7). Sensorimotor integration and learning may require the temporal coincidence of activity in presynaptic and postsynaptic neurons, which underlies plasticity in many systems (Bi and Poo 2001; Kandel 2001; Spitzer 2004). As the distance and number of synapses from the periphery increase, as in the vertebrate forebrain, the coincidence of activity in sensory and motor processing streams becomes more difficult to achieve as the result of significant synaptic delays between related motor and sensory activity. We propose that, to enable plasticity, the time gap between motor command and sensory feedback (Troyer and Doupe 2000) is bridged by ongoing activity in the motor control system during sensorimotor learning (Fig. 7A), but not during adulthood (or to a lesser degree), when the capacity for plasticity is decreased (Fig. 7B). Further, we hypothesize that prolonged bursting activity is primarily related to motor command, but is modulated by sensory feedback. According to these hypotheses, prolonged motor activity allows postsynaptic potentials from sensory feedback pathways to bias spike-timing-dependent plasticity and shape the motor pattern. The weak preference for tutor song expressed by the pre/postmotor neurons makes sense in younger finches that will utter sounds that only remotely resemble the tutor song because the best of initially poor imitations must be reinforced and shaped toward the mature song (Immelmann 1969). In addition, the thousands of motif repetitions during development combined with Hebbian activity-dependent plasticity would amplify even the smallest of synaptic biases, making a larger effect potentially detrimental to plasticity.

Three key predictions of the sensorimotor integration hypothesis described above are supported by data presented herein: 1) HVC activity during learned behavior is less precise during vocal plasticity, consistent with prolonged activity in the motor network; 2) single neurons that fire after vocalization in juveniles also fire before, consistent with their hypothesized role in motor planning; and 3) these same pre/postmotor neurons respond to auditory stimuli during waking, revealing a cellular site of convergence of sensory and motor signals.

We have identified single HVC neurons that appear to integrate sensory and motor information in the sensorimotor phase of vocal learning. The next key steps in understanding the role of HVC in song learning require assessment of the sensory response of HVC neurons to auditory feedback during the behavioral act of singing, characterization of the effects of these putative interneurons on HVC neuronal ensembles, and perturbation of inhibitory pathways within HVC and examination of behavioral plasticity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank M. Adam, D. Cygnar, J. Despinoy, J. Jackson, A. Kinnischtzke, M. Negia, L. Onikoro, A. D. Redish, and S. Zhang for technical assistance; J. Ashe, M. Coleman, J. Dubinsky, M. Konishi, L. Lanier, P. Mermelstein, J. L.

Peña, and R. Poppele for reviewing preliminary drafts of the manuscript; and E. Knudsen, P. Narins, and A. D. Redish for helpful discussions.

GRANTS

This work was supported by The John Merck Scholars Program, National Institute of Deafness and Other Communication Disorders Grant R01-DC-007384, and the Minnesota Medical Foundation.

