

Neural Auditory Selectivity Develops in Parallel with Song

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ABSTRACT: The zebra finch learns his song by memorizing a tutor's vocalization and then using auditory feedback to match his current vocalization to this memory, or "template". The neural song system of adult and young birds responds to auditory stimuli, and exhibits selective tuning to the bird's own song (BOS). We have directly examined the development of neural tuning in the song motor system. We measured song system responses to vocalizations produced at various ages during sleep. We now report that the auditory response of the song motor system and motor output are linked early in song development. During sleep, playback of the current BOS induced a response in the song nucleus

HVC during the song practice period, even when the song consisted of little more than repeated begging calls. Halfway through the sensorimotor period when the song was not yet in its final form, the response to BOS already exceeded that to all other auditory stimuli tested. Moreover, responses to previous, plastic versions of BOS decayed over time. This indicates that selective tuning to BOS mirrors the vocalization that the bird is currently producing. © 2004 Wiley Periodicals, Inc. *J Neurobiol* 62: 000–000, 2005

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The developing songbird uses auditory feedback to shape singing behavior (Konishi, 1965). Behavioral experiments have shown that zebra finches acquire song in two phases: a sensory phase (18–65 days posthatching) during which a song is learned from a tutor (Immelmann, 1969; Eales, 1985; Böhner, 1990) and a sensorimotor phase (35–90 days) during which vocalizations are matched to the learned sound

through comparison of auditory feedback to the tutor song memory, or template (Immelmann, 1969; Konishi, 1985). In the sensorimotor phase, dramatic changes occur in the song, which progresses from the repetition of simple calls that resemble babbling in human infants (Doupe and Kuhl, 1999) to a mature song that exhibits stable syllable structure, phrase sequence, and duration. The present study examined whether these changes in song are accompanied by parallel changes in auditory responses.

A network of brain nuclei, known as the song system, is dedicated to song learning and production (Nottebohm et al., 1976). Most of the song nuclei, including those in the vocal motor pathway, contain neurons that respond to sounds. These neurons are particularly strongly activated by playback of the individual bird's own song (BOS) under

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anesthesia and in sleep (McCasland and Konishi, 1981; Margoliash, 1983; Dave et al., 1998; Schmidt and Konishi, 1998; Mooney, 2000; Nick, 2001; Nick and Konishi, 2001).

As in adults, the song system of anesthetized young birds is responsive to auditory stimuli: during the sensorimotor phase, the BOS is the most effective activating stimulus for the nucleus HVC of white-crowned sparrows (Volman, 1993) and the lateral magnocellular nucleus of the anterior nidopallium (LMAN) (Doupe, 1997; Solis and Doupe, 1997). Interestingly, a subset of neurons in LMAN preferred tutor song or responded equally well to both BOS and tutor song (Solis and Doupe, 1997). In addition, these authors noted that the LMAN response to playback of different versions of the immature BOS varied in young animals in acute experiments. The reported variability may have been due to any number of factors, including the degree of stimulus similarity to the tutor song and/or to the current BOS. These previous studies did not examine responses to BOS relative to the time since the BOS was produced. Therefore, they could not determine whether the response to the current BOS was due to its similarity to the tutor song model or due to selective and dynamic tuning of the sensorimotor system to the sound it produces.

Since this work on anesthetized juveniles was reported, evidence has accumulated that song system responses to BOS are state-dependent in adults (Dave et al., 1998; Schmidt and Konishi, 1998; Nick and Konishi, 2001). Adult HVC neurons respond to BOS during sleep and anesthesia, but much less so during waking (Schmidt and Konishi, 1998; Nick and Konishi, 2001; Cardin and Schmidt, 2003; Rauske et al., 2003). Recent data from the sensorimotor phase indicate that HVC responds most to the tutor song during waking and BOS during sleep (Nick and Konishi, 2004), suggesting that conclusions originally drawn under anesthesia should be revisited during natural states.

In the current study, we systematically examined responses during sleep to BOS and tutor song within HVC across the sensorimotor phase. During sleep, we studied (1) responses to the tutor song and other stimuli relative to BOS and (2) variability of the BOS response with specific reference to the age of the finch and the time since the specific copy of BOS was produced. An ideal experiment would use recording of multiple songs and auditory responses during the period of vocal development in the same individual. This approach is possible only with chronically implanted electrodes. Recording neural responses to song in sleep also removes the need to anesthetize the bird frequently and any adverse effects of the anesthetic drug on song development. Systematic compar-

ison of vocal motor output and HVC stimulus tuning during the sensorimotor period of vocal learning indicates that the auditory selectivity of HVC neurons closely follows the developing song.

METHODS

Animals and Surgery

General methods were previously described (Schmidt and Konishi, 1998; Nick and Konishi, 2001). Fourteen male zebra finches (*Taeniopygia guttata*) were hatched and reared in our facility and maintained on a 12/12 light cycle. Bengalese finch foster parents reared two of these birds. Birds were implanted with electrodes [multiunit and electroencephalogram (EEG)] at several different ages (32–200 days posthatch). Songs and neural activity from nine birds were recorded at multiple ages (one of these was Bengalese-reared). Six birds were exposed during darkness to playback of many different versions of BOS from several different ages. Before surgery, young finches were either kept with their natural parents in the aviary or placed in acoustic attenuation chambers with their Bengalese foster parents. 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). The Caltech institutional animal care committee approved all procedures.

Electrophysiology and Song Playback

All multiunit electrodes and the EEG electrode were referenced to an animal ground. Multiunit electrodes were 25 μm nichrome-formvar wire plated with rhodium (Schmidt and Konishi, 1998). 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. All HVC recordings were from the right HVC. The animal ground was placed over the right hyperpallium apicale (formerly known as the hyperstriatum accessorium). The EEG electrode was placed over the right mesopallium (formerly known as the hyperstriatum ventrale). EEG traces were filtered 1–500 Hz. Differential multiunit records were filtered 300–10,000 Hz. The electrodes exhibited no sign of degradation during the periods examined in this study. Figure 1 shows that responses to the best BOS stimuli actually increased with time from electrode implantation during the sensorimotor phase. It is important to point out that, over the long time periods examined in this study, even large multiunit electrodes are subject to spatial drift. Therefore, although the electrodes remained within HVC throughout the study, it is unlikely that all of the recordings from a single electrode are from the exact same subpopulation of HVC neurons. However, as in adults (Margoliash, 1986), during sleep the activity of HVC was remarkably uniform (Sup-

