Neural Song Preference during Vocal Learning in the Zebra Finch Depends on Age and State

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ABSTRACT: **The zebra finch acquires its song by first memorizing a model song from a tutor and then matching its own vocalizations to the memory trace of the tutor song, called a template. Neural mechanisms underlying this process require a link between the neural memory trace and the premotor song circuitry, which drives singing. We now report that a premotor song nucleus responds more to the tutor song model than to every other stimulus examined, including the bird's own song (BOS). Neural tuning to the song model occurred only during waking and peaked during the template-matching period of development, when the vocal motor output is sculpted to match the tutor song. During the same developmental phase, the BOS was the most effective excitatory stimulus during sleep. The preference for BOS compared to tutor song inverted with sleep/wake state. Thus, song preference shifts with development and state.** J Neurobiol 62: 231–242, 2005

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Birds and humans learn vocal signals in two phases: a perceptual phase during which sounds are memorized and a production phase during which vocalizations are matched to the learned sounds (Konishi, 1965, 1985; Doupe and Kuhl, 1999). In zebra finches, the perceptual, or sensory, phase occurs from fledging (≈18 days) to 65 days posthatching (Immelmann, 1969; Eales, 1985; Böhner, 1990). During this phase, the tutor song template is formed. The production or sensorimotor phase begins at 35–40 days (Immelmann, 1969; Konishi, 1985). The first "songs" are slow and uniform series of sounds that resemble begging calls (Immelmann, 1969). The song changes considerably over 40 days to become the highly structured, repetitive adult form. The sensorimotor phase ends at 80–90 days with song crystallization, after which the syllable structure, phrase sequence, and song duration are fixed (Immelmann, 1969).

Songbirds have a set of interconnected brain nuclei for production and learning of song (Nottebohm et al., 1976). Neurons of the song system are precisely tuned to the bird's own song (BOS) in the anesthetized adult (Margoliash, 1983). Previous studies in anesthetized, young white-crowned sparrows have suggested that BOS is the most effective activating stimulus for the premotor song nucleus HVC of the nidopallium (acronym used as proper name) (Reiner et al., 2004) during the template-matching phase (Volman, 1993). In young birds, HVC neurons preferred BOS even

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These data were previously presented in abstract form (Nick, 2003).

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over tutor song. Few other studies have obtained neural responses to the tutor song within the song system, although the template theory predicts a tutor song memory trace. An exception is the work of Solis and Doupe (1997, 1999), who found neurons sensitive to BOS, tutor song, or both BOS and tutor song in Area X and the lateral magnocellular nucleus of the anterior nidopallium (lMAN) in anesthetized young animals (≈60 days). In every developmental study to date (all in anesthetized birds), BOS was found to be the most effective stimulus for triggering responses in the song system of young birds (Volman, 1993; Solis and Doupe, 1997).

However, recent multiunit recordings in adults show that auditory responses are state-dependent (Schmidt and Konishi, 1998; Nick and Konishi, 2001), such that auditory stimuli induce large responses during sleep and anesthesia, but very little activity in the waking HVC. These findings were confirmed in a single-unit study that reported that only a single class of interneurons responded to auditory stimuli during waking (Rauske et al., 2003). Because all developmental studies were done under anesthesia and because adult responses under anesthesia are different from those during waking, we systematically examined how HVC stimulus preference changed during song learning with special reference to wake and sleep states. We recorded with the same chronically implanted multiunit electrodes during waking and sleep from the beginning of the sensorimotor phase into adulthood.

METHODS

Animals and Surgery

General methods were previously described (Schmidt and Konishi, 1998; Nick and Konishi, 2001). Fifteen male zebra finches (*Taeniopygia guttata*) were hatched and reared in our facility and maintained on a 12:12 light cycle. Birds were implanted with electrodes at several different ages (32–200 days posthatch). Nine birds were recorded at multiple ages. Before surgery, young finches were either kept with their natural parents in the aviary or placed in soundproof chambers with their foster parents. Two of the birds reared in the aviary in separate clutches had the same tutor. All other aviary-reared birds had distinct tutors. Two birds were reared by a Bengalese finch (*Lonchura striata*) mating pair in a soundproof box. The juvenile aviary birds were visually isolated from adult males other than their father with opaque dividers, which restricts learning to the tutor in the cage with the juvenile (Eales, 1989; Solis and Doupe, 1997, 1999, 2000). An institutional animal care committee approved all procedures.

Electrophysiology and Song Playback

All multiunit electrodes and the electroencephalogram (EEG) electrode were referenced to an animal ground. Differential electrode recordings were obtained by referencing two of the multiunit electrodes within HVC to the third multiunit electrode that was placed in a less active brain area outside of HVC. Multiunit recording provides more stability, but less sensitivity relative to single-unit and intracellular investigations. The objective of this study was to record HVC activity across many days, which necessitated use of chronic multiunit techniques.

Data collection software was written by A. Leonardo using Labview (National Instruments). Songs for playback were digitized at 40 kHz and edited with Matlab (Mathworks, Natick, MA). To ensure that the recording electrodes were correctly placed in HVC, we measured premotor activity for song in awake birds with these electrodes prior to each playback recording session. Additionally, electrode placement was confirmed with Cresyl violet histology in all birds.

Playback experiments began at least 2 h after the soundproof chamber lights were turned off, during the bird's normal lights-off cycle. Sleep data were collected in a dark, quiet chamber during the bird's established sleeping period, during which finches normally sleep from ≈35 min after lights off to ≈60 min before lights on. Based on adult sleep studies that used electroencephalography to monitor sleep, zebra finches occasionally awakened during the first ≈10 playback sounds (less than two trials in this study), but then remained asleep through further stimulation (Nick and Konishi, 2001). Thus, our sleep data may have contained a small number of wake trials (0–4%).

All birds were presented with sets of 50–100 trials (referred to as "Trial Sets") consisting of at least six stimuli, in random order: silence, the tutor song (from either an adult male zebra finch or Bengalese finch), conspecific song, heterospecific song (from a Bengalese finch), and white noise (3 s). Conspecific Song 1 and Conspecific Song 2 were invariant stimuli for all animals examined. Each trial lasted 6 s. The intertrial recovery period lasted 15 s. All songs used for playback were recorded in the absence of a female (undirected). When available, the bird was also presented with multiple copies of his own song (BOS). The BOS changes rapidly and dramatically during the sensorimotor phase (Immelmann, 1969). Up to four songs for playback were collected each recording day. Only the most recent BOS stimulus that elicited the most HVC activity during sleep (recorded within 72 h of playback; Nick, 2003) was used for comparisons between BOS and non-BOS stimuli.

