Pattern of Interhemispheric Synchronization in HVc During Singing Correlates With Key Transitions in the Song Pattern

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Schmidt, Marc F. Pattern of interhemispheric synchronization in HVc during singing correlates with key transitions in the song pattern. J Neurophysiol 90: 3931–3949, 2003. First published August 27, 2003; 10.1152/jn.00003.2003. Many complex voluntary behaviors require that motor commands be tightly coordinated between cerebral hemispheres. The neural mechanisms underlying such coordination, however, remain poorly understood. Song production in birds is a highly stereotyped learned motor behavior that requires finely tuned coordination between hemispheres. In the present study, neural activity was recorded simultaneously from the song control nucleus HVc in each hemisphere of singing adult male zebra finches (Taeniopygia guttata). In all cases, the pattern of recorded multiunit activity in each hemisphere was highly correlated during short segments of the song motor pattern. These correlated segments often consisted of multiple short bursts of activity. Because of the absence of interhemispheric connections between song control nuclei, these observations suggest that HVc activity is “synchronized” by common inputs to both hemispheres. Using sliding-window cross-covariance analyses, periods of high interhemispheric synchronization were found to be time-locked to the acoustic onset of syllables and notes. In some cases, precisely synchronized bursts in both hemispheres were also observed during periods associated with the intersyllable silent interval. In all cases, activity was correlated between hemispheres independently of the recording site, suggesting that all regions of HVc may be globally synchronized during these short segments of the song. Given the anatomical organization of the song system, inputs originating from either thalamus or midbrain are proposed to act as timing signals that initiate and synchronize intrinsic motor networks within each HVc thus allowing for the precise coordination of motor commands across hemispheres.

INTRODUCTION

Many voluntary motor behaviors in vertebrates are controlled, or modulated, by motor control regions in the telencephalon (Grillner et al. 1997). Certain motor behaviors, such as language, may be primarily under the control of a dominant specialized hemisphere (Geschwindt 1970). In many cases, however, motor behavior requires the finely tuned coordination between motor patterns produced in each hemisphere. A classic example of such coordination is the production of bimanual movements (Kelso et al. 1979). Because sectioning of the corpus callosum causes deficits in bimanual coordination (Brinkman and Kuypers 1973), it has generally been assumed that interhemispheric motor coordination is primarily mediated by the corpus callosum (Donich et al. 1998). An equally complex motor behavior that requires finely tuned coordination between hemispheres is the production of song in passerine birds (Vu et al. 1998). Interestingly, the forebrain structures that control song production are not connected between hemispheres (Wild 1997) (birds do not have a corpus callosum). Therefore in contrast to mammalian motor systems, the song system provides a powerful model for investigating the mechanisms of interhemispheric motor coordination in the absence of direct callosal projections.

Song production requires the finely tuned coordination of respiratory muscles and those controlling the vocal organ (syrinx) (Goller and Suthers 1996; Suthers 1997). In addition, because the syrinx is a bilateral structure capable of producing separate independent sounds in each syringeal half (Goller and Suthers 1995; Suthers 1990), exquisite coordination is also required between the muscle commands controlling the left and right syrinx. Neural control of respiratory and syringeal muscles is ultimately controlled by a complex network of interconnected forebrain nuclei (Nottebohm et al. 1976, 1982) known collectively as the song system. This system projects to vocal and respiratory centers in the brain stem (Wild 1997) and is bilaterally organized in that each hemisphere contains anatomically identical song control nuclei (Nottebohm et al. 1976).

Nucleus HVc (also known as HVC) is a forebrain song nucleus necessary for the production of song (McCasland 1987; Nottebohm et al. 1976; Yu and Margoliash 1996). It forms an integral part of a song pattern-generating network (Vu et al. 1994) and its activity is highly stereotyped across song renditions (Hahnloser et al. 2002; Hessler and Doupe 1999; Vu et al. 1998; Yu and Margoliash 1996). Control of brain stem vocal control centers is primarily ipsilateral in nature (Wild et al. 2000) but song premotor activity in HVc appears to be highly coordinated between hemispheres (Vu et al. 1994, 1998) and this coordination is most likely achieved by feedback signals that originate in the thalamus or midbrain. Specifically, nucleus robustus archistriatalis (RA), which receives direct motor inputs from HVc, projects in turn to structures (Fig. 1A) that project (indirectly) back to HVc in both hemispheres (Striedter and Vu 1998; Vates et al. 1997). These include the dorsomedialis posterior (DMP) thalamic nucleus (Vates et al. 1997), the midbrain nucleus DM (Striedter and Vu 1998), and nucleus paramarginalis (PAm), a brain stem structure containing inspiratory bulbospinal neurons (Reinke and Wild 1998; Striedter and Vu 1998; Wild 1997). Two of these...
pathways project back to HVc by way of nucleus uvaiformis (Uva) (Striedter and Vu 1998), a thalamic nucleus that exhibits premotor activity bursts during singing and has been hypothesized to play a role in the initiation, and possible coordination, of song production (Williams and Vicario 1993).

Song-specific premotor activity has been shown to occur in both left and right HVc (McCasland 1987; Vu et al. 1998). However, because these recordings were performed at different times and often in different birds, it was not possible to assess the fine temporal relationship between activity patterns in each hemisphere and address how song motor patterns might be coordinated and synchronized between hemispheres. In the present study, HVc neural activity was recorded simultaneously from multiple electrodes implanted in each hemisphere and shown to be highly synchronized between hemispheres during short portions of the song that were time-locked to key transitions in the song. The lack of direct connections between left and right HVc suggests the existence of a common input that drives HVc activity in each hemisphere. Because zebra finch song is hierarchically organized, characterization of the source and timing pattern of the inputs that synchronize HVc activity are likely to provide important insight into the nature by which higher-order song features may be encoded in the avian song system.

**METHODS**

**Animals and electrode implantation**

Adult (>200 days posthatch) male zebra finches (Taeniopygia guttata) were obtained from the lab’s breeding colony or from a commercial breeder (Magnolia Bird Farm, Anaheim, CA and Canary Bird Farm, Old Bridge, NJ). In some cases (5/9), small silastic pellets of testosterone propionate were implanted subcutaneously to increase the frequency of singing behavior. Electrodes were chronically implanted into both the left and right HVc. In some cases (4/9), 2 electrodes (separated by 200–400 μm) were also placed in the same HVc. One electrode was also sometimes deliberately placed just outside of HVc to make sure there was no cross talk between electrodes. All electrodes for each bird were connected to a nanoconnector (Ultimate, Orange, CA or Omnetics, Minneapolis, MN) and the whole assembly was cemented (GripCement, Milford, DE) onto the bird’s skull with the nanoconnector placed several mm away from the implanted electrodes. Recording electrodes were fabricated from Formvar-insulated nichrome wires (25 μm bare diameter; A-M Systems, Seattle, WA) whose tips were electroplated with rhodium to lower the tip impedance. Typical impedances of electrodes ranged from 100 kΩ to 1 MΩ (measured at 1 kHz). A silver wire was inserted under the dura and partly cemented to the skull to ground the animal. To locate the sites of implanted electrode tips, parasagittal sections of 40 μm thickness were cut on a freezing microtome, mounted, and stained with cresyl violet as described previously (Vu et al. 1998). The location of individual recording electrodes was identified based on gliosis that formed around electrode tips, which had been implanted for 4–12 wk. Electrodes placed within the borders of HVc could be unambiguously identified as being placed within the nucleus. The exact location within HVc could only be estimated because of the gliosis scar around the electrode tip. All procedures were approved by an institutional animal care committee.