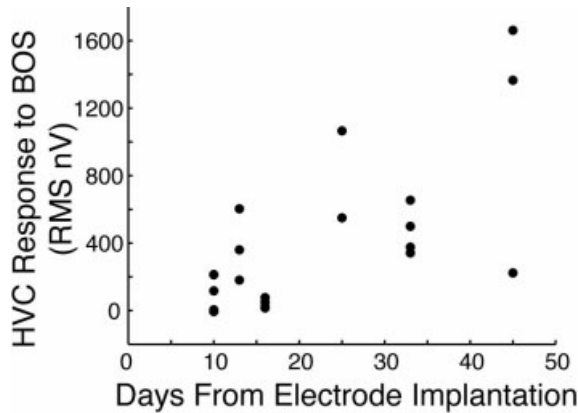


Figure 1 Electrode recordings did not degrade over time. Responses to the best BOS are plotted against the days since implantation of the electrode. Electrode degradation over time should lead to smaller voltage signals. During the sensorimotor phase, responses actually increase with days from implantation.

plementary Fig. 1). This indicates that the changes observed during sleep across development were most likely due to changes in the neural system and not to the spatial drift of electrodes.

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.

Previous data indicated that the adult HVC responds selectively to song playback during slow-wave sleep, but not during waking (Nick and Konishi, 2001). We thus focused our attention on these two states. Playback experiments began at least 2 h after the acoustic attenuation chamber lights were turned off, during the bird's normal lights-off cycle. "Darkness" data were collected in a dark, quiet chamber during the bird's established sleeping period. "Light" data were acquired in a well-lit chamber during the bird's established waking period. Birds were visually monitored during waking data collection sessions. To utilize EEG to assess slow-wave sleep/wake state, we first compared all EEG records obtained during silence trials (see below) in both Darkness and Light sessions recorded in the same 24 h period. We measured the power between 1–4 Hz in a spectrogram of the entire trace. We continued our analysis if and only if there was a significant difference between the Darkness and Light EEG power and the Darkness power was greater than the Light. If these criteria were met, we set the "waking" criterion at less than the mean Light EEG power, and the "sleep" criterion at greater than the mean Darkness EEG power. These criteria were then applied to all the trials recorded in the session. Data that did not meet the criteria were excluded from further analysis.

Exclusion of Darkness trials with little power in low frequencies most likely excluded rapid eye movement or paradoxical sleep trials. Whether the adult or juvenile HVC responds to auditory stimuli during paradoxical sleep remains an open question.

All birds were presented with sets of 50–100 trials (referred to as "Trial Sets" in the figure legends) consisting of at least six stimuli, in random order: silence, the tutor song (from either an adult male zebra finch or Bengalese finch), two different conspecific songs, heterospecific song (from a Bengalese finch), and white noise. Each trial lasted 6 s. The intertrial interval recovery period lasted 15 s. 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. To avoid bias in the selection of songs, we wrote a MATLAB function that randomized all sound recordings from a given bird. These were shown, in the randomized order, to one of us (T.A.N.) who eliminated recordings of movement and intermittent calls. The first four randomized recordings that were not eliminated were used for playback studies. For comparisons of BOS responses across development, responses to all BOS stimuli were used. Only the best BOS stimulus that elicited the most HVC activity during sleep (recorded within 72 h of playback) was used for d-prime comparisons between BOS and non-BOS stimuli (see below). All playback stimuli other than the BOS and tutor song were invariant for all animals examined.

We compared sound pressure levels between BOS and tutor song using a 1 inch condenser microphone and Type 2604 microphone amplifier (Brüel and Kjøer, 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 μ V; BOS: 204.7 ± 7.3 μ V; $p = 0.39$; $n = 8$ songs each). Amplitude modulation of BOS and tutor song stimuli was not modified to achieve normalization. Other stimuli used in this study were not normalized with regard to peak or RMS volume, because it is difficult to normalize for both parameters without altering amplitude modulation, which could be a more salient feature of song than overall volume.

Analysis

All data were analyzed with Matlab with functions included with the software or written by T.A.N. A function written in Matlab automatically discarded trials with movement artifacts (indicated by large deflections in the EEG and reference multiunit record), vocalizations, or EEG power that did not meet criteria. 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.

The RMS of the HVC voltage trace was calculated for each 5 ms bin. The RMS, in contrast to thresholding, includes all neurons recorded. Larger units will have proportionally larger effects on the RMS. For clarity in the detailed Figures 9 and 10, the RMS was smoothed with a moving 250 ms window. For statistical comparison of results, the RMS difference response was calculated by subtracting the mean RMS during a 1 s prestimulus period (RMS_{BASE}) from the mean RMS during sound presentation or a silent period of duration equal to the tutor song (RMS_{SONG}).

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

$$d'_{A-B} = \frac{2(\overline{RS}_A - \overline{RS}_B)}{\sqrt{\sigma_A^2 + \sigma_B^2}} \quad (1)$$

where \overline{RS}_A and \overline{RS}_B are the mean normalized response strength (RS) to stimulus A and B, respectively. The RS was defined as:

$$RS = \frac{RMS_{SONG} - RMS_{BASE}}{RMS_{SONG} + RMS_{BASE}} \quad (2)$$

RMS difference responses and d' values are presented in the figures. All data were compared using either a Student's t test or a one-way ANOVA with a posthoc Tukey-Kramer test for significance ($p < 0.05$). The specific tests are noted in the figure legends. Data are presented as mean \pm standard error of the mean.