We compared sound pressure levels between BOS and tutor song using a 1 in condenser microphone and Type 2604 microphone amplifier (Brüel and Kjœr, Copenhagen). Sound pressure level was measured 15 cm from the speaker with an A-type filter, 200–10,000 Hz. There was no significant difference between the peak amplitude of the best BOS stimuli (45–65 days) and the tutor songs (tutor: 90.5 ± 0.4 dB SPL; BOS 88.8 ± 0.6 dB SPL; *t* test, $p = 0.34$;

$n = 8$ songs each). Comparison of root mean square (RMS) of oscillograms of tutor song and best BOS stimuli (45–65 days) also did not reveal a significant difference (tutor: 171.2 ± 7.7 μV; BOS 204.7 ± 7.3 μV; $p = 0.39$; $n = 8$ songs each).

Analysis

All data were analyzed with Matlab functions included with the software or written by T.A.N. A function written in Matlab automatically discarded trials with movement artifacts (revealed by thresholding of large deflections in the EEG, or, in the case of Orange 9, the reference multiunit record) and trials during which the bird vocalized. Vocalizations were identified by subtraction of a filtered amplitude envelope of an exemplar trial (a sound recording of only the playback stimulus and no vocalizations) from a filtered amplitude envelope of the trial under consideration. Any increases in sound amplitude at any point in the trial, from 1 s preceding the trial to 20 ms after the playback stimulus ended, led to rejection of the trial. The same Matlab function was applied to all data. Thus, the criteria for exclusion of trials were consistent and objective. For further analysis, at least three trials had to remain after exclusion based on movement and vocalization. Consistent with previous studies of sleep in young mammals (Gramsbergen, 1976; Frank and Heller, 1997; Marshall et al., 2002), we found that EEG was not a reliable indicator of sleep in young zebra finches. Briefly, we computed the power spectral density (PSD) of the EEG voltage trace during trials when no stimulus was presented in birds aged 45–65 days. The mean of the PSD curve between 1–4 Hz was compared between sleep and wake states. There was no difference in this measure between sleep and waking ($n = 6$ birds; wake: 106.2 ± 0.1 μV^2; sleep: 105.9 ± 0.1 μV^2; N.S.). As in prior finch studies (Dave et al., 1998; Dave and Margoliash, 2000; Rauske et al., 2003), we used behavioral criteria to determine wake state. To be classed as "awake", the birds had to have open eyes and feathers that lay close to the body and were not ruffled. Birds were not visually observed during sleep, but movement artifacts and vocalizations were monitored and used to discard trials in which the animal awakened and moved.

For comparison of responses to BOS during different developmental periods and states, the most recent BOS that induced the greatest response in HVC was used (Nick, 2003). This best BOS was also used for comparisons of responses to BOS with those to other stimuli. In cases when more than 1 day during a developmental period was sampled from the same bird and recording site, only data from the oldest day were used. Thus, each recording site only contributed one data set per graph. The data were divided into two developmental groups: sensory/early sensorimotor (35–69 days) and late sensorimotor/adult (≥70 days).

The RMS of the HVC voltage trace for each trial was calculated for each 5 ms bin. This resulted in a two-dimensional matrix of RMS values with trial number and time within trial as axes. The RMS, in contrast to thresholding, includes all neurons recorded. Larger units will have proportionally larger effects on the RMS. The HVC response versus time was calculated by subtracting the mean RMS of all 5 ms bins (across all trials) during a 1 s prestimulus period ($\mathrm{RMS}_{\mathrm{BASE}}$) from the mean RMS of each 5 ms bin (across all trials). The RMS response for each trial was calculated by subtracting the mean RMS of all 5 ms bins during a 1 s prestimulus period ($\mathrm{RMS}_{\mathrm{BASE}}$) from the mean RMS of all 5 ms bins during sound presentation, or, in the case of the "silence" stimulus, a silent period of duration equal to the tutor song ($\mathrm{RMS}_{\mathrm{SONG}}$). Recording sites contributed one mean RMS response value for each condition for the HVC response comparisons shown in bar graphs.

The preference for stimulus A over stimulus B was described as (Green and Swets, 1966; Solis and Doupe, 1997)

$$d'_{A-B} = \frac{2(\overline{RS_{\mathrm{A}}} - \overline{RS_{\mathrm{B}}})}{\sqrt{\sigma_{\mathrm{A}}^2 + \sigma_{\mathrm{B}}^2}} \tag{1}$$

where d'_{A-B} indicates preference for stimulus A over stimulus B, σ_{A}^2 and σ_{B}^2 are the variances of responses to stimulus A and B, respectively, and $\overline{RS_{\mathrm{A}}}$ and $\overline{RS_{\mathrm{B}}}$ are the mean normalized response strength (*RS*) to stimulus A and B, respectively. The *RS* was defined as:

$$RS = \frac{\mathrm{RMS}_{\mathrm{SONG}} - \mathrm{RMS}_{\mathrm{BASE}}}{\mathrm{RMS}_{\mathrm{SONG}} + \mathrm{RMS}_{\mathrm{BASE}}} \tag{2}$$

All data were compared using either a Student's *t* test or a one-way ANOVA with a posthoc Tukey-Kramer test for significance ($p < 0.05$, unless otherwise noted). The specific test is noted for each figure. Data are presented as mean ± standard error of the mean.

RESULTS

HVC Responds to Tutor Song Playback during Waking in the Juvenile

During waking, playback of the tutor song induced robust activity in the HVC of young birds [Fig. 1(a)]. This contrasts with the adult HVC, which responds very little to auditory stimuli during waking (Schmidt and Konishi, 1998; Nick and Konishi, 2001). In some trials, premotor activity, which precedes and drives vocalizations, was recorded within milliseconds of tutor song playback [Fig. 1(b–d); Supplementary Video 1]. Thus, responses to tutor song occurred when the bird was awake, as indicated by vocalizations, and the electrodes were in HVC, as indicated by the premotor activity.