**Neural recording**

Several days after surgery, birds were placed in custom-designed Plexiglas recording chambers (manufactured by Herb Adams, Caltech Machine Shop) and attached to a liquid mercury commutator by a flexible cable made from a braided bundle of 7–9 ultraflexible PVC-coated 38-gauge copper wires (Cooner Wire, Chatsworth, CA). The recording chamber was itself placed in a sound-attenuating chamber (Industrial Acoustics Company, Bronx, NY). The flexible cable/commutator arrangement allowed the bird full freedom of movement while providing electrical connections between the electrode and the recording amplifier. In most cases, a female zebra finch was placed in

![FIG. 1. Bilateral recording of HVc neural activity during singing. A: multiple electrodes are implanted in both left and right HVc and neural activity is recorded simultaneously from each electrode site while bird sings. HVc forms part of a bilaterally symmetrical descending motor pathway controlling song production. Interhemispheric connections between song control nuclei are absent at level of forebrain. Several bilateral projections situated below forebrain that project back to HVc have been described. For clarity, only the bilateral feedback pathway from DM to Uva is shown. Two other major feedback pathways: [HVc → RA → PAm → Uva → HVc] and [HVc → RA → DMP → m-MAN → HVc] are described in text. DM, dorsal medial nucleus of the intercollicular region; DMP, dorsomedial posterior nucleus of thalamus; HVc, used here as a proper name; m-MAN, medial portion of magnocellular nucleus of anterior neostriatum; NIf, nucleus interstitialis; RA, nucleus robustus archistriatalis; RAm, nucleus retroambiguus; PAm, nucleus paramedialis; Uva, nucleus uvaformis; Xlls, tracheosyringeal branch of the hypoglossal nerve. B: example of simultaneous recording of premotor activity in left and right HVc during singing of an adult zebra finch. Top: spectrogram (sound frequency spectrum vs. time) of bird’s song, which typically consists of several introductory notes followed by one or more repetitions of a motif (see Fig. 2A for details on zebra finch song structure). Bottom: multiunit neural activity recorded from HVc in each hemisphere shows that activity is generally elevated in both hemispheres during entire song bout. This example is from bird ZF054.](image)
an adjacent cage to induce directed singing. Birds were provided with unrestricted access to food, gravel, and water. Although birds could remain electrically connected for several weeks at a time, activity was typically recorded from each individual bird over only a 1- to 4-day period. In all cases, birds displayed normal feeding, singing, and courtship behavior and showed no signs of discomfort in this recording setup. To test the effect of auditory feedback deprivation on neural activity in HVc, some implanted birds were deafened by cochlear removal (Konishi 1964).

The recording cable was attached to a low-noise, LinCMOS, operational amplifier (TLC27LA, Texas Instruments, Dallas, TX) that was connected to the bird’s head. This amplifier served as a unity-gain voltage follower providing a low-impedance path from the bird’s head to the main recording amplifier, thus greatly reducing movement artifacts from the signal. Auditory and neural records were amplified (4-channel differential AC amplifier, A-M Systems, Everett, WA or a BioAMP, Tucker-Davis Technologies, Gainesville, FL), band-pass filtered between 300 Hz and 10 kHz (7-pole antialiasing filter FT6–7, Tucker-Davis Technologies), and digitized at 20 kHz with a 100-MHz, 16-bit data acquisition (DAQ) board (PCI-MIO-16XE-10 from National Instruments, Austin, TX). Acquisition software (Labview, National Instruments, Austin, TX) was custom-written (A. Leonardo, Caltech, Pasadena, CA) and allowed the simultaneous recording of one sound channel and up to 4 neural channels. Individual files were collected automatically by triggering off of specified features (e.g., amplitude, spectral profile) of the bird’s vocalization. Great care was taken to ensure that song files used for analysis were completely devoid of vocalizations from the neighboring female. In many cases this meant that a large number of songs could not be used for analysis and explains why only 10–20 songs were analyzed for each bird. Neural and sound files were analyzed post hoc using custom-written Matlab scripts (Mathworks). All data were collected and analyzed using a Dell computer.

Data analysis

SONG ANALYSIS AND ALIGNMENT. The highly stereotyped nature of syllables in zebra finch song allows precise alignment of neural traces across many renditions of the same syllable. To construct a profile of neural activity for individual syllables, syllables of a given type (e.g., syllable A) were aligned to the syllable onset and the corresponding rectified HVc neural traces were smoothed (see following text) and averaged. Syllable onset time was determined manually using a custom-written Matlab script. Similar methods were used to align syllables based on their acoustic offset. In some cases, alignment was performed using the calculated cross-correlation time lag between a reference syllable and individual renditions of that syllable. This method of syllable alignment yielded identical results to those obtained using the manual alignment method. Because the time delay between premotor activity and syllable onset is about 45 ms (Yu and Margoliash 1996; present study), syllable-specific neural records typically started 100 ms before syllable onset and ended at syllable offset. In cases such as the 1st syllable of the song, where the 100-ms period preceding the acoustic onset of the syllable might have included an introductory note, care was taken to reduce this period to 60 ms so as not to include premotor activity associated with the introductory note. The variable duration of intersyllable intervals inevitably caused individual records to contain small portions of the premotor neural trace from adjacent syllables. In all figures, song is represented as either the spectrogram or the derivative of that spectrogram (Tchernichovski et al. 2000). Spectrograms were calculated using scripts written either in Matlab (Leonardo and Konishi 1999) or C++ (Tchernichovski et al. 2000) using a sliding-window (5–8 ms) method in which each time point consisted of the direct multitaper estimate of the power spectrum.

ANALYSIS OF NEURAL ACTIVITY. To assess syllable-specific neural traces across electrode sites, neural waveforms for each rendition of the syllable were rectified and smoothed by convolving traces with Gaussian filters of variable widths (SD = 1, 5, or 10 ms). These different smoothing parameters differentially preserve the temporal structure of the premotor neural trace (Fig. 3A). The similarity between smoothed waveforms was assessed by calculating the linear correlation (Pearson’s correlation coefficient). Correlation coefficients for all syllable comparisons were presented as means ± SD and were calculated for each syllable.

In some cases, to visualize neural activity associated with an entire sequence of syllables, such as in a motif, profiles of premotor activity were constructed for the first 2 motifs of the song. To construct these profiles, a canonical song was used as a template to concatenate neural activity profiles obtained from individual song syllables (Fig. 2B). These “whole song” neural activity profiles were then normalized by dividing by the maximum activity level for each recording site. It should be emphasized that these “whole song” profiles were used for visual display only and were never used for quantification purposes.

To quantify the dynamic nature of coincident activity in HVc between electrode pairs, a sliding-window cross-covariance method (using the Matlab function xcov.m) was used to compute a profile of song-related coincident neural activity. This method of analysis was only ever performed on neural traces smoothed with a narrow Gauss-

![Fig. 2.](http://jn.physiology.org/)

Comparison of smoothed song premotor patterns. A: representation of a typical zebra finch song highlighting highly stereotyped nature of this behavior. A given song bout is typically preceded by one or more introductory notes (i) followed by a stereotyped sequence of syllables (S1 → S4), known as motifs, which are repeated several times (2 in this example). In some cases, as exemplified by syllable S4, individual syllables are made up of several distinct subsyllabic elements, known as notes. B: comparison of premotor activity patterns across hemispheres and across motifs. Top: spectrogram of 1st motif of a canonical song used as reference to compute profiles of song premotor activity. Middle and bottom: average neural activity levels (“Neural Activity Profile”) shown for 1st motif from neural traces recorded simultaneously in left (gray) and right (black line) HVc. Each “Neural Activity Profile” has been smoothed with a wide Gaussian filter (SD = 10 ms; see Fig. 3A for details) and normalized to maximum firing rate. This figure illustrates similarity in smoothed neural pattern across hemispheres as well as motifs. Each histogram represents neural activity recorded from 22 songs over a 2-day period from bird ZF036.
ian function (SD = 1 ms). Cross-covariance, which is simply the cross-correlation of both traces whose means are subtracted, was computed for individual windows (20-ms-long segments), which were then moved piecewise in 4-ms steps. For a typical neural trace of 200 ms, the syllable would therefore be represented by 48 overlapping 20-ms windows. The specific window size was optimized to capture rapid synchronous onsets and burst events on the time scale of those shown in Figs. 5 and 14. The correlation coefficient $r$ for each time lag ($k$) was then obtained by normalizing each cross-covariance by the product of each autocovariance at time lag 0 ms such that

$$r_{RL(k)} = \frac{g_{RL(k)}}{\sqrt{g_{RR(k)} g_{LL(k)}}}$$

where $g_{RL(k)}$ is the cross-covariance function between right and left traces and $g_{RR(k)}$ and $g_{LL(k)}$ represent the autocovariance function at time lag 0 ms (Diggel 1990). A value of $r = 1$ indicates that both waveforms are perfectly correlated, whereas a value of $r = 0$ means waveforms are not correlated. Negative $r$-values describe waveforms that are anticorrelated. The sliding cross-covariance matrix calculated for each syllable rendition was then averaged for all renditions of that syllable to obtain a matrix where each row presented the mean $r$-value at any given time and time lag. Cross-covariance matrices were color coded to illustrate the different $r$-values with red representing high levels of correlation ($r > 0.5$) and blue representing either negative $r$-values or $r$-values near zero as shown in Fig. 6.

To correlate neural patterns in both hemispheres with individual acoustic features of the song, matrices were transformed into vectors by taking the maximal $r$-values within the time-lag range of ±1 ms (see Fig. 8A). These vectors are referred to as covariation profiles. The Pearson correlation was used to compare the similarity between “covariation profiles” of the same syllable in different motifs. Correlation coefficients for all syllable comparisons were presented as means ± SD. Peaks in the “covariation profile,” which were arbitrarily defined to be significant if $r > 0.5$, were then compared with acoustic features that occurred 45 ms after these peaks. This delay is the average premotor delay obtained from all birds in this study.