RESULTS

EEG Is Not a Reliable Indicator of Sleep in Very Young Finches

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 very young zebra finches (<50

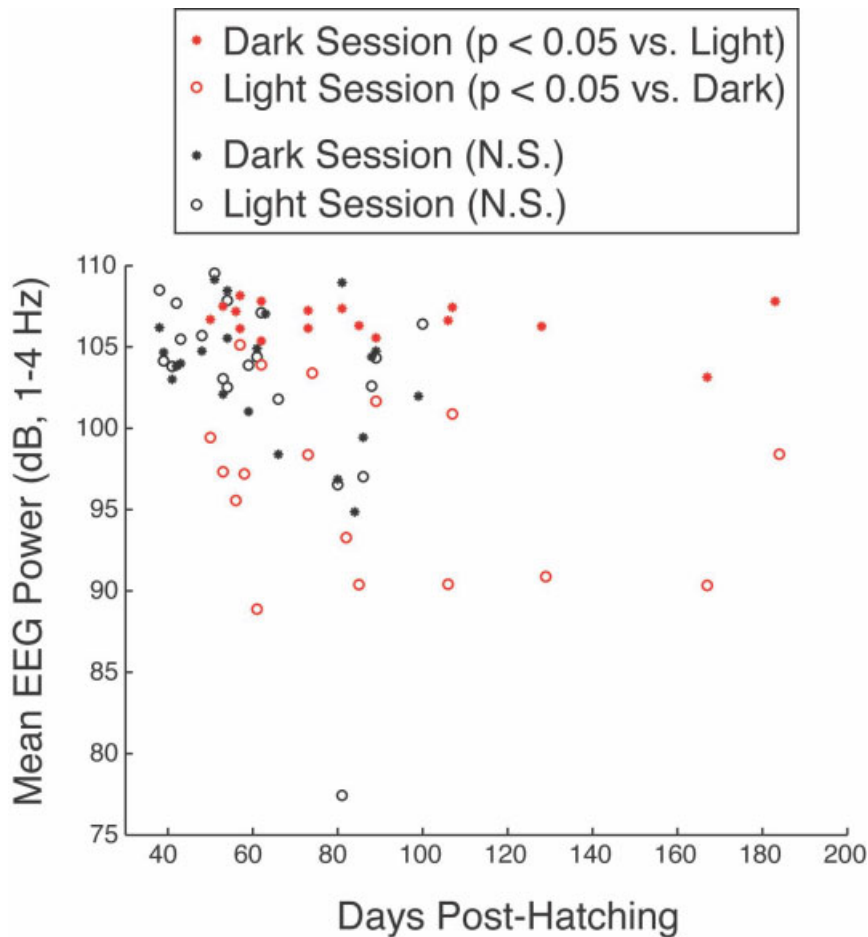


Figure 2 EEG recordings during development. A subset of recordings yielded darkness EEG power (solid circles) significantly greater than waking EEG power (hollow circles) within the same 24 h period (indicated by red color). Black indicates that the criterion was not met. Before the age of 50 days, this criterion was not met in any recording, suggesting that EEG is not a reliable indicator of sleep prior to 50 days posthatching.

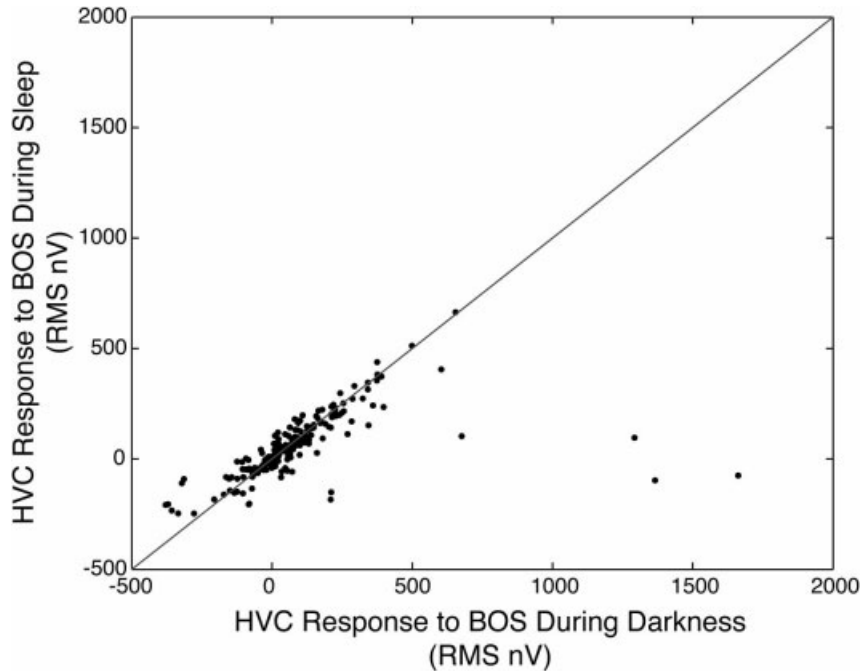


Figure 3 HVC responses to BOS during darkness are representative of responses during sleep. Plotted are HVC BOS responses during darkness versus responses during the same session with strict EEG criteria applied. The solid line indicates $x = y$. Except for three outliers (lower right), responses during darkness correlate well with responses during sleep and can be fit well with a linear regression ($y = 0.74439x + 8.0129$; $R^2 = 0.76$; $F = 799$; $p < 0.0001$).

days; Fig. 2). After the age of 50 days, more EEG reliability was observed, such that this measurement could be used to eliminate any potential waking trials in darkness data sets. To assess the level of potential waking contamination in darkness data sets, we plotted BOS responses during darkness versus those measured in the same session only during EEG-defined sleep trials (Fig. 3). After the removal of three outliers, we found a significant correlation between darkness and sleep ($R^2 = 0.76$; $p < 0.0001$; age 50–90 days), indicating that responses during darkness are representative of responses during EEG-defined sleep. Nevertheless, we used strict EEG criteria to define sleep/wake state. All data presented below as “sleep” or “waking” met the requirements that (1) the power of the EEG 1–4 Hz was significantly greater during Darkness than during Light; and (2) the power of the EEG 1–4 Hz for each included “sleep” trial was greater than the mean Darkness power and that for each included “wake” trial was less than the mean Light power.

Similar to Adults, the Juvenile HVC Responds More to BOS during Sleep Than during Waking

The HVC of juveniles responds more to BOS playback during sleep than during waking (Fig. 4), as in

adults (Nick and Konishi, 2001). These data indicate that state-dependent shifts in HVC responses are evident from the earliest ages that can be reliably investigated using EEG criteria.