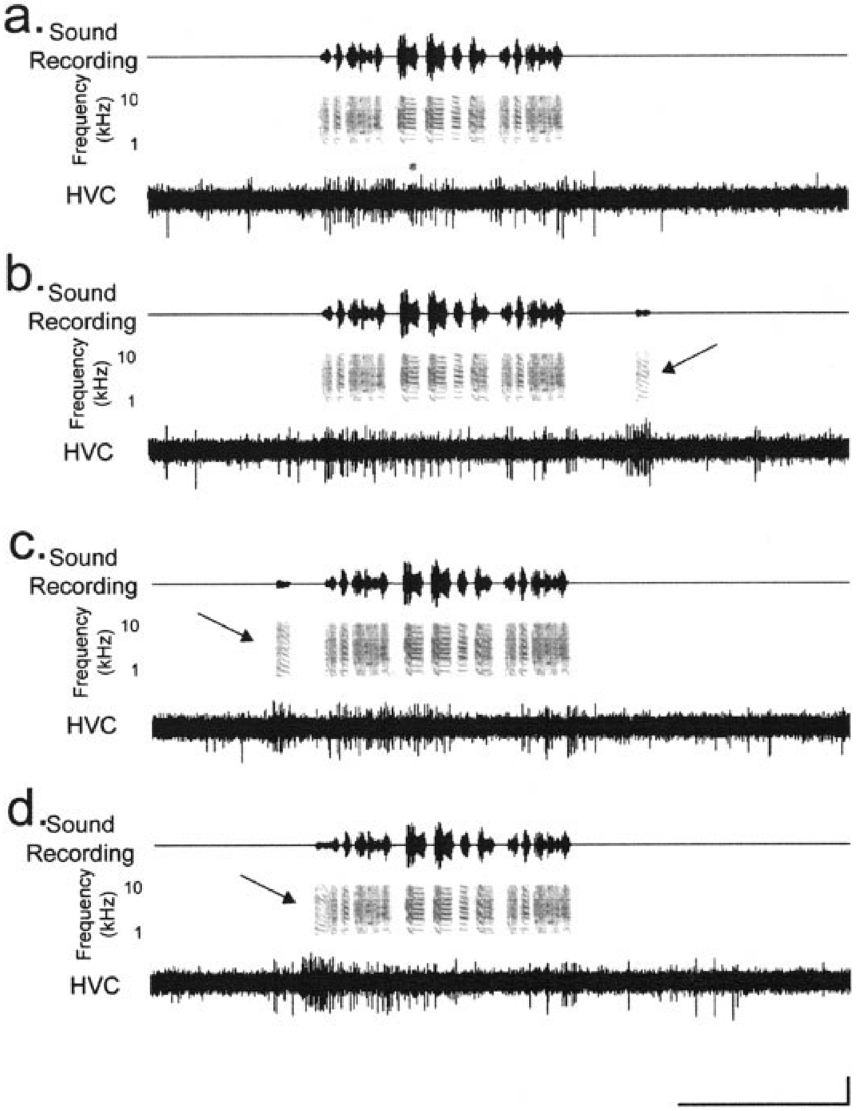

Figure 1 During vocal development, waking responses to the tutor song occurred in the song nucleus HVC. (a–d) Top panel: oscillogram of a sound recording of all sounds in the recording chamber. This includes tutor song playback and any vocalizations. Middle panel: spectrogram of sound recording. Bottom panel: multiunit HVC activity recorded simultaneously with sound. (a) Tutor song playback elicited neural activity in HVC. The juvenile did not vocalize during this trial, so there was no premotor activity. (b–d) The juvenile finch vocalized near the time of tutor song playback. Simple calls are indicated with arrows in the spectrograms. Both premotor and auditory activity were evident in HVC. Note that premotor activity in HVC preceded the onset of the calls. All data are from finch Green 17, 57 days. Scale bar: 25 μV, 1 s.

Waking Response Occurs Specifically during the Early Sensorimotor Phase

The waking HVC response to tutor song declined with age (Fig. 2). Recordings from the same electrode in the same HVC revealed that waking responses to tutor song were much greater during the late sensory/early sensorimotor phase than in the late sensorimotor phase [Fig. 2(a,b)]. The lack of responses late in development was specific to waking, because robust responses during sleep were induced by playback of BOS [Fig. 2(c)]. To determine the reproducibility of this finding, 12 recording pairs, each set from the same electrode, were compared from the early sensorimotor period (35–69 days) and from the late sensorimotor phase (70–90 days). The HVC response to tutor song was much greater during the early sensorimotor period than later in development