In some cases, the sliding cross-covariance matrix was calculated between neural traces shuffled across renditions. Specifically, neural traces recorded from the left HVC during production of a given syllable (rendition $n$) were correlated to neural traces recorded from the right HVC during a previous rendition of the same syllable (rendition $n - 1$). Correlation values obtained using this analysis method were then compared with those obtained using the standard trial-by-trial correlation analysis. Because sliding cross-covariance analysis yields multiple correlation values for any given syllable, shuffled and trial-by-trial correlations were compared in 2 different ways. First, the total number of segments (each 20 ms long) with values of $r > 0.5$ were compared. Second, similarity of the “covariation profiles” obtained with the trial-by-trial method or the shuffled method were compared using Pearson’s correlation coefficient.

**RESULTS**

*Slowly varying HVC song motor patterns are highly correlated across motifs*

To study the relationship between song motor patterns in each hemisphere, multiple electrodes (2–4) were implanted in left and right HVC of adult male zebra finches (Fig. 1A). Neural activity was monitored from multiunit clusters of neurons in each hemisphere during singing and quiet non-vocalizing episodes. Neurons in HVC generally exhibited little spontaneous activity during quiet periods but showed a dramatic increase in activity 40–70 ms before song onset (Fig. 1B). With the exception of the first few introductory notes, where onset and offset were clearly demarcated, neural activity remained generally elevated during the entire song (Fig. 1B), although clear modulations in firing patterns could be readily observed (Figs. 2B and 14). After song termination, activity usually rapidly returned to baseline levels, and in some cases (Fig. 1B), was even suppressed for several hundred ms thereafter. The general pattern of HVC song premotor activity was similar to patterns obtained from multiunit recordings in previous studies (McCasland 1981; Vu et al. 1998; Yu and Margoliash 1996).

A defining feature of zebra finch song is its highly stereotyped production. A typical song consists of several introductory notes, which are followed by a stereotyped sequence of syllables, known as motifs, that are repeated several times throughout a given song bout (Sossinka and Boehner 1980) (Fig. 2A). Individual syllables can often be subdivided into smaller song elements known as notes (Immelmann 1969; Zann 1996). To analyze premotor activity patterns, neural records obtained for each song rendition were aligned to the onset of individual song syllables. Neural traces were first rectified and smoothed before being averaged across all renditions to create a “neural activity profile” for each syllable. These activity profiles were then used to compare syllable-specific neural activity patterns across motifs and electrode pairs.

A graphic representation of the similarity of HVC song premotor activity patterns across motifs as well as across hemispheres is shown in Fig. 2B. In this example, neural activity profiles computed for individual syllables were smoothed with a wide Gaussian filter (SD = 10 ms) and then concatenated to provide a graphic representation of the “neural activity profile” for the entire motif (see METHODS). Smoothing the neural waveform with this filter retains the slowly varying modulations in firing pattern while smoothing out rapidly modulated events (see Fig. 3A). This figure illustrates the highly stereotyped nature of smoothed song premotor firing patterns in HVC between motifs (Fig. 2B). A similar sized smoothing filter was previously used to illustrate the similarity of song premotor patterns in HVC across syllable renditions (Vu et al. 1998).

Quantitative comparison of smoothed neural waveforms (SD = 10 ms) associated with individual syllables shows that the neural pattern is highly correlated for the same syllables produced in the 1st and 2nd motifs ($r = 0.73 \pm 0.07$; $n = 34$ syllable pairs from 9 birds; Fig. 3B gray bars). No significant correlation is observed when activity is compared between different syllables ($r = 0.05 \pm 0.09$; $n = 34$ syllable pairs in 9 birds). In contrast, comparisons of syllable-specific neural traces smoothed with a narrower filter (SD = 1 ms; Fig. 3B, black bars) or no filter (Fig. 3B, white bars) give much lower correlation values $[F(2,31) = 175; P < 0.001$; one-way ANOVA]. Average correlation values are 0.39 ± 0.09 and 0.06 ± 0.02, respectively. These results suggest that the similarity observed between the same syllables produced in different motifs is high only when analyzing slowly varying modulations in the firing pattern.

*Slowly varying HVC song motor patterns are highly correlated across hemispheres*

In all cases in which recordings were monitored in both hemispheres ($n = 9$ birds), neural activity profiles in HVC during singing were remarkably similar across hemispheres. In
Fig. 2B, smoothed (SD = 10 ms) HVc premotor activity profiles from the left (solid gray) and right (black line) side, for both the 1st and 2nd motifs of the song, are superimposed to show this similarity. Quantitative comparison of smoothed neural activity for individual syllables shows a high level of correlation between left and right HVc (r = 0.73 ± 0.06; n = 34 syllable pairs from 9 birds). No significant correlation is observed when activity in left and right HVc is compared between different syllables (r = 0.06 ± 0.08; n = 34 syllable pairs from 9 birds). In several cases multiple electrodes were successfully implanted in HVc in the same hemisphere (separated by 200–400 μm). Neural activity in ipsilateral electrode pairs showed correlation levels similar to those measured between hemispheres (r = 0.72 ± 0.11; n = 17 syllable pairs from 4 birds). A summary of correlation values for each recorded bird is shown in Fig. 3.

Similar to comparisons made between identical syllables across motifs, correlations of smoothed neural activity between hemispheres decrease significantly as the size of the smoothing filter is narrowed (F(2,24) = 402; P < 0.001; one-way ANOVA). Average correlation values of neural traces smoothed with a narrow filter (SD = 1 ms) or no filter at all are 0.41 ± 0.06 and 0.07 ± 0.02, respectively, for contralateral HVc electrode pairs and 0.47 ± 0.07 and 0.11 ± 0.04 for ipsilateral electrode pairs. Because electrodes pairs are placed at different sites within HVc, the similarity between ipsilateral or bilateral electrode pairs suggests that HVc neurons exhibit the same slowly varying motor pattern independent of placement within the nucleus. This similarity in activity between recording electrodes is not caused by cross talk between electrode pairs because: 1) placing one of the 2 electrodes a short distance outside of HVc (300–500 μm) fails to show any vocalization-specific neural activity (data not shown), and 2) electrode pairs are only correlated when neural activity is smoothed with a wide filter.

The present results suggest that slowly varying premotor patterns are nearly identical in both hemispheres for the entire duration of the syllable. However, because correlation values are low (0.41 ± 0.06) when neural traces are smoothed with a narrow window (SD = 1 ms), these results also suggest that the fine temporal patterns in the premotor trace are significantly different between electrode pairs. The low correlation values obtained for whole syllable comparisons, however, might potentially mask the existence of short segments in the neural trace that are highly correlated across hemispheres. Neural traces associated with each syllable were therefore divided into short 20-ms segments and the linear correlation between left and right HVc was calculated for each segment. The distribution of correlation values obtained from whole syllables (white bars; n = 987 renditions of 21 syllables in 4 birds) and those obtained from the segment correlations (black bars; n = 21,322 segments obtained from all syllables in 4 birds) are shown in Fig. 4. The distribution of correlation values obtained from the short 20-ms segments is broader than that obtained for the whole syllable.
Interhemispheric synchronization of song motor activity

The existence of short segments of highly correlated neural activity between electrode pairs suggests that neural activity may be correlated between hemispheres during precisely timed segments in the song neural trace. Figure 5 illustrates an example of such temporally precise correlated premotor activity. Neural activity is elevated during most of the song but displays clear periods where the modulations in the firing pattern are nearly identical (gray boxes labeled 1 and 2) in each hemisphere (left HVC trace in black and right HVC trace in red) and are highly stereotyped across renditions of the syllable. These highly correlated segments of activity are often flanked by periods of activity where the neural discharge pattern appears quite different between the left and right HVC (dotted boxes labeled a and b). Activity during these periods is also less stereotyped across renditions. In this example, the portion of the neural traces highlighted in gray is characterized by discrete bursts of activity that occur at precisely the same time at each electrode. Similar patterns of synchronized and desynchronized neural activity are observed in bilateral and ipsilateral electrode pairs.

To quantify the relationship between activity patterns in each hemisphere during singing, a sliding-window cross-correlation method was used to compute a profile of synchronous neural activity between electrode pairs. The cross-covariance, which is simply the cross-correlation of both traces whose means are subtracted, is computed for individual windows (20-ms-long segments), which are then moved piecewise in 4-ms steps. The specific window size is optimized to capture correlated burst events on the same time scale of those shown in Fig. 5. Neural traces are rectified and smoothed with a narrow filter (SD = 1 ms) and the cross-covariance function for each segment is normalized to the autocorrelation function of that segment at 0-ms time lag (Diggle 1990) (see METHODS) such that the value of the cross-covariance function at any given time lag represents the correlation coefficient $r$. In the present study, synchronous is defined as events, in the rectified smoothed multiunit neural trace, that are highly correlated ($r > 0.5$) between hemispheres using this method.