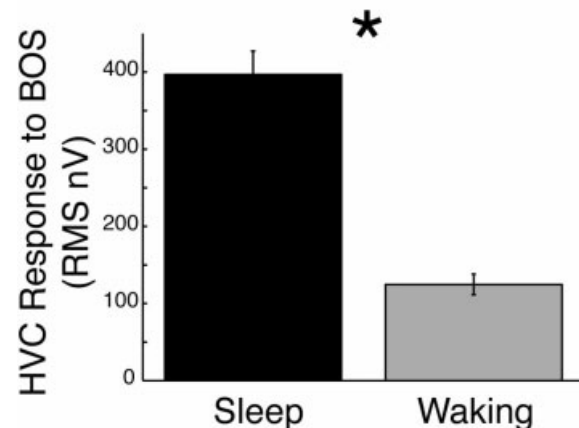


Figure 4 As in adults, the HVC response to BOS is greater during sleep than during waking in young animals ($*p < 0.05$, paired t test; sleep: $n = 16$; waking: $n = 16$). Sleep and waking were defined by EEG criteria during the sensorimotor phase (50–90 days). Error bars represent the SEM.

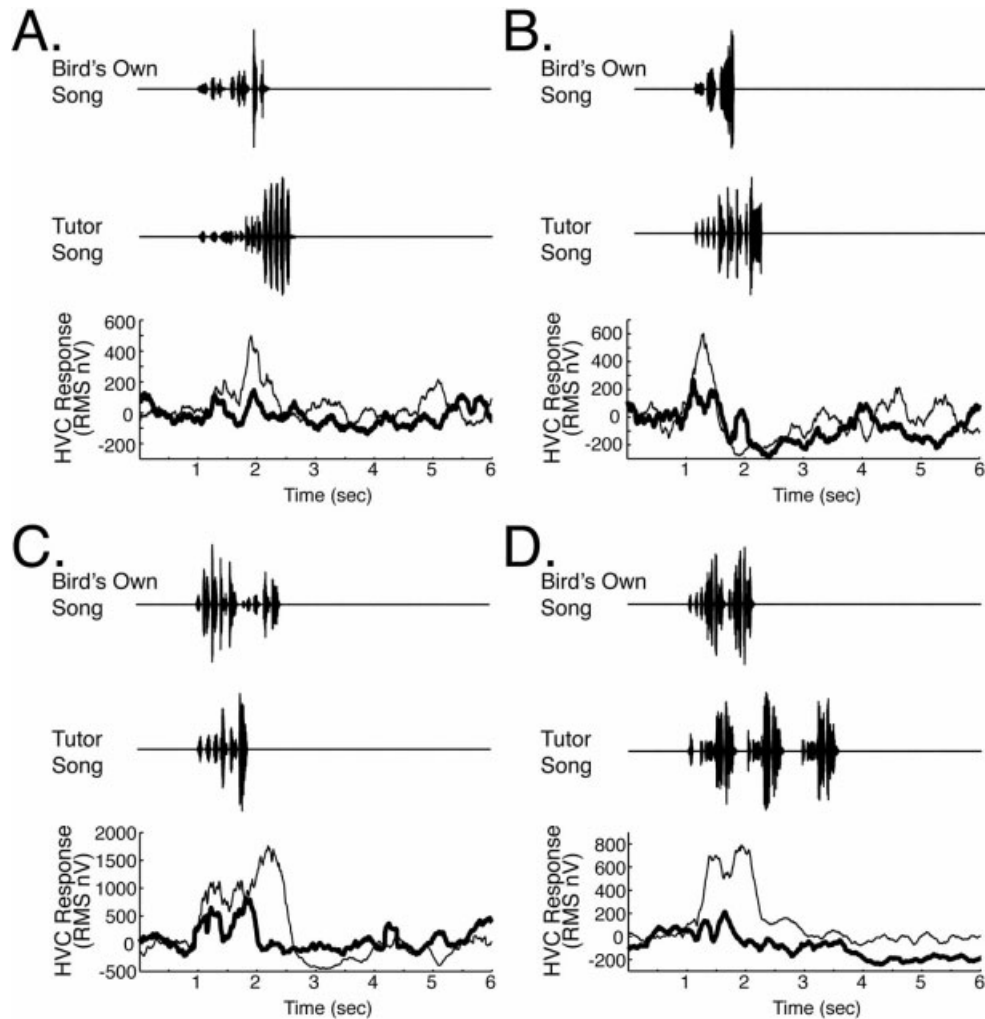


Figure 5 The HVC response to BOS is greater than that to tutor song during sleep in the sensorimotor phase. Data from the four young finches that passed the EEG criteria for sleep are shown (A–D). Oscillograms for BOS (top) and tutor song (middle) are shown temporally aligned with the HVC response below. In each case, the HVC RMS response to BOS (bottom, thin line) exceeded that to tutor song (bottom, bold line).

Juvenile HVC Responds More to BOS Than to Tutor Song during Sleep

BOS is the most effective activating stimulus in the HVC of sleeping young finches (Figs. 5–7). Vocalizations and HVC voltage records were obtained from zebra finches from the early sensorimotor phase into adulthood. The BOS changes rapidly during the sensorimotor phase, transforming from simple calls to a mature song resembling the tutor model (Immelmann, 1969; Tchernichovski et al., 2001). We used chronic multiunit recording to stably assess activity in the motor song nucleus HVC during a protracted time period of up to 200 days. To assess the level of activity, a running RMS (5 ms bins) was calculated

for each trace. This method measures the total power of the trace and includes all neurons recorded, weighting them relatively according to the measured action potential amplitude. In the sensorimotor phase during sleep (Fig. 5), HVC responded more to playback of the current BOS (thin line) than to tutor song (bold line). We found that even when the BOS was a simple series of calls [e.g., Fig. 5(B)], the response to these autogenous sounds exceeded that to the tutor song. Sonograms of these songs are shown in Supplementary Figure 2.

We have found that the HVC response to BOS significantly exceeds responses to a variety of other stimuli even during the sensorimotor phase (Fig. 6).