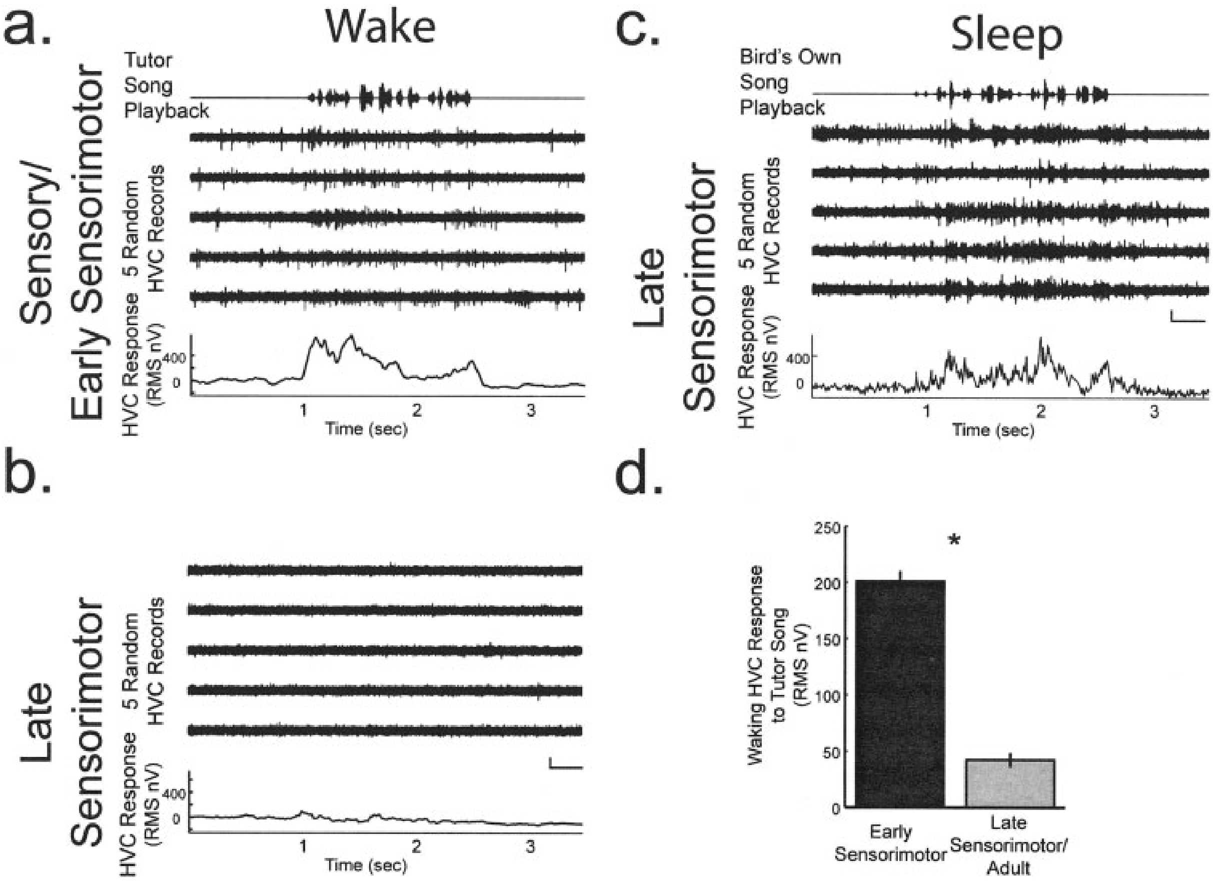

Figure 2 Tutor song responses peaked during a specific period of development. (a) During the early sensorimotor phase of song acquisition (35–69 days; exemplar Green 17, 57 days), HVC responded to tutor song playback during waking. Top: tutor song playback is temporally aligned with all HVC records and mean RMS plots below [in (a) and (b)]. Middle: five randomly selected multiunit HVC records. Bottom: mean RMS response (spontaneous-subtracted) was calculated for each trial in 5 ms bins. Plotted here is the mean for each 5 ms bin across trials ($n = 66$ trials; mean HVC response: 297.24 ± 2.02 RMS nV). (b) During the late sensorimotor phase (70–90 days; exemplar Green 17, 80 days), tutor song playback did not induce activity in HVC during waking ($n = 15$ trials; mean HVC response: 7.25 ± 6.87 RMS nV; t test HVC response 57 days vs. 80 days: $p < 0.0001$). (c) During the late sensorimotor phase (70–90 days; exemplar Green 17, 80 days), HVC responded robustly to playback of the bird's own song during sleep ($n = 43$ trials; mean HVC response: 263.71 ± 0.78 RMS nV). (d) Group data: the waking HVC response to tutor song declined during the sensorimotor phase (t test; $^*p < 0.0004$; number of birds = 6; number of sites = 12). Scale bar: 25 μV, 250 ms.

[Fig. 2(d); spontaneous activity is subtracted in all HVC RMS response data]. All 12 electrodes showed a decreased response to tutor song with development, whereas 8 of 10 responses to the BOS increased with development (Fig. 3; the early sensorimotor BOS was not available in two cases because the birds had not yet sung).

Tutor Song Is the Best Stimulus during Waking in Juveniles

A range of stimuli activated HVC during waking in the early sensorimotor phase (Figs. 4 and 5). The d′ metric (Green and Swets, 1966) has been used to compare neural activity in response to two stimuli (Solis and Doupe, 1997). d'_{A-B} values near zero indicate no preference for stimulus A over B, and vice-versa. Positive d'_{A-B} values indicate preference for stimulus A over B, whereas negative d'_{A-B} values indicate preference for stimulus B over A. "Selectivity" is defined by d′ values greater than +0.5 or less than −0.5, indicating preference for stimulus A or B, respectively. When compared to the response to several other auditory stimuli, tutor song (specific for each bird) induced the greatest response during

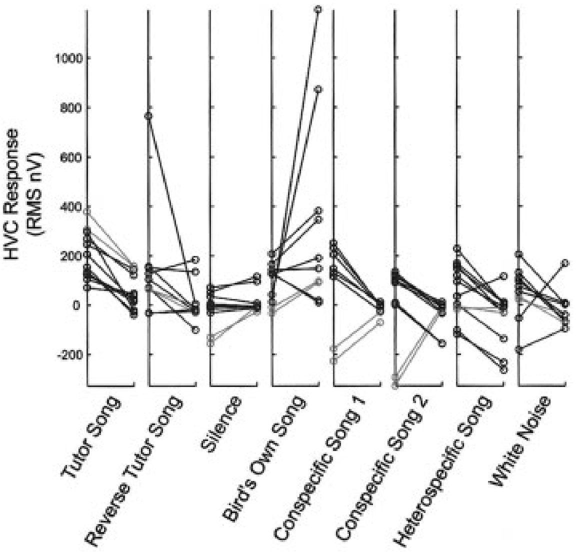

Figure 3 The decrease in responses to tutor song was consistent across electrodes. Each graph presents the waking response of all electrodes to the stimulus written below. The left point indicates the response during the early sensorimotor phase (30–69 days), whereas the right point indicates the late sensorimotor phase (70–90 days). Only electrodes that were recorded at both developmental phases are shown. Gray lines indicate Bengalese-reared.

waking in the early sensorimotor phase, with $d'_{\text{Tutor Song}-\text{Other}}$ approximating +0.5 when compared to BOS, silence, reversed tutor song, two conspecific songs (which were the same for every finch), heterospecific song (Bengalese finch), and white noise. The conspecific data indicate that experience with a tutor, and not just species-specificity, underlies the HVC response. d′ values can be misleading if inhibition of activity occurs in response to one of the stimuli compared. RMS responses to tutor song and BOS in young, awake finches were consistent with HVC activation by both tutor song and BOS, with tutor song the more effective stimulus (Supplementary Fig. 1). Two zebra finches in this study were reared by a Bengalese finch. These two birds, as with those reared by zebra finches, preferred the song of their heterospecific tutor to both their own song ($d'_{\text{Tutor Song}-\text{BOS}}$: Black 12: 1.66; Red 4: 1.09) and conspecific (zebra finch) songs (Black 12, $d'_{\text{Tutor Song}-\text{Conspecific 1}}$: 1.01, $d'_{\text{Tutor Song}-\text{Conspecific 2}}$: 1.39; Red 4 conspecific data were not available).