A pseudocolor representation of $r$-values, obtained from cross-correlating neural traces obtained from left and right HVC, is shown in Fig. 6. This quantitative representation of synchronous activity, shown for both the 1st and 2nd motifs of the song, reveals a pattern of highly correlated activity (red peaks) that is remarkably conserved between motifs. Cross-covariance analysis re-
veals strongly conserved patterns of synchronous activity be-
 tween motifs (see quantification in following section) in all
recorded ipsilateral \((n = 4)\) and bilateral \((n = 9)\) electrode
pairs. This stereotypy of interhemispheric synchrony suggests
a possible direct relationship between coincident HVc activity
patterns and the production of specific song features.

From a sample of 9 implanted birds, 20 syllables from 8 of
these birds (see Table 2) were carefully chosen to compare
neural patterns with song acoustic features. All chosen sylla-
bles had sharp acoustic onsets that allowed reliable syllable
alignment and were \( \geq 50 \) ms long. Although the pattern of
coincident activity clearly differs for each individual syllable,
2 general features emerge that are common to all syllables.
First, all correlations occur at a 0-ms time lag, suggesting that
both hemispheres are driven by a common synchronizing in-
put. Second, neural activity is maximally correlated about 45
ms before syllable onset.

Cross-covariance matrices for all syllables were summed to
represent the common features that could be extracted from the
correlation analysis between left and right HVc. In Fig. 7A,
maximal correlation values at all time lags (\( \gamma \)-axis, conditional
that they be \( \geq 0.5 \)), are shown for each time window (\( x \)-axis) of
the sliding cross-covariance matrix. The overall distribution of
these conditional maximal correlations shows that all of these
values occur within a time lag of \( \pm 2 \) ms, with the majority of
them centered at 0-ms time lag (Fig. 7B). In the time axis (Fig.
7C), the temporal distribution of these correlation values (thick
black line) reveals a prominent peak at about 45 ms before
syllable onset (peak occurs at \(-43.4\) ms; Fig. 7C). In the
interval between this initial peak and a secondary peak that
occurs about 10 ms after syllable onset, activity is largely
uncorrelated between hemispheres. The initial peak likely rep-
resents the onset of premotor activity because it is temporally
related with the rise in activity (Fig. 7C; filled gray histo-
gram, smoothed with a Gaussian filter whose \( SD = 1 \) ms).

Because all high correlations (i.e., \( r > 0.5 \)) were centered
around the 0-ms time lag, individual cross-covariance matrices
were collapsed into vectors that were constructed by taking the
maximal \( r \)-value in the time-lag range of \(-1 \) to +1 ms. These
vectors will be referred to as covariance profiles. A representa-
tive example of such a “covariance profile” is shown in Fig.
8A for the same syllable produced in 3 different motifs. For
reference, a control “covariance profile” (\( \pm 2 \) SD) obtained
from correlating normal left HVc activity with the reversed
neural trace obtained from the right HVc is also shown in this
figure (Fig. 8A; gray area near the 0 correlation value). A direct
comparison of the relationship between raw neural activity and
“covariance profiles” is shown in Fig. 5. This example illus-
trates the tight correspondence between visually apparent
patterns of coincident activity and the corresponding high \( r \)-values
obtained from the “covariance profile.”

Comparing “covariance profiles” between the 1st and 2nd
motifs, for the 20 syllables whose acoustic onset could be
reliably defined, shows that these profiles are highly stable
across renditions of the same syllable. The linear correlation
obtained for left/right HVc “covariance profiles” in the 1st and
2nd motifs is \( 0.78 \pm 0.15 \) (\( n = 20 \) syllables in 8 birds). These
correlation values are similar to those obtained from ipsilateral
paired recordings (\( r = 0.75 \pm 0.19; n = 11 \) syllables in 4
birds). In contrast, “covariance profiles” between different
syllables in the 1st and 2nd motifs are uncorrelated (\( r =
-0.010 \pm 0.22; n = 20 \) syllables in 8 birds). A summary of
average correlation values for each bird is shown in Fig. 8B.

To investigate whether the pattern of correlated activity
between hemispheres is linked to the produced vocal output,
correlation values were compared under normal conditions and
under conditions where neural traces were shuffled across
renditions (Fig. 9A). Briefly, neural traces aligned to a given
syllable were shuffled pairwise such that the neural trace from
the left HVc was correlated with the neural trace from the right
HVc from a previous rendition of that syllable. The “covi-
arance profile” obtained from shuffled data were then compared
with those obtained under normal conditions. Qualitatively,
these profiles appeared remarkably similar (Fig. 9B) with a
general tendency for the size of the peaks to be smaller when
the data were shuffled (Fig. 9B; dotted line). Quantitatively,
comparison of the correlation coefficient obtained from cal-
culating the linear correlation of the “covariance profile” for
normal data between the 1st and 2nd motifs was not signifi-
cantly different (\( P > 0.05; \) paired \( t \)-test) from correlations
obtained between the shuffled 1st motif and a normal 2nd
motif (Fig. 9C). The linear correlation coefficient was, re-
spectively, 0.77 \pm 0.10 for the shuffled data (white bar; \( n =
11 \) syllables in 4 birds) and 0.82 \pm 0.09 for the normal data

FIG. 6. Quantitative representation of synchroniza-
tion of HVc song premotor activity across hemispheres.
Top: spectrogram of 1st motif of a canonical song used
as reference to compute sliding cross-covariance matrix.
Middle and bottom: color representation of sliding win-
dow cross-covariance matrix (see text and Methods for
details) for song premotor activity in 1st motif (middle)
and 2nd motif (bottom). Each cross-covariance matrix is
normalized to auto-covariance to show correlation value
\((r)\) for each time lag. Each matrix is color-coded to
illustrate different \( r \)-values with red representing high
 correlation levels (\( r > 0.5 \)) and blue representing neg-
ative \( r \)-values and values near zero. Comparison of
cross-covariance matrix between 1st and 2nd motifs
show that overall correlation pattern between hemi-
 spheres is highly conserved across motifs. All high
correlation values (shown in red) occur at or near 0-ms
time lag and occur in a discrete syllable-specific pattern.
Sliding cross-covariance matrices for 1st and 2nd motifs
represent mean from 22 different songs sung over a
2-day period (bird ZF036).
FIG. 7. Summary of common features in HVc premotor pattern. Data are presented for 20 different syllables (n = 8 birds) whose sharp onset allowed reliable syllable alignment. A: summary representation of maximal correlation values and their corresponding time lag for each time window in the sliding cross-covariance. Time lag (y-axis) for each time window (x-axis) where maximal correlation value is recorded is assigned a gray color value. Multiple occurrences at a given time lag/time window coordinate results in darkening of pixel at that coordinate. Because cross-covariance matrices are calculated for a total of 20 syllables, maximum number of pixels for any given time window in x-axis is 20. B: distribution of maximal correlations as a function of time lag shows that periods of highly correlated activity occur within a time lag of ±2 ms with the majority of them centered around 0-ms time lag. C: distribution of maximal correlations as a function of time reveals a prominent peak at about 45 ms before syllable onset (peak occurs at ~43.4 ms). This peak is temporally aligned with onset of "neural activity profile" peak (filled gray histogram smoothed with SD = 1 ms). This profile represents the average of all "neural activity profiles" from 20 syllables.

Interhemispheric synchronization occurs in the absence of auditory feedback

Although HVc exhibits premotor activity during singing, the existence of auditory-responsive neurons within HVc (McCasland and Konishi 1981; Schmidt and Konishi 1998; Yu and Margoliash 1996) suggests that the present observations could result in part from neural responses caused by auditory feedback of the bird’s own song. To test this possibility directly, several birds (n = 3 birds; see Table 1) were deafened by cochlear removal to prevent auditory feedback. An example of the “covariance profile” calculated from the same left/right HVc electrode pair before (black line) and after deafening (gray line) is shown in Fig. 10. In this example, because the bird tended to sing little in the days after cochlear removal, a 4-wk period separated recordings obtained before and after deafening. The large number of peaks in the “covariance profile” with values of r > 0.5 illustrates that activity is highly correlated between hemispheres in both normal and deafened birds. It also reveals that the overall pattern of synchronous activity remains stable after deafening. Linear correlation of “covariance profiles” generated before and after deafening reveals that the pattern of synchronous activity between left and right HVc is stable in all 3 birds (r = 0.58 ± 0.11; n = 9 syllables with sharp acoustic onsets in 3 deafened birds).
Correlated HVC activity defines key timing elements such as syllable and note onset

Activity recorded during production of all analyzed syllables (20 syllables; 8 birds) showed left/right HVC “covariance profiles” that were highly stereotyped from one motif to the next (Fig. 8B). In general, the amount of time where activity was highly correlated across hemispheres (i.e., \( r > 0.5 \)) for a given syllable was positively correlated with syllable duration (Fig. 11; regression = 0.57). This suggests that each syllable-specific motor pattern contains more than simply a segment that is correlated with the acoustic onset of the syllable. Because syllable length is likely related to syllable complexity, this relationship suggests that synchronization may be associated with features that specify syllable substructure. In the sections that follow, representative syllables will be used to illustrate the relationship between neural patterns, “covariance profiles,” and the associated vocal acoustic features in 3 different categories of syllables. These categories are 1) syllables with simple harmonic structures (Fig. 12), 2) syllables that are composed of 2 clearly distinguishable notes (Fig. 13), and finally 3) complex syllables with multiple note elements (Fig. 14).