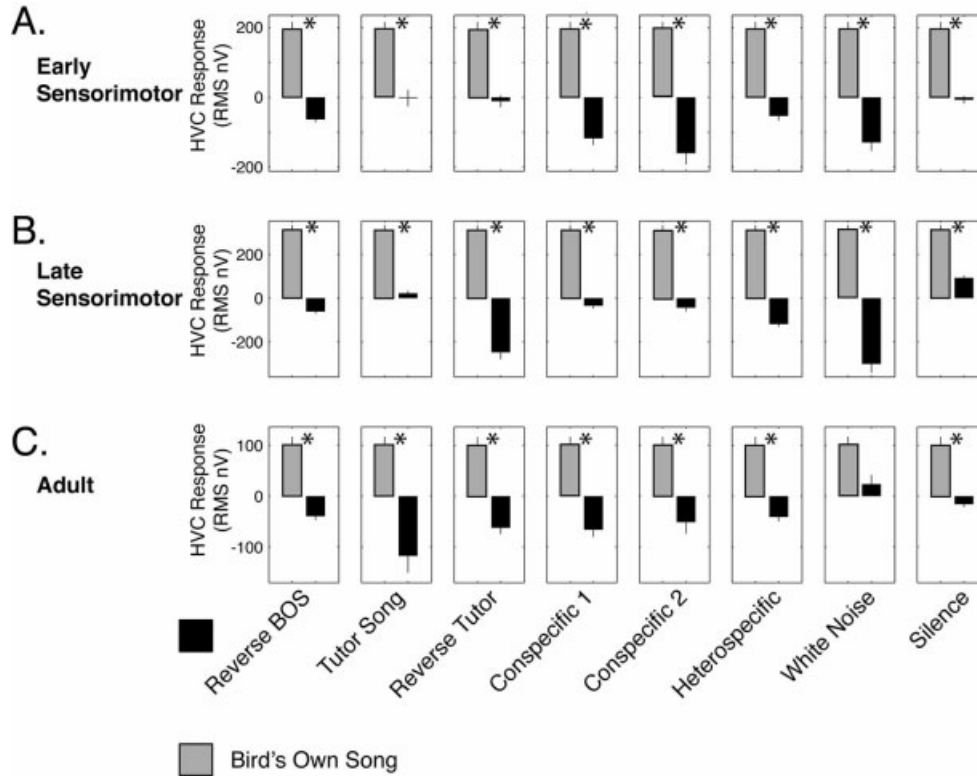


Figure 6 The HVC response to BOS during sleep is greater than that to all other stimuli examined. The y axis indicates the HVC response above baseline. Negative values indicate decrement of activity during playback relative to baseline. (A) During the early sensorimotor phase (50–70 days), HVC of sleeping finches responded more to BOS than to other stimuli [# recording sites left to right = 14, 14, 14, 14, 10, 14, 14, 14; * $p < 0.05$ paired t test for (A–C)]. (B) The same was true of the late sensorimotor phase (70–90 days) (# recording sites = 18, 18, 14, 14, 14, 14, 14, 18). (C) Consistent with previous results, BOS induced the most activity in the HVC of the sleeping adult (# sites = 10, 10, 10, 10, 8, 10, 10, 10). Error bars represent the SEM.

These stimuli include the songs of adult zebra finches, notably the tutor song.

Juvenile HVC Responds Selectively to BOS during Sleep

The d' metric (Green and Swets, 1966) has been used to examine relative responses to various song stimuli (Solis and Doupe, 1997). A d' value of 0.5 or greater was used previously to categorize neural activity as “selective” (Solis and Doupe, 1997). Using this criterion, we found that HVC was selectively activated by BOS over all stimuli during the sensorimotor phase (Fig. 7). Moreover, $d'_{\text{BOS}-x}$ values increased with development, indicating that the preference for BOS increased. The increase from the early (50–70 days) to the late sensorimotor phase (71–90 days) was significant in several cases. A $d'_{\text{BOS}-x}$ greater than 0 does not reveal whether there is increased activity to BOS, decreased activity to x , or both. To inves-

tigate this, we plotted the response to BOS versus all stimuli (Fig. 8). We found that BOS reliably increased activity, although some stimuli decreased HVC activity as well.

Responses to Older Versions of BOS Decline during Song Learning

HVC responds less to prior versions of the BOS as the song matures. In Figures 9 and 10, the HVC response to each song is plotted according to the day BOS was recorded (columns) versus the day it was played back (rows). Figure 9 shows recordings from the HVC of one finch during the sensorimotor phase and adulthood. A decline in responding to the day 66 song can be noted when comparing the response to the identical stimulus played back on days 66, 89, or 303. Sonograms of the playback stimuli are shown in Supplementary Figure 3. The overall decline in responding

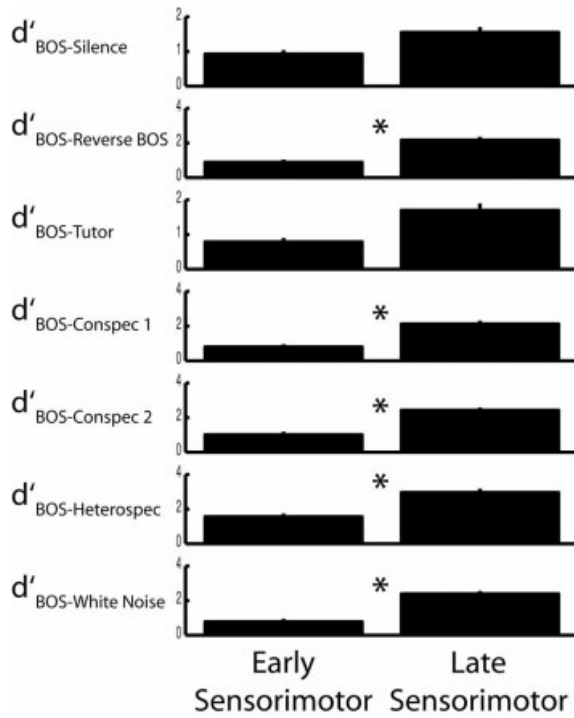


Figure 7 During sleep in the sensorimotor phase, d' values indicate that BOS preference increases with development in HVC (* $p < 0.05$, paired t test; # birds = 4; # electrodes = 8). Error bars represent the SEM.