Tutor Song Response Declines with Age

In contrast to earlier in development, $d'_{\text{Tutor Song}-\text{Other}}$ values during waking in the late sensorimotor/adult periods did not approach 0.5 (Fig. 5). This age-dependent change in preference for the tutor song compared to other stimuli was significantly different in several cases. Notably, d′ indicated that the preference for tutor song compared to BOS flipped during development, with tutor song preferred early in the sensorimotor period, and BOS preferred later. Scatter plots of raw RMS responses to tutor song versus all other stimuli also indicate a shift in relative responses with song development (Supplementary Fig. 2). In addition, RMS values show a decline of tutor song responses and concomitant increase in BOS responses with development (Fig. 3). This apparent contradiction of previous findings with multiunit recordings of HVC in awake adults that HVC exhibited little response to stimuli during waking (Schmidt and Konishi, 1998; Nick and Konishi, 2001) may be explained by the age of our animals (the oldest here are <90 days) and/or the increased sensitivity of our current analysis protocol, which normalized response strength across sites and animals and employed the d′ metric.

Tutor Song Response Is Not Due to Similarity with BOS

The response to tutor song may result from the acoustic similarity of the tutor song to BOS. If so, the results would be confounded by the bird's performance. To clarify this issue, we examined the similarity of tutor song and BOS of the subjects of this study with the Sound Analysis 3 program (Tchernichovski et al., 2000). Consistent with previous studies (Immelmann, 1969; Tchernichovski et al., 2001), we found that the similarity of BOS and tutor song increased with age [Fig. 6(a)]. During the early sensorimotor period (35–69 days), the HVC response to tutor song during waking was not correlated with the similarity of tutor song and BOS [Fig. 6(b)]. Thus, the tutor song response is not due to its acoustic similarity to BOS, but more likely a result of the experience with the tutor.

Changes in Spontaneous Activity Cannot Explain the Decline in the Response

Changes in spontaneous activity may play a role in increased responsiveness, although it alone cannot explain the change in stimulus preference. To examine whether increases in spontaneous activity correlate with HVC responsiveness, we compared ongoing activity during silence in sleep and waking during the early sensorimotor and late sensorimotor/adult phases. We found no differences among the groups

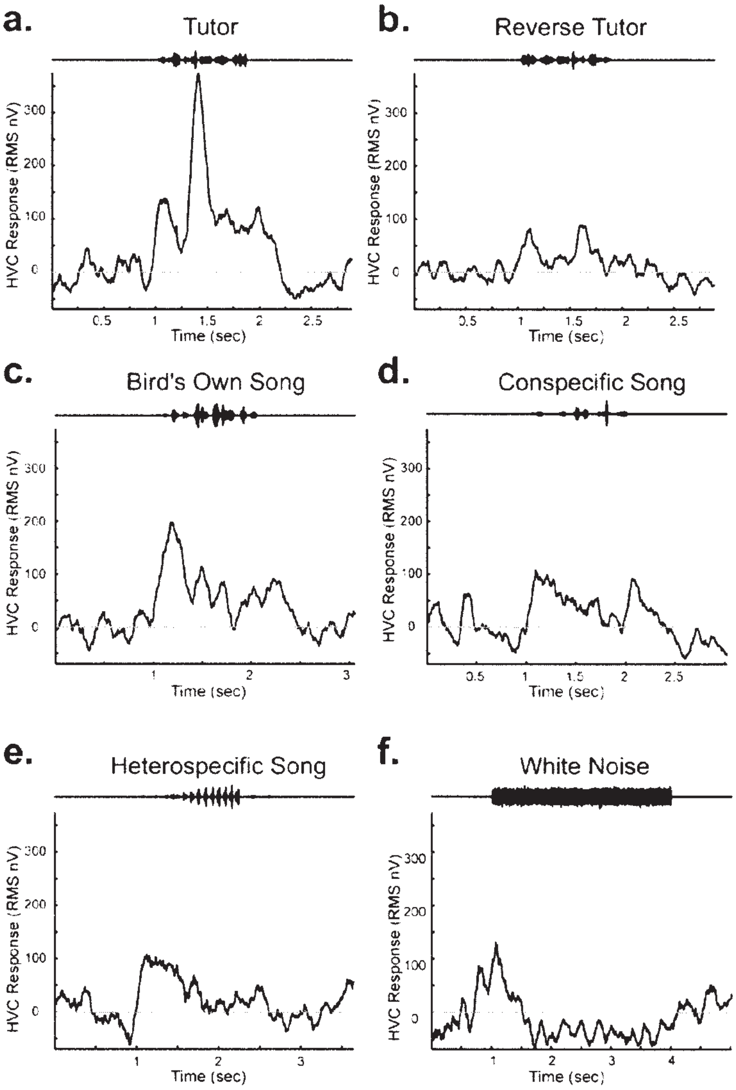

Figure 4 HVC responses to a variety of stimuli in an awake young bird. (a–f) Representative data from a single animal (Orange 9, 48 days). The response to tutor song playback exceeds that to all other auditory stimuli examined: reverse tutor, the bird's own song (BOS), conspecific song, heterospecific song, and white noise.

examined in this study: early sensorimotor—sleep (35–69 days; $n = 20$; 3.61 ± 0.02 RMS μV), early sensorimotor—waking (3.65 ± 0.03 RMS μV), late sensorimotor/adult—sleep ($\geq$70 days; $n = 14$; 3.23 ± 0.08 RMS μV), and late sensorimotor/adult—waking (3.41 ± 0.11 RMS μV) (N.S., one-way ANOVA with posthoc Tukey-Kramer). Interestingly, we did observe a decrease in spontaneous activity when comparing the entire sensorimotor period (35–89 days) to adulthood ($\geq$90 days) both in sleep and waking (Fig. 7). Spontaneous activity during sleep versus during waking was not significantly different within any age group that we examined. The HVC baseline activity was not correlated with the days since implantation (Fig. 8), which indicates that the age-dependent decrease was not a result of electrode degradation.