References

- Aoki N, Nick TA. Sensorimotor integration in the song system: a neuronal analysis. *Soc Neurosci Abstr* 2006;32:818–19.
- Arnold AP. The effects of castration on song development in zebra finches (*Poephila guttata*). *J Exp Zool* 1975;191:261–278. [PubMed: 1113072]
- Ashmore RC, Wild JM, Schmidt MF. Brainstem and forebrain contributions to the generation of learned motor behaviors for song. *J Neurosci* 2005;25:8543–8554. [PubMed: 16162936]
- Bi G-Q, Poo M-M. Synaptic modification by correlated activity: Hebb's postulate revisited. *Annu Rev Neurosci* 2001;24:139–166. [PubMed: 11283308]
- Bolhuis JJ, Gahr M. Neural mechanisms of birdsong memory. *Nat Rev Neurosci* 2006;7:347–357. [PubMed: 16760915]
- Bottjer S, Miesner E, Arnold A. Forebrain lesions disrupt development but not maintenance of song in passerine birds. *Science* 1984;224:901–903. [PubMed: 6719123]
- Brainard MS, Doupe AJ. Interruption of a basal ganglia-forebrain circuit prevents plasticity of learned vocalizations. *Nature* 2000;404:762–766. [PubMed: 10783889]
- Crandall SR, Nick TA. Development of timing precision in a sensorimotor circuit. *Soc Neurosci Abstr* 2006;32:818.18.
- Deregnacourt S, Mitra PP, Feher O, Pytte CL, Tchernichovski O. How sleep affects the developmental learning of bird song. *Nature* 2005;433:710–716. [PubMed: 15716944]
- Fagioli M, Hensch TK. Inhibitory threshold for critical-period activation in primary visual cortex. *Nature* 2000;404:183–186. [PubMed: 10724170]
- Fee MS, Kozhevnikov AA, Hahnloser RHR. Neural mechanisms of vocal sequence generation in the songbird. *Ann NY Acad Sci* 2004;1016:153–170. [PubMed: 15313774]
- Goller F, Cooper BG. Peripheral motor dynamics of song production in the zebra finch. *Ann NY Acad Sci* 2004;1016:130–152. [PubMed: 15313773]
- Hahnloser RHR, Kozhevnikov AA, Fee MS. An ultra-sparse code underlies the generation of neural sequences in a songbird. *Nature* 2002;419:65–70. [PubMed: 12214232]
- Harris KD, Henze DA, Csicsvari J, Hirase H, Buzsáki G. Accuracy of tetrode spike separation as determined by simultaneous intracellular and extracellular measurements. *J Neurophysiol* 2000;84:401–414. [PubMed: 10899214]
- Immelmann, K. Song development in the zebra finch and other estrilid finches. In: Hinde, RA., editor. *Bird Vocalizations*. Cambridge, UK: Cambridge Univ. Press; 1969. p. 61–74.
- Jarvis ED, Scharff C, Grossman M, Ramos JA, Nottebohm F. For whom the bird sings: context-dependent gene expression. *Neuron* 1998;21:775–788. [PubMed: 9808464]
- Kandel ER. The molecular biology of memory storage: a dialogue between genes and synapses. *Science* 2001;294:1030–1038. [PubMed: 11691980]
- Kao MH, Doupe AJ, Brainard MS. Contributions of an avian basal ganglia-forebrain circuit to real-time modulation of song. *Nature* 2005;433:638–643. [PubMed: 15703748]
- Katz LC, Gurney ME. Auditory responses in the zebra finch's motor system for song. *Brain Res* 1981;221:192–197. [PubMed: 6168335]
- Konishi M. The role of auditory feedback in the control of vocalization in the white-crowned sparrow. *Z Tierpsychol* 1965;22:770–783. [PubMed: 5874921]
- Margoliash D. Acoustic parameters underlying the responses of song-specific neurons in the white-crowned sparrow. *J Neurosci* 1983;3:1039–1057. [PubMed: 6842281]
- McCasland JS. Neuronal control of bird song production. *J Neurosci* 1987;7:23–39. [PubMed: 3806194]