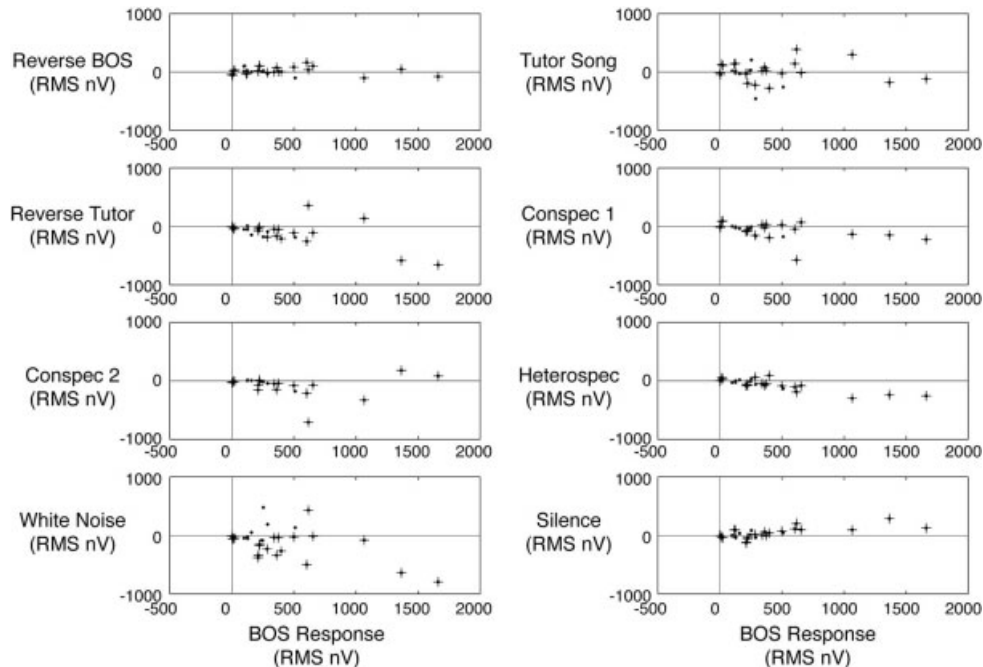


Figure 8 HVC responses to BOS versus responses to other stimuli during the sensorimotor phase (plus signs) and adulthood (solid circles). Activation by BOS is indicated by points to the right of zero, whereas activation by other stimuli is indicated by points above zero. The general trend in the data was activation by BOS, with some decreases in HVC activity induced by several stimuli. Data from all trial sessions are shown, such that each electrode may contribute more than 1 day's data to the graph.

to BOS in adulthood relative to the sensorimotor phase may be related to a decline in HVC activity with maturation (Nick and Konishi, 2004). Alternatively, the overall decline in responding could be due to a nonspecific degradation of the electrodes. This is unlikely, because the electrodes exhibited no sign of degradation during the periods examined in this study (Fig. 1). As an additional test of electrode stability, we compared the response to BOS during darkness in adults that had been implanted either less than or greater than 5 weeks. (This specific time value allowed us to divide the total number of adult recordings in half.) We found that there was no significant difference in the BOS response between these two groups (implant < 35 days: 200.8 ± 53.7 nV, $n = 4$; > 35 days: 213.5 ± 54.6 , $n = 4$; t test, N.S.).

Figure 10 shows recordings from another zebra finch, which was reared by a Bengalese finch pair. Sonograms of the playback stimuli are shown in Supplementary Figure 4. Raw voltage traces from these recording sessions are shown in Supplementary Figures 5–9. As in Figure 9, during the sensorimotor phase, the response to each BOS decreased with the time from recording. For example, the HVC responses shown in the “Day BOS Recorded” column “50” were

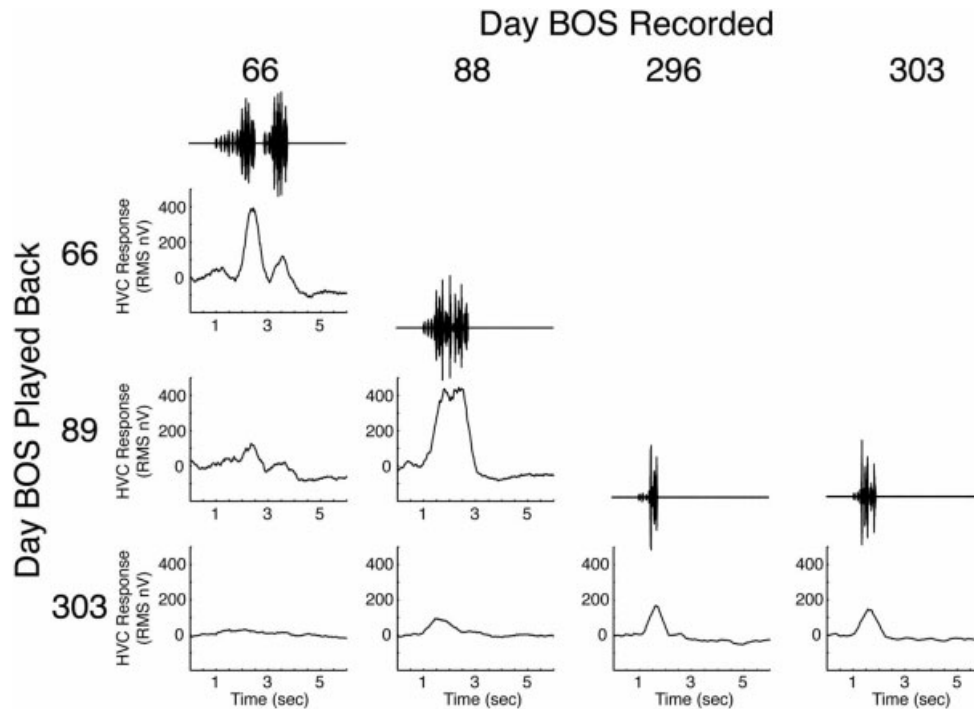


Figure 9 Responses to previous versions of the BOS decline during the sensorimotor phase of song acquisition. COLUMNS: The day that each BOS was recorded. The oscillogram of each BOS is shown at the top of the column. The HVC response during sleep is shown underneath. ROWS: The day that each BOS was played back to the bird. The sensorimotor phase ends at approximately 90 days. Afterward, the response magnitude to each copy of BOS appeared to stabilize. All data are from bird Orange 11 during darkness.

largest on day 53, less than 72 h after the song was recorded. The response to the day 50 song subsequently decreased on day 73 and was imperceptible by day 85. The response on day 85 to the day 84 song was larger than responses to two other versions of BOS that had been previously effective. The decline in response with time from recording was not due to a degradation of the recording electrodes, because newer versions of BOS were capable of inducing large responses on the same days that older versions of BOS failed to cause a response. Later in development, the magnitude of the response to past and current versions of BOS stabilized (Rows 106 and 183). This particular zebra finch was reared by Bengalese finches, which may have delayed crystallization. Interestingly, even though two syllables were dropped from the song between days 128 and 181, the peak response to these two songs on day 183 was similar. In both Figures 9 and 10, during adulthood the magnitude of the BOS response stabilized, even when we used stimuli that appeared radically different with regard to duration and amplitude modulation. That is, versions of BOS produced later in develop-

ment activated HVC regardless of the time from recording.