Song Preference Inverts According to Sleep-Wake State

Song preference is state-dependent in the early sensorimotor phase (Fig. 9). With the same chronic electrode, in the same 24 h period, the tutor song was preferred over BOS during waking, whereas BOS was preferred over the tutor song during sleep. Tutor song and BOS playback trials were randomly interleaved with at least five other stimuli. Thus, the results for

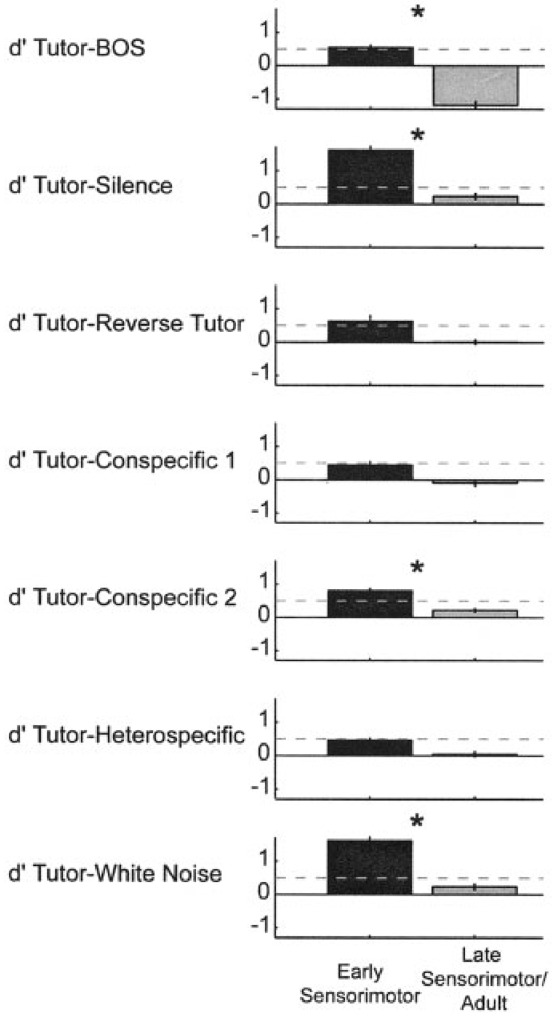

Figure 5 d′ values indicate that tutor song was preferred during waking over all other stimuli during the early sensorimotor period (35–69 days), but not later in development (≥70 days). d′ values greater than 0 indicate preference (Green and Swets, 1966), whereas d′ values of +0.5 or greater indicate selectivity (Solis and Doupe, 1997) for tutor song. Early in the sensorimotor phase, d′ values approximated or exceeded +0.5 for tutor song versus all stimuli examined. During the late sensorimotor phase and into adulthood, tutor song was not strongly preferred over any stimulus. The change in d′ values with development is significant in several cases, including $d'_{\text{Tutor Song-BOS}}$. (*$p < 0.05$, paired *t* test; number of birds for each graph, top-to-bottom = 5, 6, 5, 5, 5, 6, 6; number of sites = 10, 12, 10, 10, 10, 12, 12).

each stimulus were not the result of time-varying arousal or other unrelated physiological change.

Comparison of data from 15 recording electrodes in eight young finches revealed that, within a 24 h time period, the same recorded area of HVC changes significantly from preferring tutor song to preferring BOS [Fig. 10(a)]. When each electrode was examined independently [Fig. 10(b)], one-third of all HVC recordings (5 of 15) were found to shift from selectivity for tutor song (d′ ≥ +0.5) during waking to selectivity for BOS (d′ ≤ −0.5) during sleep, even though some of the sleep data may have been contaminated with waking trials (see Methods). Twelve of fifteen (80%) had d′ values that were positive, indicating response preference for tutor song during waking.

The heterogeneity of responses to tutor song led us to examine the spatial coherence of responses to BOS and tutor song. The amount of activity recorded from two spatially distinct electrodes in response to song playback in different areas of the same HVC is correlated during sleep and waking in young and older zebra finches [R-squared ≥ 0.61 for all combinations of stimulus (tutor song/BOS), state (sleep/wake), and age (35–69/≥70 days); Supplementary Fig. 3].Interestingly, the tutor song consistently induced less correlated activity than BOS.

DISCUSSION

Previous studies reported selectivity for both tutor song and BOS in the song system of anesthetized young zebra finches (Volman, 1993; Solis and Doupe, 1997, 1999, 2000). However, selective tuning to tutor song was rare. In adult finches, song system neurons prefer BOS under anesthesia or in sleep with the exception of one HVC interneuron type that responds to BOS in the awake state (Rauske et al., 2003). Nothing was known about the possible changes in stimulus selectivity with age or the phase of song development. These previous studies differ from our work in several ways: we used unanesthetized birds; we used chronic electrodes in order to record from the same bird and HVC site during the entire period of song development; we noted behavioral states, wake or sleep, during stimulus presentation and neural recording; and we collected and analyzed population activity with multiunit electrodes. This set of methods showed how responses to tutor song and BOS evolved during different phases of song development and systematically varied with behavioral states. BOS was the best stimulus during sleep, and tutor song was the best during waking. However, waking birds preferred tutor song only during the initial stages of vocal development, with preference for BOS during waking expressed later in development. Collectively, these data provide evidence of tutor song selectivity in the premotor song system that drives singing, describe the developmental expression pattern of tutor song selec-

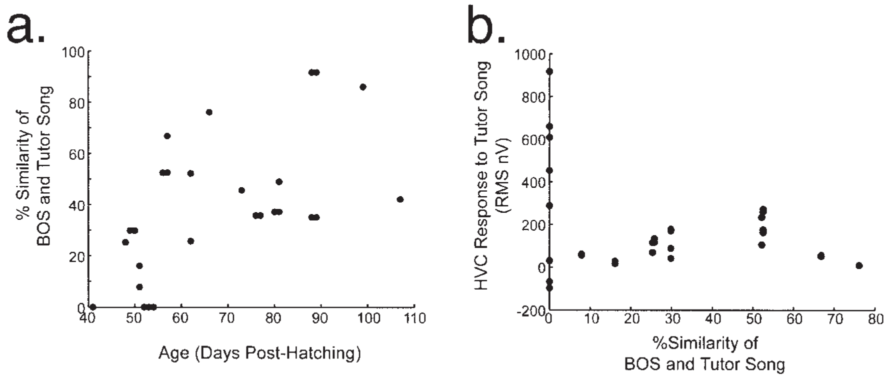

Figure 6 Similarity between BOS and tutor song does not explain the HVC response. (a) The similarity of BOS and tutor song increases with development. (b) There is no correlation between the response to tutor song and the similarity of BOS and tutor song in awake finches 30–69 days.

tivity in an identified song nucleus, and show age- and state-dependent shifts in song preference.