**Simple Syllable.** In the population of 9 birds recorded for the present study, 4 birds produced songs that contained simple harmonic syllables. All 4 of these syllables showed the same general pattern and 2 of these are shown in Fig. 12 to illustrate the general relationship between neural activity patterns in both hemispheres and syllable morphology. In these examples, the smoothed (SD = 1 ms) neural activity profile (middle) shows a sudden increase in activity in both the left (gray) and right (black) HVC about 45 ms before the syllable’s acoustic onset. This correlated increase in activity in both hemispheres is reflected by the first large peak [Peak 1 in the left (\( r = 0.71 \)) and right (\( r = 0.68 \)) syllable] in the “covariance profile” (bottom). The delay between the correlated increase in activity and the acoustic onset of the syllable is consistent with the delay calculated from the population analysis (Fig. 7C) and likely represents the premotor onset response for this syllable. After the initial increase in activity, neural discharge levels, although modulated, remain relatively elevated and uncorrelated for another 40 ms. Interestingly, about 50 ms after the initial correlated increase in activity in both hemispheres, there is a 2nd correlated increase in activity [Peak 2 in the left (\( r = 0.62 \)) and right (\( r = 0.67 \)) syllable].

The relationship between this 2nd correlated increase in activity and the syllable is difficult to assess because shifting the peak by the premotor delay of 45 ms places the pattern of activity squarely in the middle of the syllable. Interestingly, a similar pattern is observed in the complex syllable shown in Fig. 14 where the 1st component of the syllable consists of a simple harmonic note similar in its acoustic structure to the syllables described here. Shifting the 2nd peak in the complex syllable by the same premotor delay of 45 ms also aligns it to the middle of the note (2nd dotted line in Fig. 14). This similarity in neural patterns obtained for acoustically similar elements from different recordings in different birds suggests a possible common encoding scheme for the production of acoustically simple syllables.

**Two-Note Syllable.** Given the striking relationship between correlated increases in activity in both hemispheres and the acoustic onset of syllables, it was of interest to investigate whether a similar relationship could be observed for the acoustic onset of notes. Although many syllables are made up of 2 or more notes, it is sometimes difficult to determine the precise transition between the notes that make up these syllables. In a few cases, however, clear transitions can be identified. In the 9 birds that were recorded, 4 syllables from 4 different birds were identified as having clear syllable transitions. These syllables were analyzed in detail and 2 of these are shown in Fig. 13. All 4 syllables showed the same general pattern.

In the examples shown below, activity is, as in previous
examples, highly correlated with the acoustic onset of the syllable [Peak 1 on the “covariance profile” for both the left ($r = 0.59$) and right ($r = 0.53$) syllable]. In both syllables, however, a 2nd correlated increase in activity, shown by the large covariance peak [Peak 2 ($r = 0.68$) in the left panel and Peak 3 ($r = 0.54$) in the right panel], occurs 60 ms (left syllable) and 98 ms (right syllable), respectively, after the initial increase in activity. Assuming a premotor lead time of 45 ms, these correlated increases in activity correspond almost exactly to the acoustic onset of the 2nd note in the syllable (dotted line). In one of the syllables (ZF16 Syllable D), there is also a correlated decrease in activity followed by a correlated burst in both hemispheres (Peak 2; $r = 0.51$). This correlated pattern of activity falls precisely in the middle of the syllable’s 1st note and is reminiscent of the pattern observed for the simple harmonic syllables. Taken together, these examples suggest that both syllable and note onset are temporally linked to the correlated activation of HVc activity in both hemispheres.

**COMPLEX SYLLABLE.** In the previous examples, activity was modulated during production of the syllable but emphasis was directed at correlated activity associated with either the acoustic onset of the syllable or the notes that make up the syllable. In this example, analysis of the neural patterns associated with long (235-ms) complex syllables reveals that the premotor pattern can be highly modulated and that this modulated firing pattern is highly correlated across hemispheres. In Fig. 14, neural activity (top panel) recorded simultaneously from left and right HVc is shown for 22 different renditions of this complex syllable. In this pseudocolor representation of premotor activity, neural traces have been rectified and smoothed with a narrow filter (SD = 1 ms). Red represents high levels of activity and dark blue represents the absence of neural activity. Particularly striking in this example is the relatively large number of discrete neural events characterized by short bursts of activity that are highly stereotyped across renditions. Many of these bursts are quite short (about 5–10 ms in duration) and are reminiscent both in their precision of firing as well as in their duration to the sparse bursts produced by HVc projection neurons during singing (Hahnloser et al. 2002). Because electrodes sample neural activity from multiple neurons, these

**TABLE 1. Summary of songs and recording protocols**

<table>
<thead>
<tr>
<th>Bird ID</th>
<th>Song Structure</th>
<th>Songs Analyzed</th>
<th>Recording Period</th>
<th>Electrode Pairs</th>
<th>Deafening</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZF003</td>
<td>i CDE ABCDE</td>
<td>14</td>
<td>Over 4 days</td>
<td>CONTRA &amp; IPSI</td>
<td>No</td>
</tr>
<tr>
<td>ZF012</td>
<td>i ABCD ABCD</td>
<td>5</td>
<td>Over 4 days</td>
<td>CONTRA only</td>
<td>No</td>
</tr>
<tr>
<td>ZF015</td>
<td>i ABCDE ABCDE</td>
<td>14</td>
<td>Over 4 days</td>
<td>CONTRA &amp; IPSI</td>
<td>YES</td>
</tr>
<tr>
<td>ZF016</td>
<td>i ABCDEF ABCDEF</td>
<td>15</td>
<td>Single day</td>
<td>CONTRA &amp; IPSI</td>
<td>YES</td>
</tr>
<tr>
<td>ZF036</td>
<td>i ABCD ABCD</td>
<td>22</td>
<td>Over 2 days</td>
<td>CONTRA &amp; IPSI</td>
<td>No</td>
</tr>
<tr>
<td>ZF054</td>
<td>i CDE ABCDE ABCDE</td>
<td>11</td>
<td>Single day</td>
<td>CONTRA only</td>
<td>No</td>
</tr>
<tr>
<td>ZF110</td>
<td>i ABCDEF ABCDEF ABCDEF</td>
<td>16</td>
<td>Single day</td>
<td>CONTRA only</td>
<td>YES</td>
</tr>
<tr>
<td>ZF122</td>
<td>i XCDE ABCDE</td>
<td>20</td>
<td>Single day</td>
<td>CONTRA only</td>
<td>No</td>
</tr>
<tr>
<td>ZF128</td>
<td>i BCDE ABCDE ABCDE</td>
<td>18</td>
<td>Single day</td>
<td>CONTRA only</td>
<td>No</td>
</tr>
</tbody>
</table>

Birds were deafened within 2–3 days after the initial recordings. Neural recording in deafened birds was typically performed 1–4 wk after cochlear removal.
bursts could consist of multiple neurons activated in near synchrony or single neurons producing a single burst or both. Although the neural pattern is for the most part nearly identical across hemispheres, a few differences (highlighted by the small red arrows in Fig. 14) are apparent. Equally striking in this example are the brief periods of nearly complete suppression (shown in dark blue) of activity in both hemispheres.

Analysis of the “covariance profile” reveals 7 distinct peaks whose $r > 0.5$. Small black arrows numbered 1–7 show the temporal relationship between the raw neural trace and each associated peak. Shifting each peak by the premotor delay of 45 ms aligns the 1st peak (Peak 1) with the onset of this complex syllable. The 2nd peak (discussed in the previous section) aligns to the middle of the first note in the syllable. Peaks 3 and 4 align, respectively, to the offset of the 1st note and the onset of the 2nd note. Peak 5 appears to be loosely correlated with a possible note transition in this complex syllable, whereas Peak 6 does not seem to be correlated to any obvious feature of the syllable. Peak 7 may be associated with the acoustic offset of the syllable but the exact relationship is complex, given that this peak corresponds to a correlated increase in activity rather than a correlated decrease in activity. Taken together, the relationship between peaks of correlated interhemispheric activity and syllable transitions in this example suggests that synchronous activation of neural activity in both hemispheres may represent timing signals that define transitions in these complex syllables. Some of these transitions may be obvious (Peaks 1, 3, 4, and 5), whereas others (Peaks 2, 6, and 7) may not.