Decline in Responses to Older Versions of BOS Is Reliable across Birds and Recording Sites

The decline in responding during the sensorimotor phase was noted across birds and recording sites. Figure 11(A) shows HVC responses to BOS from each recording site for each BOS version that was played back at least twice before 90 days of age. The first point of each line is the day that a particular BOS version was first played back. Note that in almost all cases, the juvenile response declines with time from the day the bird sang that particular song. A similar decline in responding to particular songs with time was not observed in adults [Fig. 11(B)]. In several obvious cases, two lines parallel each other almost exactly [Fig. 11(A)]. These were recordings of responses to the same BOS version from different sites in the same HVC nucleus. The amount of BOS-induced activation in the sleeping adults was very low

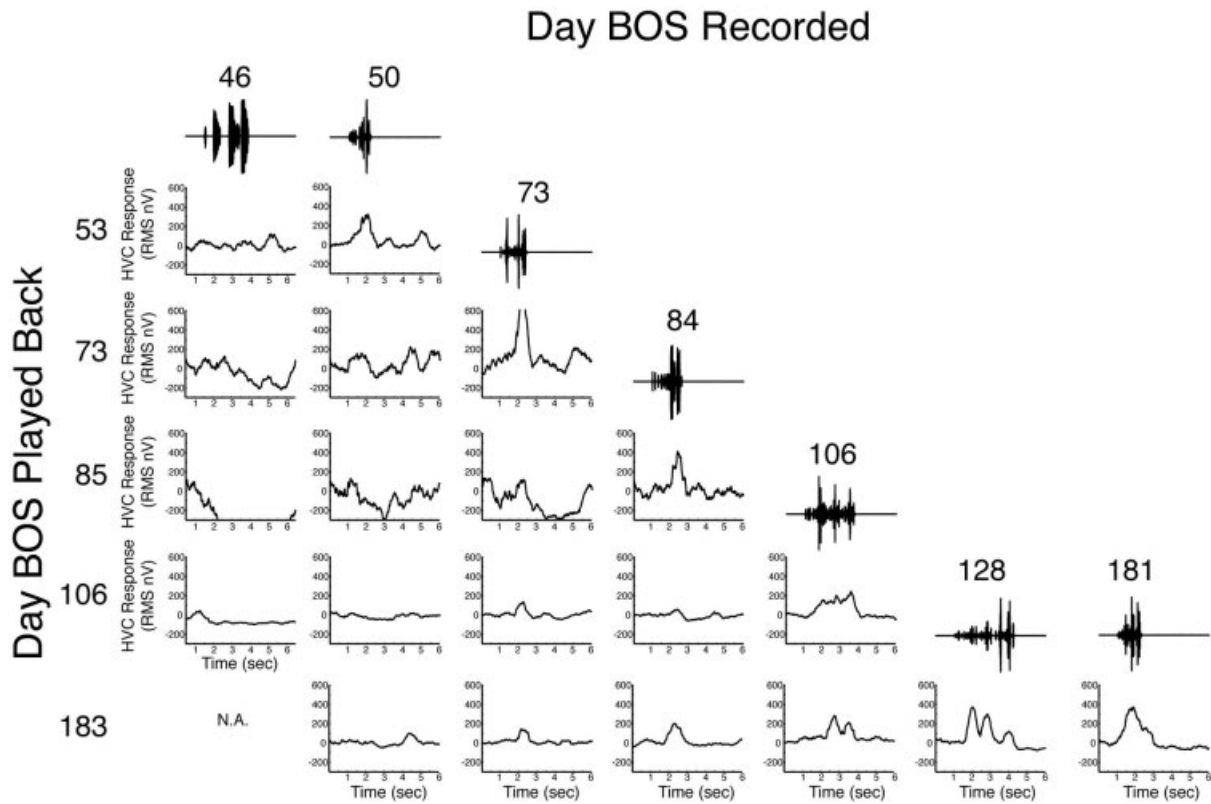


Figure 10 Another example of the decline of BOS responses during the sensorimotor phase of song acquisition. COLUMNS: The day that each BOS was recorded. The oscillogram of each BOS is shown at the top of the column. The HVC response during sleep is shown underneath. ROWS: The day that each BOS was played back to the bird. The sensorimotor phase usually ends at approximately 90 days, but may end later in this bird due to rearing by Bengalese finches. During adulthood, the response magnitude to each copy of BOS appeared to stabilize. All data are from bird Black 12 during sleep.

compared to juveniles [Fig. 11(B)] and, in some cases, BOS playback decreased activity below baseline levels. This is consistent with our previous ob-

servations in sleeping adults (Nick, 2001) and may not replicate previous reports executed under anesthesia.

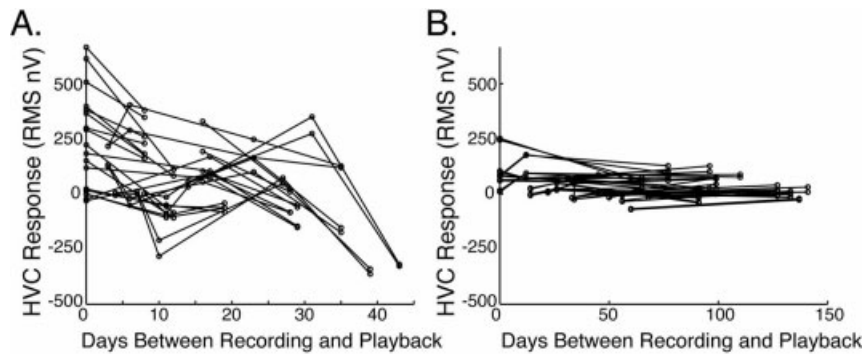


Figure 11 During sleep, HVC responses to different versions of BOS decline with time from recording during the sensorimotor phase (A). Similar declines are not seen in HVC recordings from adults (B). Each line represents responses recorded from one electrode to a given version of BOS. Songs from four birds were used in (A) and three birds in (B).