The timing of the increased tutor song response coincides with the phase of development during which the young bird must match its vocal output to the tutor song template through auditory feedback (early sensorimotor phase; 35–69 days) (Konishi, 1965, 1985). In the developing finch brain, two types of information must be compared: the memorized vocalization (the tutor song memory) and the current vocalization (auditory feedback of BOS). Further, the result of the comparison of these two must be generated within or conveyed to the song control system to guide the behavior toward a correct copy. During the overlap of the sensory and sensorimotor phases, a range of stimuli activated the song nucleus HVC in the wake state, and tutor song induced the largest response.

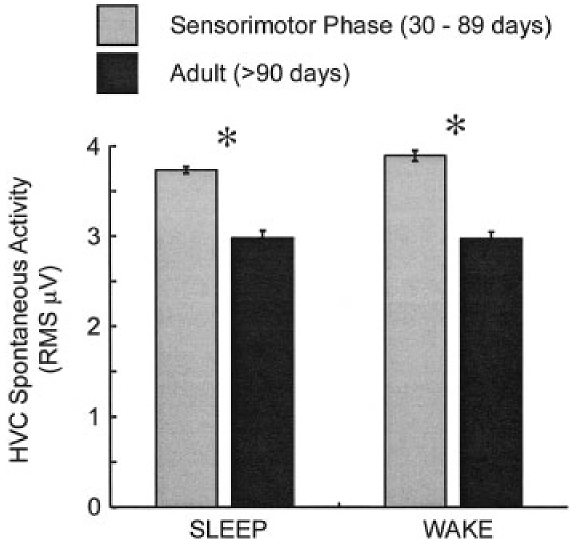

Figure 7 Spontaneous HVC activity is greater in the sensorimotor phase than in adulthood ($p < 0.05$; one-way ANOVA, posthoc Tukey-Kramer). Within each age group, spontaneous activity during waking and sleep was not significantly different.

Defining the origin of the HVC inputs selective for tutor song may reveal the locus of template storage and of the comparator, which is a behaviorally defined brain space that compares auditory feedback to the tutor song template. These two neural modules may be contained within HVC. Alternatively, candidate locations include all of the diverse brain areas that project to HVC (Fortune and Margoliash, 1995; Vates et al., 1996). Expression patterns of the immediate early gene *zenk* (Mello et al., 1992; Mello and Clayton, 1994) combined with electrophysiological studies (Stripling et al., 2001) suggest that the caudal medial nidopal-

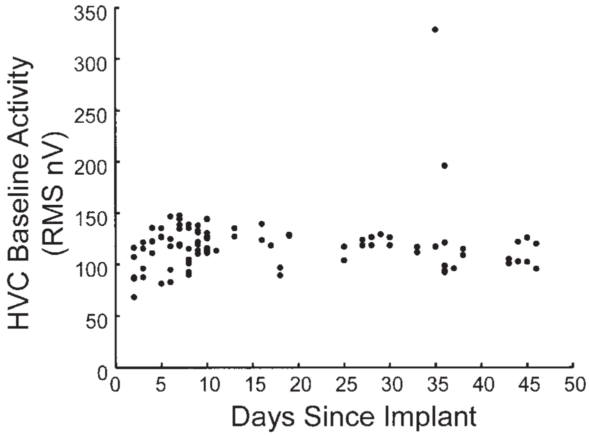

Figure 8 There is no correlation between the days since the implantation of electrodes and the amount of recorded HVC spontaneous activity. These data suggest that there is little degradation in the recording electrodes.

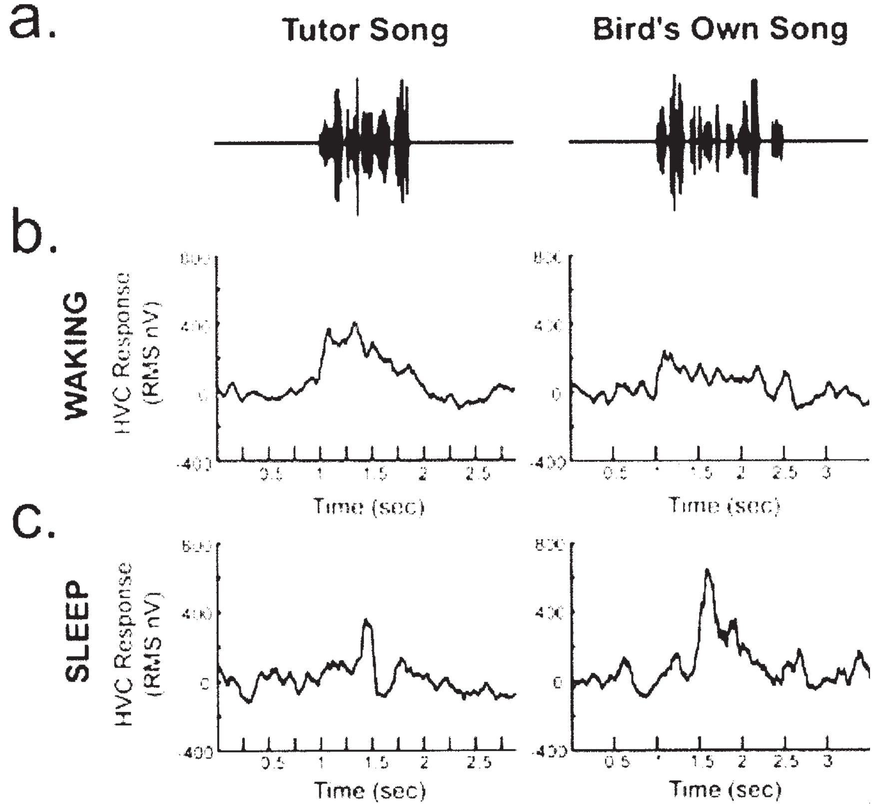

Figure 9 During the early sensorimotor phase, the relative HVC response to the tutor song and the bird's own song (BOS) inverted with state change. (a) Tutor song (left) and BOS (right) are temporally aligned with the mean RMS response traces below. (b) During waking, playback of tutor song (left; $n = 45$; 268.32 ± 2.54 nV) induced more HVC activity than BOS (right; $n = 36$ trials; 141.40 ± 4.28 nV; t test, waking, tutor vs. BOS: $p < 0.004$). (c) Conversely, during sleep, BOS (right; $n = 20$; 157.71 ± 3.76 nV) induced more HVC activity than tutor song (left; $n = 16$; 16.49 ± 3.70 nV; sleep, tutor vs. BOS: $p < 0.004$). All data from bird Orange 9, 52 days.