Acoustic offset is not strongly associated with a correlated offset of activity in both hemispheres

The relationship between acoustic offset and neural activity is often difficult to assess. Because syllables within a motif precede other syllables, it is hard to determine the end of the premotor activation period for one syllable and the onset of activity for the next syllable. This problem is clearly illustrated in Figs. 12 and 13 for the rightmost syllables where the large correlated increase in activity at the end of the trace is not associated with the syllable shown in the figure but rather with the syllable that directly follows it. To circumvent this problem, the relationship between premotor activity and acoustic offset was analyzed only during production of the last syllable of the bird’s song bout.

On average, neural activity decreases slowly toward baseline about 40 ms before acoustic offset of the syllable (Fig. 15A; filled gray histogram, top panel). This slow decrease in activity is in sharp contrast to the rapid synchronized increase in activity observed at syllable offset (see Fig. 7C). One problem with this average representation of premotor activity is that it smooths out the considerable variability that is observed across syllables (see examples in Fig. 15, B and C). Some syllables will show rapid decreases in activity about 40 ms before the acoustic offset, whereas others will continue to exhibit bursts of activity past the expected premotor offset period (Fig. 15B). In some cases, these premotor bursts in HVc may even occur past the acoustic offset of the syllable (see Fig. 16).

In addition to the general variability of the premotor pattern associated with syllable offset, variability is also observed in the degree to which activity is correlated across hemispheres.

FIG. 10. Effect of deafening on interhemispheric correlation of HVc activity. A: sonogram of same syllable recorded before (left) and after (right) deafening by cochlear removal. B: “covariance profile” calculated from same left/right HVc electrode pair before (black line) and after deafening (gray line) reveals that activity remains highly correlated after deafening. In addition, it also shows that overall pattern of “covariance profile” remains remarkably stable despite 4-wk interval between collection of PRE- and POST-deafening records. This example is from bird ZF110.

FIG. 11. Absolute time of correlated activity between hemispheres in-
This variability is shown in the “covariance profiles” for each of the analyzed syllables (Fig. 15A, bottom). In 7/10 birds, correlated activity is observed during a 20-ms period (−35 to −55 ms) centered around the expected premotor offset (double line in Fig. 15A). In 5 of these birds, however, this period of correlated activity is followed by at least one additional period of correlated activity during the period spanning −35 ms and the acoustic offset (dotted line in Fig. 15A) of the syllable (i.e., 0 ms). In the remaining 3 syllables, correlated activity is absent during the expected premotor onset period. Individual examples are shown in Fig. 15, B and C. The 1st syllable (Fig. 15B) shows a correlated decrease in premotor activity in both hemispheres. It is difficult to estimate, however, what part of the neural trace is truly representative of acoustic termination because there is a correlated decrease in activity 38 ms before the syllable offset (Peak 3) and then another correlated decrease in activity about 20 ms later (Peak 4). In the second example (Fig. 15C), activity generally decreases in both hemispheres but is completely uncorrelated. This is illustrated by the absence, in the “covariance profile,” of any peaks >0.5 during the 100 ms that precede the syllable’s acoustic offset. Remarkably, even though this syllable is aligned at its acoustic offset, neural activity associated with the syllable onset (Peak 1) and the note transition (Peak 2) is still highly correlated. Taken together, these data suggest that the relationship between HVC premotor activity and syllable onset is strikingly different from that observed for syllable offset. During the onset of syllables and notes, premotor activity rises rapidly and is highly correlated across hemispheres. In contrast, during syllable termination premotor activity patterns are highly variable between syllables. In general, premotor activity does not decrease sharply at the expected premotor offset and the timing of synchronized interhemispheric activity is poorly correlated with syllable offset.

Evidence for correlated bursts associated with song termination

The difficulty in identifying clear premotor offsets may be caused in part by the existence in HVC of activity patterns that are not necessarily directly linked to syllable production. These could be caused by auditory feedback signals of the bird’s own song or by activity in HVC that specifies motor commands not associated with syllable production. Previous work has suggested the existence of large bursts of activity, referred to as “super bursts,” that occur in Uva at the offset of song bouts and possibly motifs (Williams and Vicario 1993). Because Uva projects directly to HVC, it was of interest to investigate whether similar bursts also occur in HVC and whether they are correlated between hemispheres. To ensure that these bursts...
are not simply the result of neural responses to auditory feedback of the bird’s own song, neural activity patterns in left and right HVc were analyzed in deafened birds. In addition, they were confined to neural traces associated with the last syllable of the bird’s last motif in the song bout to distinguish between the existence of a “super burst” and a burst simply associated with the onset of the next syllable.

Figure 16 shows a syllable from a deafened bird that exhibits correlated bursting after acoustic termination of the syllable. In this example, there is a sharp increase in HVc premotor activity about 50 ms before syllable onset that is highly correlated across hemispheres (Peak 1 in bottom panel; \( r = 0.89 \)). Then, about 40 ms before syllable offset, neural activity decreases back to rest levels in both hemispheres. This decrease in activity is correlated between hemispheres (Peak 2; \( r = 0.52 \)). About 40 ms after premotor activity returns to baseline levels, there is a large, highly synchronized (Peak 3; \( r = 0.85 \)) burst of activity that coincides almost exactly with the acoustic termination of the syllable (Fig. 16, black arrow). This peak is followed by a smaller but significant peak (Peak 4; \( r = 0.75 \)) 30 ms later. Although the significance of this song offset burst is not known, it may well be the HVc equivalent of the “super burst” observed in Uva. Correlated bursts after termination of the last syllable of the bird’s song bout were observed in 6/13 syllables (10 syllables from normal-hearing birds and 3 syllables from deafened birds). In these syllables, bursts occurred within 30 ms of syllables’ termination (range = −10 to 30 ms relative to acoustic offset) and had peaks in the “covariance profile” that had values of \( r > 0.5 \).

**Significance of correlated bursts that occur during the intersyllable silent period**

The existence of “super bursts” in HVc associated with song termination suggests the possibility that synchronized bursts in the premotor trace might be associated with motor gestures, such as inspiration, that occur during the intersyllable silent interval (Franz and Goller 2002; Wild et al. 1998). The duration of intersyllable intervals varies for each syllable pair (ranging from 15 to 60 ms in the set of syllables analyzed in the present study; see Table 2) but is quite stereotyped across renditions of these syllables (Sossinka and Boehner 1980).

To analyze HVc neural activity patterns during syllable intervals, neural activity levels measured during these intervals were compared with the mean neural activity recorded during quiescent non-singing periods. The neural equivalent of the syllable silent interval was considered as the period between the acoustic offset of one syllable minus the 45-ms premotor delay and the acoustic onset of the following syllable minus the same 45-ms premotor delay (see schematic in Fig. 17A). For all

**Two-Note Syllables**

![Figure 13](http://jn.physiology.org/)

**FIG. 13.** Patterns of synchronized activity in HVc correlate with both syllable and note onset. This figure is organized like Fig. 12 and illustrates relationship between premotor activity and note transitions in 2 representative syllables from different birds. In these examples, HVc activity is highly correlated (Peak 1) 45 ms before acoustic onset of each syllable. In addition, HVc activity is also highly correlated 45 ms before acoustic transition between 2 notes that make up these syllables. This is shown by Peak 2 in left syllable and Peak 3 in right syllable. In syllable from bird ZF16, there is an additional peak (Peak 2) that does not appear to correlate to any obvious transition in syllable. High level of activity at end of rightmost syllable (ZF16) corresponds to premotor onset of syllable that follows that shown in figure.
During the silent interval was correlated across hemispheres, not represented in HVc by an absence of premotor activity. The intersyllable silent interval were higher than background activity, suggesting that the silent interval between syllables analyzed (20/20), neural activity levels during the silent interval was correlated across hemispheres.

To investigate whether HVc premotor activity observed during the silent interval was correlated across hemispheres, r-values obtained from “covariance profiles” were measured during these intervals. In over half of the syllables (13/20 syllable pairs; Table 2), one or 2 discrete bursts of activity were observed during the intersyllable silent interval. A subset of these (7/20) had correlation values > 0.5 during these silent intervals. Two striking examples of such correlated bursts from 2 different birds are shown in Fig. 17B. In these examples, discrete bursts lasting 5–10 ms are observed about 60 (bird ZF128) and 70 ms (bird ZF110) before syllable onset. Each burst is precisely synchronized with the contralateral burst with a correlation value of 0.82 and 0.79, respectively, for birds ZF128 and ZF110.