Juvenile Responses to BOS Are Correlated at Different Sites within the Same HVC

To further explore the correlation of HVC activity during development, we made paired recordings of responses to playback of the same BOS versions in four animals under the age of 90 days (Supplementary Fig. 1). The records from each electrode were significantly correlated ($R^2 = 0.73$; $p < 0.0001$). These data indicate that HVC activity is highly correlated during sleep in the sensorimotor phase, as in adulthood (Margoliash, 1986).

Decline in Responses to Older Versions of BOS Is Significant

The decline in responding to individual versions of BOS was analyzed quantitatively using data from the four juvenile animals that passed the strict EEG criteria for sleep. During the sensorimotor period, songs that were played back 0–9 days after recording induced significantly more HVC response during sleep than the same songs played back 10–100 days after recording (Fig. 12, left; $p < 0.05$, paired t test). Thus, during the sensorimotor phase, the response to a given version of BOS declined with time from recording. Once the song crystallized into the mature form in adulthood, the response stabilized as well, with no significant changes in HVC responding over many days (Fig. 12, right).

DISCUSSION

We have found that, during sleep, the current BOS induces the greatest response in the developing song nucleus HVC. Further, we have found that responses to prior versions of the BOS decline during the period of greatest vocal plasticity. These data indicate that sensory activity during sleep and motor behavior are tightly linked early in the developmental onset of vocalization.

We recorded auditory responses in HVC because this nucleus is the key station in which the neuronal selectivity for BOS is largely established. Nucleus Interfacialis (Nif) (Vates et al., 1996), the main source of auditory input to HVC, is selective for BOS and reverse BOS (played backward) (Janata and Margoliash, 1999; Coleman and Mooney, 2004). However, intracellular recordings in anesthetized adult HVC showed that HVC neurons filter out subthreshold postsynaptic potentials responding to stimuli other than forward played BOS (Mooney, 2000; Mooney et

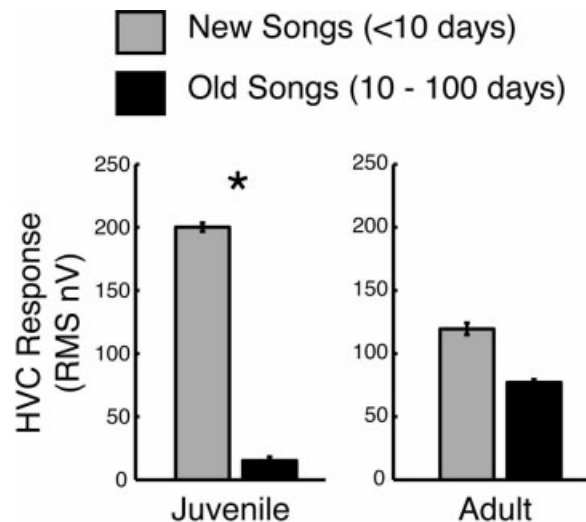


Figure 12 During sleep, the response to prior BOS versions declines during the sensorimotor phase (50–90 days; $*p < 0.05$ paired t test; # birds = 4; # sites = 8; # trial sets: 96, 66). Comparison of recordings during adulthood (>90 days; N.S.; # birds = 3; # sites = 6; # trial sets: 24, 38) indicates that once the BOS crystallizes, the response to BOS is more stable. Error bars represent the SEM.

al., 2001). Thus, the observed parallel changes in song and auditory selectivity in HVC during song development may originate in HVC itself instead of being relayed from its afferent sources. HVC also occupies an important nodal site within the song control system. It sends signals to both the posterior premotor pathway to RA and the anterior forebrain pathway, which is essential for song plasticity (Bottjer et al., 1984; Williams and Mehta, 1999; Brainard and Doupe, 2000).

The Template Theory (Konishi, 1965) postulates that (1) during the sensory phase, a memory of the tutor song, or template, is formed and (2) during the sensorimotor phase, auditory feedback is used to shape the bird's own vocalization through comparison with the template. Physiological investigation of the process described by the Template Theory requires an understanding of the function and mechanism of auditory feedback in the neural song system. During the sensorimotor phase, two critical pieces of information must be gleaned from auditory signals and conveyed to the song system: (1) the degree of similarity between the current vocalization and the template; and (2) the relationships between the patterns of neural firing and the sounds produced. The response to the current BOS reflects the information in (2). As the bird learns the vocal-auditory relationships, it uses the song template (1) to guide the development of the final BOS.

Premotor and auditory inputs may be coconstructed during song learning. This would explain why the auditory responses within HVC during sleep track the vocal motor output (BOS), but it does not explain why there is an auditory response during sleep. In adults (Schmidt and Konishi, 1998; Nick and Konishi, 2001) as well as juveniles (Nick and Konishi, 2004), auditory responses within HVC are gated according to sleep/wake state. State-dependent auditory gating may result from the lack of selective pressure to suppress auditory responses critical for song performance and maintenance during sleep, when the recorded bird would not sing and other conspecific birds would not likely sing.

The data presented here show that circuits within or afferent to HVC actively change with song learning. Other recent work indicates that a putative tutor song matching signal is conveyed to HVC during song learning (Nick and Konishi, 2004). Collectively, these data suggest that the brain sites for storage of the tutor song memory and for comparison of this memory to auditory feedback are within or afferent to HVC. However, the tutor song memory used to shape song during development is not simply sculpted into a BOS memory that can be used to reconstruct perturbed adult song. Data from actively learning juveniles that show state-dependent segregation of stimulus preference within HVC for either BOS (current study) or tutor song (Nick and Konishi, 2004) suggest two separate auditory processing streams that converge afferent to or within HVC: one for learning that responds best to tutor song transiently during development and one for maintenance that responds best to BOS throughout development.

An accurate auditory representation of BOS is necessary for song maintenance. All oscine songbirds studied so far need auditory feedback to develop and maintain song. Recent studies indicate that the template of the tutor song may guide song development and then regress or cease to function (Nick and Konishi, 2004), leaving an accurate representation of the current BOS encoded in the network of synapses that carry auditory information within the song circuitry. This BOS representation would be responsible for song maintenance and error detection in the adult zebra finch, possibly through efference copy (Troyer and Doupe, 2000). We assume that the same neural circuits process BOS whether it comes from an external source or the bird's own mouth directly. However, we still do not know if the auditory feedback signals reach the song system during singing. Solution of these important issues awaits further studies.

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