- McCasland JS, Konishi M. Interaction between auditory and motor activities in an avian song control nucleus. *Proc Natl Acad Sci USA* 1981;78:7815–7819. [PubMed: 6950421]
- Mooney R. Different subthreshold mechanisms underlie song selectivity in identified HVC neurons of the zebra finch. *J Neurosci* 2000;20:5420–5436. [PubMed: 10884326]
- Mooney R, Prather JF. The HVC microcircuit: the synaptic basis for interactions between song motor and vocal plasticity pathways. *J Neurosci* 2005;25:1952–1964. [PubMed: 15728835]
- Nick TA. Neural auditory feedback in the song nucleus HVC. *Soc Neurosci Abstr* 2005;31:203.3.
- Nick TA, Konishi M. Dynamic control of auditory activity during sleep: correlation between song response and EEG. *Proc Natl Acad Sci USA* 2001;98:14012–14016. [PubMed: 11717459]
- Nick TA, Konishi M. Neural auditory selectivity develops in parallel with song. *J Neurobiol* 2005a;62:469–481. [PubMed: 15616963]
- Nick TA, Konishi M. Neural song preference during vocal learning in the zebra finch depends on age and state. *J Neurobiol* 2005b;62:231–242. [PubMed: 15459895]
- Nick TA, Ribera AB. Synaptic activity modulates presynaptic excitability. *Nat Neurosci* 2000;3:142–149. [PubMed: 10649569]
- Nottebohm F. The road we travelled: discovery, choreography, and significance of brain replaceable neurons. *Ann NY Acad Sci* 2004;1016:628–658. [PubMed: 15313798]
- Nottebohm F, Stokes TM, Leonard CM. Central control of song in the canary, *Serinus canarius*. *J Comp Neurol* 1976;165:457–486. [PubMed: 1262540]
- Olveczky BP, Andalman AS, Fee MS. Vocal experimentation in the juvenile songbird requires a basal ganglia circuit. *PLoS Biol* 2005;3:0902–0909.
- Perkel DJ. Origin of the anterior forebrain pathway. *Ann NY Acad Sci* 2004;1016:736–748. [PubMed: 15313803]
- Rauske PL, Shea SD, Margoliash D. State and neuronal class-dependent reconfiguration in the avian song system. *J Neurophysiol* 2003;89:1688–1701. [PubMed: 12626633]
- Sakata JT, Brainard MS. Real-time contributions of auditory feedback to avian motor control. *J Neurosci* 2006;26:9619–9628. [PubMed: 16988032]
- Schmidt MF, Konishi M. Gating of auditory responses in the vocal control system of awake songbirds. *Nat Neurosci* 1998;1:513–518. [PubMed: 10196550]
- Schmitzer-Torbert N, Jackson J, Henze D, Harris K, Redish AD. Quantitative measures of cluster quality for use in extracellular recordings. *Neuroscience* 2005;131:1–11. [PubMed: 15680687]
- Solis MM, Doupe AJ. Compromised neural selectivity for song in birds with impaired sensorimotor learning. *Neuron* 2000;25:109–121. [PubMed: 10707976]
- Solis MM, Perkel DJ. Rhythmic activity in a forebrain vocal control nucleus *in vitro*. *J Neurosci* 2005;25:2811–2822. [PubMed: 15772341]
- Spitzer NC. Coincidence detection enhances appropriate wiring of the nervous system. *Proc Natl Acad Sci USA* 2004;101:5311–5312. [PubMed: 15067118]
- Suthers RA, Zollinger SA. Producing song: the vocal apparatus. *Ann NY Acad Sci* 2004;1016:109–129. [PubMed: 15313772]
- Tchernichovski O, Mitra PP, Lints T, Nottebohm F. Dynamics of the vocal imitation process: how a zebra finch learns its song. *Science* 2001;291:2564–2569. [PubMed: 11283361]
- Tchernichovski O, Nottebohm F, Ho CE, Pesaran B, Mitra PP. A procedure for an automated measurement of song similarity. *Anim Behav* 2000;59:1167–1176. [PubMed: 10877896]
- Theunissen FE, Doupe AJ. Temporal and spectral sensitivity of complex auditory neurons in the nucleus HVC of male zebra finches. *J Neurosci* 1998;18:3786–3802. [PubMed: 9570809]
- Troyer TW, Doupe AJ. An associational model of birdsong sensorimotor learning. I. Efference copy and the learning of song syllables. *J Neurophysiol* 2000;84:1204–1223. [PubMed: 10979996]
- Volman SF. Development of neural selectivity for birdsong during vocal learning. *J Neurosci* 1993;13:4737–4747. [PubMed: 8229196]
- Vu ET, Mazurek ME, Kuo Y-C. Identification of a forebrain motor programming network for the learned song of zebra finches. *J Neurosci* 1994;14:6924–6934. [PubMed: 7965088]
- Williams H. Birdsong and singing behavior. *Ann NY Acad Sci* 2004;1016:1–30. [PubMed: 15313767]