**DISCUSSION**

In the present study, neural activity in HVc was recorded from multiple electrodes, placed either in the same hemisphere or in separate hemispheres, while the bird was singing. The present findings support 2 major conclusions. First, there is a lack of obvious spatial organization of song representation in HVc, given that multiunit recordings from small populations of neurons sampled throughout the structure are active during all song syllables and exhibit the same slowly varying firing pattern at each electrode site. Second, song premotor activity is mostly uncorrelated between electrode sites except for short periods in the song when HVc activity is highly correlated both within and across hemispheres. The acoustic onset of syllables and notes is tightly associated with the occurrence of these periods of synchronized HVc activity. These periods of synchronous activity are also strongly associated with the short silent intervals that occur between syllables.

*Song is represented globally within HVc by the slowly varying premotor pattern*

Vocal output results from the combined action of syringeal muscles as well as the muscles that control respiration (Goller and Suthers 1995; Suthers 1997; Wild 1997). In songbirds, the left and right halves of the syrinx act as independently controlled sound-generating structures that are primarily under the control of ipsilateral song control nuclei (Wild et al. 2000). Early pioneering studies by Nottebohm demonstrated that each syringeal half contributes differently to the bird’s song (Nottebohm et al. 1990; Nottebohm and Suthers 1995; Suthers 1997; Wild 1997). In songbirds, the left and right halves of the syrinx act as independently controlled sound-generating structures that are primarily under the control of ipsilateral song control nuclei (Wild et al. 2000). Early pioneering studies by Nottebohm demonstrated that each syringeal half contributes differently to the bird’s song (Nottebohm et al. 1990; Nottebohm and Suthers 1995; Suthers 1997; Wild 1997). The source of this lateralization may originate in part from hemispheric differences between forebrain song control nuclei (Wild et al. 2000).

FIG. 14. Complex syllables contain multiple periods where HVc activity is highly correlated across hemispheres. In this example, neural activity patterns (top panel) recorded simultaneously from left and right HVc are shown during production of a complex syllable (sonogram shown in middle panel). *Top panel:* neural traces for 22 renditions of this syllable have been rectified and smoothed with a narrow filter (SD = 1 ms) with red representing high levels of activity and dark blue representing absence of neural activity. “Covariance profile” for this syllable is shown on bottom panel and peaks with r > 0.5 are numbered 1 through 7. Timing of each of these peaks relative to raw neural trace is shown by numbered arrows in top portion of figure. Dotted black lines are drawn for each peak and are shifted by premotor delay of 45 ms to show their relationship to acoustic features of syllable. Two red arrows indicate clear examples where a burst of activity in one hemisphere is not accompanied by activity in contralateral hemisphere. See text for details regarding figure interpretation.

syllables analyzed (20/20), neural activity levels during the intersyllable silent interval were higher than background activity, suggesting that the silent interval between syllables is not represented in HVc by an absence of premotor activity. To investigate whether HVc premotor activity observed during the silent interval was correlated across hemispheres,
Multiple sites in HVC either in the same hemisphere, or in different hemispheres, suggested a lack of any obvious spatial segregation of song-related neural activity. In fact, comparison of neural records smoothed with a wide filter (SD = 10 ms) to capture only the slowly varying changes in the firing rate revealed that neural firing patterns were highly correlated across recording sites in all birds. In some cases the similarity of the neural firing pattern was quite remarkable even at the level of the fine structure of the motor pattern. Figures 5 and 14 provide examples of neural records obtained simultaneously from each hemisphere where short segments of the neural record are nearly identical at both electrodes. At first glance, these data may suggest that song-related firing patterns are not different between hemispheres and that song is encoded in a globally distributed manner. Caution should be exercised in making such conclusions, however, because with the exception of short segments at precise periods in the song, non-smoothed neural traces were usually not correlated between recording sites. In fact, raw neural traces were just as likely to be uncorrelated whether they were in the same hemisphere, separated by 300–400 µm, or in different hemispheres. These data thus suggest that individual neuron clusters in HVC, although constrained in their firing pattern by extrinsic inputs (see next section), exhibit for the most part their own spatially distinctive firing patterns.

HVC contains 3 distinct classes of neurons that are thought to be distributed heterogeneously throughout the nucleus (Fortune and Margoliash 1995; Gahr 1990; Margoliash et al. 1994). It has recently been shown that interneurons tend to be active during production of all song syllables, whereas RA- and X-projecting neurons are much more sparse in their firing patterns, producing in many cases only a single burst of action potentials during a given song motif (Hahnloser et al. 2002). Thus although all 3 of these neuron types may contribute to multiunit traces, it is likely that much of the neural signal in the present study represents inter-
hemispheric differences cannot be explained by comparing the causes a resetting of the song pattern. This has been used as commands across hemispheres for synchronization in the coordination of motor

...pattern of individual identified neurons in each hemisphere remains an open question. neuron activity. Irrespective of the exact constitution of the multiunit trace, however, it is clear that—in zebra finches at least—hemispheric differences cannot be explained by comparing the slowly varying firing pattern in each hemisphere. Whether lateralization can be explained by studying the collective population pattern of individual identified neurons in each hemisphere remains an open question.

**Role for synchronization in the coordination of motor commands across hemispheres**

Stimulation of HVc in either hemisphere during singing causes a resetting of the song pattern. This has been used as evidence to implicate HVc as part of a song pattern–generating network (Vu et al. 1994). Because song is controlled by anatomically identical vocal control nuclei in each hemisphere, resetting of the song pattern also suggests that song motor commands are tightly coordinated across hemispheres (Vu et al. 1994, 1998). It was previously proposed that HVc motor patterns in each hemisphere are continuously being compared, possibly at the level of the midbrain or thalamus, and that stimulation in one hemisphere causes a mismatch between sides that results in a resetting of the motor pattern (Vu et al. 1998). Interestingly, identical stimulation in RA resets the motor pattern only in about 10% of cases (Vu et al. 1994).

In the present study, HVc neural activity patterns are shown to be highly correlated between hemispheres during brief periods in the song associated with syllable and note onset but are mostly uncorrelated during the remainder of the motor trace. These results suggest that motor output from each hemisphere may be compared only during the periods when activity is correlated. Because of intrinsic properties in the motor network, brief stimuli in HVc might cause prolonged disruptions in motor output that inevitably disrupt activity during periods where both hemispheres are synchronized. Vu et al. (1998) proposed that such disruption might cause an error mismatch that results in a song reset. Identical stimuli in RA would cause only brief disruptions in the motor trace that rarely overlap with periods where both hemispheres are synchronized and would thus only rarely result in song resets. A specific prediction of this idea is that stimulation in RA should result in song resetting if stimuli are timed to precisely overlap with the portion of the motor trace that is synchronized between hemispheres.

**TABLE 2. Summary of correlated syllable onsets and inter-syllable silent intervals**

<table>
<thead>
<tr>
<th>Bird ID</th>
<th>Syllable ID*</th>
<th>Pre-Syllable Silence Interval</th>
<th>Syllable</th>
<th>Duration, ms†</th>
<th>Correlated bursts‡</th>
<th>Duration, ms†</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZF003</td>
<td>D</td>
<td>36 ± 2</td>
<td>YES (r &gt; 0.5)</td>
<td>74 ± 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZF015</td>
<td>B</td>
<td>43 ± 3</td>
<td>NO</td>
<td>250 ± 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>21 ± 1</td>
<td>NO</td>
<td>158 ± 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>33 ± 3</td>
<td>NO</td>
<td>239 ± 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZF016</td>
<td>B</td>
<td>42 ± 2</td>
<td>YES (r = 0.42)</td>
<td>224 ± 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>33 ± 3</td>
<td>YES (r = 0.48)</td>
<td>97 ± 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>32 ± 2</td>
<td>NO</td>
<td>184 ± 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>32 ± 2</td>
<td>weak (r = 0.30)</td>
<td>144 ± 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZF036</td>
<td>C</td>
<td>20 ± 3</td>
<td>YES (r = 0.37)</td>
<td>286 ± 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>43 ± 3</td>
<td>NO</td>
<td>235 ± 2</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>ZF054</td>
<td>C</td>
<td>29 ± 2</td>
<td>YES (r &gt; 0.5)</td>
<td>97 ± 3</td>
<td></td>
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<tr>
<td>D</td>
<td>20 ± 2</td>
<td>YES (r = 0.41)</td>
<td>223 ± 3</td>
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<tr>
<td>ZF110</td>
<td>C</td>
<td>36 ± 3</td>
<td>YES (r &gt; 0.5)</td>
<td>108 ± 4</td>
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<tr>
<td>D</td>
<td>29 ± 3</td>
<td>YES (r &gt; 0.5)</td>
<td>167 ± 3</td>
<td></td>
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<tr>
<td>E</td>
<td>34 ± 2</td>
<td>YES (r &gt; 0.5)</td>
<td>141 ± 2</td>
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<tr>
<td>ZF122</td>
<td>D</td>
<td>25 ± 3</td>
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<td>153 ± 2</td>
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<tr>
<td>ZF128</td>
<td>C</td>
<td>38 ± 3</td>
<td>YES (r &gt; 0.5)</td>
<td>166 ± 5</td>
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<tr>
<td>D</td>
<td>35 ± 4</td>
<td>NO</td>
<td>102 ± 4</td>
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<tr>
<td>E</td>
<td>32 ± 4</td>
<td>YES (r = 0.46)</td>
<td>133 ± 3</td>
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</tr>
</tbody>
</table>