lium (NCM) has a role in template acquisition. An intervening nucleus between NCM and HVC, the caudomedial mesopallium (CMM; formerly known as the caudomedial hyperstriatum ventrale) (Reiner et al., 2004), exhibits experience-dependent representational plasticity of songs in anesthetized birds after operant training (Gentner and Margoliash, 2003). Thus, the NCM-CMM-interfacial nucleus of the nidopallium (NIf)-HVC pathway provides a set of candidate locations for template storage and the comparator. Another set of possible nuclei involved in template comparison is the anterior forebrain pathway (AFP), which has a role in song learning (Bottjer et al., 1984) and adult plasticity (Brainard and Doupe, 2000). Interestingly, the developmental period during which HVC responds selectively to tutor song (the early sensorimotor phase; current study) is also the time window during which AFP lesions are most effective at perturbing song development (Bottjer et al., 1984). Template-matching within the AFP would require back-projections or fast retrograde signaling to HVC, because the AFP is efferent to HVC and tutor song selectivity is observed within HVC. The medial magnocellular nucleus of the anterior nidopallium (mMAN), which is closely juxtaposed to the AFP and has projections to HVC, could have a role in this feedback pathway. Speculation aside, further examination of the source of the tutor song selective inputs to HVC will clarify the respective roles of these brain areas in song learning.

The best stimulus for HVC during sleep was the current BOS. This indicates that sleep responses reflect the song that the bird is currently producing. In contrast, during waking, HVC responded maximally to playback of the stimulus that most closely resembled the memorized template, the tutor song itself. During development, the BOS is shaped to match the template through auditory feedback (Konishi, 1965). The HVC responses to song during waking may have

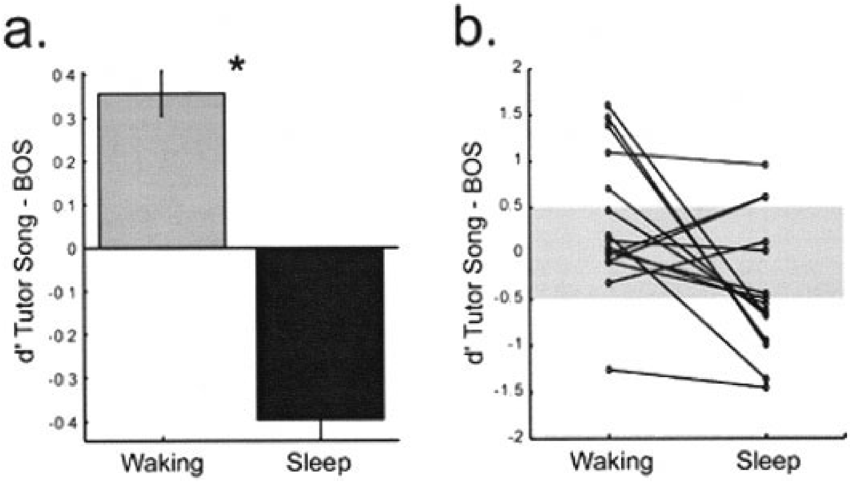

Figure 10 During the early sensorimotor phase, d′ values indicate an inversion of stimulus preference with state change. (a) Group data: $d'_{\text{Tutor Song-BOS}}$ changed significantly between sleep and waking [number of birds (35–69 days) = 8; number of sites = 15; *t* test, $p < 0.02$]. (b) Examination of the same group data on a site-by-site basis reveals heterogeneity in the sensitivity of $d'_{\text{Tutor Song-BOS}}$ to state. The gray bar indicates d′ values that do not indicate selectivity. In 80% of sites, $d'_{\text{Tutor Song-BOS}}$ was greater during waking than during sleep. In one-third of sites, the d′ value changed from indicating selectivity for tutor song during waking ($d' \geq +0.5$) to indicating selectivity for BOS during sleep ($d' \leq -0.5$).

a role in this process. It is important to note that the data presented here were responses to passive stimulation. Testing of the hypothesis that the waking responses have a role in song learning through auditory feedback will require observation of auditory responses during singing. The multiunit activities that we observed in this work may have consisted of responses from two types of projection or relay neurons and several types of interneurons in HVC. Our data do not allow us to discriminate between different neuron types, because multiunit methods record neural population activity. All neurons respond to BOS in adult birds under anesthesia (Mooney, 2000), but whether anesthesia in any way reflects waking activity remains to be determined. Data from adult finches during quiet waking suggest that only one type of interneuron responds to auditory stimuli (Rauske et al., 2003). However, only single neuron recordings from awake, singing juveniles will critically determine the role of auditory feedback within HVC in song learning.

Previous work suggests that patterns of spontaneous and auditory-evoked activity during sleep in the robust nucleus of the arcopallium (RA; efferent to HVC) match the patterns of activity produced during singing (Dave and Margoliash, 2000). Whether waking responses to the tutor song also match activity patterns produced spontaneously or during singing remains a question for future study. Examination of the relative activity patterns of HVC and RA showed that HVC transmits a sparse code to RA (Hahnloser et al., 2002). That is, HVC neurons that project to RA fire only once during each song motif. The neurons that respond to tutor song in the awake, juvenile HVC appear to fire more often, although only data from single neurons can conclusively show this. The heterogeneity of responses to tutor song in the same HVC suggests that only a subset of HVC neurons may respond. Previous work has shown that only a single subtype of interneuron responds in the awake adult HVC (Rauske et al., 2003). Individual neurons may show transient selectivity for the tutor song during a critical period of development and then undergo programmed cell death (Alvarez-Buylla and Kirn, 1997) or change their song preference and/or activity patterns. Identification of the neurons that respond to tutor song and what happens to them during development will provide insights into the mechanisms of song learning.

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