- Williams H, Mehta N. Changes in adult zebra finch song require a forebrain nucleus that is not necessary for song production. *J Neurobiol* 1999;39:14–28. [PubMed: 10213450]
- Zann, RA. *The Zebra Finch: A Synthesis of Field and Laboratory Studies*. Oxford, UK: Oxford Univ. Press; 1996.

FIG. 1.

Vocal-associated activity is developmentally modulated in the song system. In the HVC of juvenile zebra finches, activity can begin hundreds of milliseconds to seconds before vocalization and continue seconds after (A; bird Blue-81, age 67 days). HVC activity in adults is tightly locked to the vocalization (B; bird Black-161, age >200 days). A and B: sonogram of vocal behavior is overlaid with the HVC multiunit recording to facilitate comparison of the timing of activity. For comparison of pre- and postmotor activity, each *inset* shows amplitude envelopes of electrode (black) and vocal (red) data in an expanded timescale (total frame 1.8 s). Dotted line in the *inset* indicates baseline activity. Gray shading indicates the timing of the vocalization. Scale bar: 100 μ V for A, 75 μ V for B; 1 s.

FIG. 2.

Brain activity that drives learned vocal behavior changes during development. In juveniles, the HVC neural activity during behavioral song motifs (*A*; example finch Blue-46, age 65 days) is longer in duration and lower in frequency than that of adults (*B*; example finch Black-161, age >200 days). *A* and *B*: all 5 graphs are temporally aligned. From the *top*: 1) sonogram of the canonical behavioral song motif used to identify developing motifs through cross-correlation; 2) amplitude envelopes of the canonical behavioral motif (red) and all behavioral motifs that fell within 1 SD of the mean amplitude envelope for that day (black); 3) a 3-dimensional (3D) plot of the behavioral vocalization amplitude during the first 116 motifs with *x*-axis time, *y*-axis motif number, and *z*-axis amplitude; white indicates the highest amplitude,

black the lowest; 4) mean amplitude envelope of HVC population neural activity (red) and of all motifs shown in 2 (black) (dashed yellow line indicates the mean of all motif activity for that day); and 5) 3D plot of the HVC neural activity associated with the first 116 motifs with x -axis time, y -axis motif number, and z -axis amplitude. For illustration purposes only, green bars indicate bursts. For ease of comparison across birds, all data in *A* and *B* were limited to the maximum number of motifs of either bird (adult in *B*, $n = 116$). Scale bar: 100 ms. *C* and *D*: group comparison of bursts (peaks above mean; 2 each are indicated in *A* and *B* by green bars) across HVC activity motifs reveals that the mean duration of HVC activity bursts decreases, whereas the mean rate of bursts increases with development [Juvenile finches (62–72 days): $n = 5$ birds, 6 electrodes; Adult finches (141 to >200 days): $n = 4$ birds, 6 electrodes; $P < 0.02$, Wilcoxon rank-sum test]. These statistics were obtained motif by motif and thus do not depend on the temporal alignment of motifs across any time period.

FIG. 3.