* These syllables were chosen for detailed analysis because of their sharp acoustic onset and because they had a duration of at least 50 ms. † The duration (in ms) of each syllable and the silent interval that immediately precedes it are described by their mean and standard deviation. These values are based on 12–20 renditions of each syllable. ‡ When bursts of activity are observed in both hemispheres during the syllable silent interval, these are categorized by either YES, yes or weak depending on the r-value calculated from the “covariance profile.” YES, r > 0.5; yes: 0.4 > r > 0.5; weak: 0.3 > r > 0.4. A correlation value smaller than 0.3 is indicated as NO and signifies that no correlated bursts were observed during this interval.
spheres. An additional consideration for different effects in HVC and RA is the different spatial organization of both structures. HVC neurons that project to RA project indiscriminately to all regions of RA (Fortune and Margoliash 1995; Margoliash et al. 1994). RA, in contrast, is divided into functionally distinct regions (Vicario 1994, 1991). One of these regions, the dorsal RA, projects directly to bilaterally connected structures in the midbrain, thalamus, and brain stem (Fig. 1B; Reinke and Wild 1998; Striedter and Vu 1998; Vates et al. 1997; Vicario 1991) and is, anatomically at least, well placed to relay signals that may be used to compare activity between hemispheres (Schmidt and Konishi 1999). To get reliable resetting of song from RA, stimuli may have to be delivered both at the right time as well as in the correct part of RA.

Hierarchical encoding of song features: role of structures in the midbrain or thalamus

Acoustic onset of individual syllables, and notes, was tightly associated with correlated increases in HVC neural activity in both hemispheres. The time delay between acoustic onset and this correlated increase in neural activity was about 45 ms, a value that approximates previous estimates of premotor delay in HVC (Yu and Margoliash 1996). In contrast to acoustic onset, the relationship between acoustic offset and HVC premotor activity was more difficult to interpret. In some cases premotor offset was correlated between hemispheres whereas in others it clearly was not.

In addition to the tight correlation between acoustic onset and interhemispheric synchronization, precisely synchronized bursts of neural activity were observed during periods, as estimated by the premotor delay, that were not associated with any acoustic event but rather with the intersyllable silent interval. The significance of these bursts is at present not clear but could fulfill possible functions. First, because manipulating activity in RA causes the bird to vocalize during the intersyllable silent period (Vicario and Raksin 2000), these bursts may specify the duration of the intersyllable silent interval. Second, these bursts may act to synchronize the timing of the short inspiratory breaths (Franz and Goller 2002; Wild et al. 1998) known to occur before the production of most zebra finch syllables (Goller and Daley 2001). Finally, it is possible that these synchronized bursts play a role in linking syllables together into larger functional units. It is known from previous work, for example, that syllables can be functionally “chunked” together (Cynx 1990; Williams and Staples 1992) and that such “chunking” may be an intermediate step, between syllables and motifs, in the overall organization of zebra finch song.

In the zebra finch, song is produced in an ordered and stereotyped manner (Scharff and Nottebohm 1991; Sossinka and Boehner 1980) and its overall hierarchical organization [song bout → motif → [syllable chunk] → syllable → note] make it plausible that the brain structures that control song production may also be organized hierarchically. Based on studies of song-related neural patterns in HVC and RA, it has
premotor load time (45 msec)

Previously been shown that RA activity is most strongly associated with note production, whereas HVC is more tightly linked to syllable identity (Margoliash 1997; Yu and Margoliash 1996). While these results have been used as evidence for a hierarchical representation of syllables and notes in the song motor pathway, little is known regarding the representation of higher-order song features such as motif and song bout structure. The existence of 2 input pathways into HVC with known song motor involvement [DM → Uva → [NIf] → HVC] (Vu and Coleman 2001; Williams and Vicario 1993) and (DMP → m-MAN → HVC) (Vu and Coleman 2001) suggest a possible role for these “ascending motor pathways” in determining some of these higher-order song features. A third bilaterally organized pathway originating from the brain stem inspiratory control nucleus (PAm → Uva → [NIf] → HVC) may also contribute to these higher-order song features.

In the present study, activity was not directly recorded from the structure in the “ascending motor pathway” but the recorded observations speak directly to the types of song features that may be represented in these pathways. Specifically, the lack of interhemispheric connections between forebrain song control nuclei (Striedter and Vu 1998; Wild 1997) make it likely that the pattern of synchronized song premotor activity in left and right HVC is caused by common inputs originating from bilaterally organized structures in the midbrain or thalamus. The bilateral organization of these “ascending motor pathways” described above make them ideal candidates for sending timing signals that serve to synchronize neural activity in left and right HVC. It is likely that the Uva pathway acts as the primary synchronizer of HVC activity because unilateral lesions of Uva (Vu and Coleman 2001; Williams and Vicario 1993), but not m-MAN (Foster and Bottjer 1993; Vu and Coleman 2001), cause significant deficits in song output.

Based on the results from this study, I propose the following model (summarized in Fig. 18). Neural signals originating in a bilaterally connected structure in the Uva pathway provide timing signals to HVC in both hemispheres that specify both the onset of syllables and notes as well as features of the intersyllable silent interval. Because of the absence of interhemispheric connections between song control nuclei and the primarily ipsilateral nature of the vocal motor pathway in each hemisphere (Wild et al. 2000), the synchronized timing signals reaching HVC in both hemispheres would act to establish the overall song temporal pattern as well as ensure the precise coupling and coordination between respiration and motor commands in the left and right syrinx.

Although synchronization between hemispheres is most likely caused by common inputs into both left and right HVC, some of the observed patterns of synchronous activity could certainly also be caused by common intrinsic firing patterns in each hemisphere. Rapid succession of synchronized bursts in each hemisphere, for example, may be caused by intrinsic network oscillatory patterns in HVC (Solis and Perkel 2002) initiated by a common input rather than by a rapid succession of extrinsic timing inputs to both hemispheres. Similarly, short periods of suppressed activity that occur simultaneously in each hemisphere, as shown in Fig. 14, might be caused by intrinsic inhibitory mechanisms known to exist in HVC (Dutar et al. 2000; Schmidt and Perkel 1998) rather than by extrinsic inputs that cause a suppression of HVC activity. Although these intrinsic properties are likely to contribute to the observed similarity in neural patterns in both hemispheres, it seems likely that the precise temporal alignment of neural events in both hemispheres is achieved by extrinsic inputs common to each hemisphere. A mechanism based largely on intrinsic mechanisms would likely cause the observed synchronization between hemispheres to drift with time. If each syllable is synchronized at its onset, such drift may possibly explain the lack of clearly correlated activity associated with syllable offset.

Taken together, the present observations suggest that thalamic and/or midbrain vocal-control nuclei play an important role in ensuring a coordinated vocal output by synchronizing HVC activity in both hemispheres. In addition, it also provides support for the idea that higher-order features of birdsong may be encoded by structures that lie afferent to HVC. Finally, it should be emphasized that the present findings might also have important implications for song learning. It is known that syllable sequencing and timing are rapidly learned after presentation of the tutor song (Tchernichovski et al. 2001). Because of their proposed role in determining song timing, nuclei in midbrain or thalamus may therefore also play an important role in learning and storing certain higher-order song features.

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FIG. 18. Schematic description of current model supported by this study. Common inputs into left and right HVC cause activity in both hemispheres to be synchronized at onset of each song syllable. These inputs also synchronize onset of notes that make up complex syllables (large black arrows). In many cases synchronized bursts of activity (small dotted arrows) are observed during the intersyllable silent interval. These results suggest that synchronizing inputs that specify syllable and note onset may also specify characteristics of the silent interval. Because of lack of connectivity between song control nuclei across hemispheres, the source of these synchronizing timing signals is likely to reside in bilaterally connected structures of thalamus or midbrain.
REFERENCES