Premotor activity during learned vocal behavior is developmentally regulated. Continuous recording throughout the sensorimotor phase reveals the development of neural activity underlying learned song motif behavior (data shown are from a single bird, Blue-70, age 72–111, $n = 22,527$ motifs). **A:** sonogram of canonical motif used to retrieve song motifs across development is aligned with 3D plots below for temporal comparisons. **B and C, left, top:** amplitude envelopes of the canonical motif (red) and all motifs that fell within 1 SD of the mean amplitude envelope for that day (black). **Left, bottom:** 3D plot of the amplitude envelopes of the first 200 vocalizations for the given day with x-axis time, y-axis motif number, and z-axis amplitude. White indicates the highest amplitude, black the lowest. **Right, top:** mean amplitude envelopes of HVC population activity (red) and of all motifs shown in *top left* (black)

(Dashed yellow line indicates the mean of all motif activity for that day.) *Right, bottom*: similar to *bottom left*, but amplitude envelopes of HVC neural population activity are shown. Scale bar: 100 ms. *D* and *E*: data across all 37 days of recording reveals a shift in neural bursting activity with regard to duration and rate at the end of the sensorimotor phase.

FIG. 4.

Changes in HVC bursting activity correlate with song features. *A*: as previously reported, a descriptive feature of a single learned syllable—entropy variance—changed during song learning (Deregnaucourt et al. 2005). The developmental timeline of changes in entropy variance (red line) matched corresponding changes in HVC bursting activity during learned motifs (gray dotted line) in the developing finch shown in Fig. 3. *Insets*: representative sonograms of the analyzed syllable at 3 different ages. Entire frame of each *inset*: *x*, 250 ms; *y*, 0.5–8 kHz. *B*: correlation between entropy variance and HVC motif burst duration was significant for this finch (Pearson $r = 0.61$; $P < 0.0001$).

FIG. 5.

Single-unit recordings reveal that the same neurons are pre- and postmotor during singing in the sensorimotor phase. *A*: sonogram of juvenile vocalizations is temporally aligned with data below. Red color indicates increased amplitude. All data are from bird Orange-273, age 60 days, implanted 3 days. *B*: raw HVC multi-electrode voltage traces (each of 3 electrode recordings shown separately). *C*: reference electrode recording confirms that recorded activity was neural (not movement) and that it emanated from HVC and not the reference area. Scale bar: 100 μ V, 1 s. *D*: spikes of a single neuron as computed by spike-sorting techniques. (This spike cluster was well separated: L-ratio = 0.0125; isolation distance = 60.3.) Gray bars indicate timing of the vocalizations. *E*: 3D plot of 3 of the 9 parameters used to cluster the spikes shown in *D*. Black dots were not part of the well-separated cluster, whereas the red dots were included. The large cloud of black dots consists of spikes and noise that were just above the inclusion threshold. *F*: mean (red) and 50 randomly selected (black) waveforms of spikes contained in the red cluster in *E*. Electrodes shown in *B* top to bottom are here left to right. Reference is on the far right. Scale bar: 100 μ V, 1 ms.

FIG. 6.

Juvenile HVC neurons that are active before and after vocalizations also respond to playback of auditory stimuli. *A*: sonogram of a 15-s time segment of recording chamber sounds during song playback and vocalization. The sonogram is temporally aligned to the clustered spikes below. *B*: activity of 3 single units identified by spike-sorting techniques. Red: putative fast-spiking interneuron; green: putative slower-spiking interneuron; blue: putative projection neuron. Note that the green neuron fires during song playback, before call vocalizations, and after calls. Color codes indicate the same single units throughout this figure. *C–E*: mean (in color) and 50 randomly selected (black) waveforms of single units shown in *B*. For each panel, *left* and *middle* waveforms are recordings from a stereotrode. *Right* waveform is the reference. Note that although in some cases the overall waveform is complicated by a 2nd spike, only the 1st 16 bits of these 48-bit waveforms were used for clustering. Scale bar: 100 μ V, 1 ms. *F–H*: vocal-associated activity of each of the three units. Red unit was premotor only, the green unit was both pre- and postmotor, and the blue unit did not change its firing pattern before or after vocalizations. *I–K*: auditory responses of the single units shown above (* $P < 0.05$ vs. silence, + $P < 0.05$ vs. tutor, ANOVA with post hoc Tukey–Kramer).

FIG. 7.

The finding that, during learning, activity in a premotor area outlasts the behavior suggests a simple model to overcome the problem of sensory-motor delays: during song learning (A), but not in adulthood (B), motor command activity lasts long enough to enable overlap of ongoing motor activity and sensory feedback.

TABLE 1
Properties of the spikes of single neurons in vocalizing juvenile zebra finches

	Peak-to-peak Spike width, ms	25% Positive peak Spike width, ms	ISI, ms	Baseline Spike Rate Hz,	Premotor Spike Rate, Hz	Postmotor Spike Rate, Hz	Auditory Response	N Spikes
Pre/Post-motor	0.21 ± 0.00 0.15 ± 0.00 0.14 ± 0.00 0.13 ± 0.00 0.17 ± 0.00 0.13 ± 0.00 0.14 ± 0.00 0.22 ± 0.00 0.21 ± 0.00 0.26 ± 0.00 0.18 ± 0.00 0.36 ± 0.00 0.35 ± 0.00 0.37 ± 0.00 0.43 ± 0.00 0.42 ± 0.00	0.30 ± 0.00 0.08 ± 0.00 0.07 ± 0.00 0.10 ± 0.00 0.17 ± 0.00 0.09 ± 0.00 0.10 ± 0.00 0.33 ± 0.00 0.34 ± 0.00 0.29 ± 0.00 0.22 ± 0.00 0.49 ± 0.00 0.51 ± 0.00 0.53 ± 0.00 0.62 ± 0.00 0.60 ± 0.00	160.3 ± 1.1 150.8 ± 0.8 209.2 ± 1.2 143.1 ± 0.6 557.8 ± 6.2 804.0 ± 15.6 330.1 ± 2.6 2455.3 ± 299 422.3 ± 20.8 201.8 ± 1.1 45.1 ± 0.1 809.0 ± 9.1 1465.8 ± 21.8 407.5 ± 2.0 448.4 ± 2.6 664.5 ± 5.6	2.2 ± 0.6 4.3 ± 0.4 3.2 ± 0.3 7.2 ± 0.5 1.2 ± 0.3 0.9 ± 0.3 2.8 ± 0.4 0.1 ± 0.0 1.4 ± 0.4 4.4 ± 0.4 16.1 ± 0.9 1.2 ± 0.4 0.7 ± 0.2 2.1 ± 0.1 2.0 ± 0.2 1.4 ± 0.1	40.8 ± 1.8 41.8 ± 1.7 36.9 ± 1.4 17.2 ± 1.2 69.0 ± 2.8 42.3 ± 7.0 54.1 ± 2.8 54.1 ± 2.2 70.0 ± 2.1 12.8 ± 0.9 25.5 ± 1.2 0.8 ± 0.3 0.4 ± 0.2 0.3 ± 0.1 0.8 ± 0.1 0.8 ± 0.1	12.2 ± 0.9 16.0 ± 1.0 25.9 ± 1.4 13.2 ± 1.0 0.4 ± 0.2 0.0 ± 0.0 0.0 ± 0.0 0.0 ± 0.0 0.1 ± 0.1 5.9 ± 0.6 13.6 ± 1.0 0.2 ± 0.1 0.5 ± 0.2 0.0 ± 0.0 0.0 ± 0.0 0.1 ± 0.1	YES YES YES NA NO NO NO NO NO NO YES, decrement NO NA NA NA NA	45,380 66,523 59,908 69,863 13,272 11,567 24,674 2,406 10,002 49,644 221,900 8,974 6,856 36,795 33,491 22,453

Values are means ± SE. "Pre/Postmotor" cells had significant activity above baseline both before and after vocalization (50-ms window). "Premotor" cells only had significant activity above baseline in the premotor time window. "Other" cells had no pre- or postmotor activity above baseline. Auditory neurons had a significant response above or below baseline to at least one auditory stimulus. Sound playback data were available for four of six finches. NA, not available.