

# Interaction between auditory and motor activities in an avian song control nucleus

(motor system/sensorimotor integration/avian vocalizations/chronic recording/learning and memory)

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**ABSTRACT** Discrete telencephalic nuclei HVC (hyperstriatum ventrale, pars caudale) and RA (nucleus robustus archistriatalis) have been implicated by lesion studies in the control of vocalization in songbirds. We demonstrate directly the role of HVC in vocalization by presenting neuronal recordings taken from HVC of singing birds. Intracellular recordings from anesthetized birds have shown that many neurons in HVC respond to auditory stimuli. We confirm this result in the extracellular recordings from awake-behaving birds and further demonstrate responses of HVC neurons to playback of the bird's own song. The functional significance of these responses is not yet clear, but behavioral studies show that auditory feedback plays a crucial role in the development of normal song. We show that the song-correlated temporal pattern of neural activity persists even in the deaf bird. Furthermore, we show that in the normal bird, the activity pattern correlated with production of certain song elements can be clearly distinguished from the pattern of auditory responses to the same song elements. This result implies that an interaction occurs in HVC of the singing bird between motor and auditory activity. Through experiments involving playback of sound while the bird is singing, we show that the interaction consists of motor inhibition of auditory activity in HVC and that this inhibition decays slowly over a period of seconds after the song terminates.

The discovery of a set of discrete brain nuclei implicated in the control of vocalization in songbirds (1) was an important step toward understanding the neural basis for this complex behavior. A series of lesion studies by Nottebohm and colleagues demonstrated severe song deficits after damage to either of the forebrain nuclei HVC (hyperstriatum ventrale, pars caudale) or RA (nucleus robustus archistriatalis). Behavioral studies (2–4) have shown that the timing and spectral characteristics of song elements are learned by males from adult birds and that this learning process occurs in two phases that can be temporally distinct: an auditory phase in which a model of the tutor song is stored in the brain and a motor phase in which the bird's own vocalizations are progressively matched to the song model. A bird deafened before the onset of singing cannot vocally reproduce the stored song model (5), suggesting that there should be a regulatory connection between the auditory and the vocal motor control systems.

Katz and Gurney (6) have recently demonstrated, using an intracellular recording technique, that many neurons in HVC of the zebra finch (*Poephila guttata*) respond to auditory stimuli. They also observed auditory responses in cells of the neostriatal shelf area underlying HVC, which receives afferents from the avian forebrain auditory area known as field L (7). Although the significance and specificity of auditory responses in HVC are not yet clear, the fact that auditory information is available to the

motor system controlling song production carries an obvious suggestion of involvement in song learning. We report the development of a technique for recording neuronal activity in freely moving vocalizing birds. Using this technique, we directly examined the roles of auditory and motor activity in song control nuclei.

## MATERIALS AND METHODS

A total of 19 male birds of three species—14 canaries (*Serinus canarius*, of the Belgian Wasserschläger and Gloucester varieties), 3 white-crowned sparrows (*Zonotrichia leucophrys*), and 2 zebra finches (*Poephila guttata*) were used. The birds were housed in sound attenuation chambers (Industrial Acoustics, Bronx, NY). We found that neural recordings of multiple-unit activity were well suited to the purposes of this study, because they reliably show the activity of many neurons and the temporal pattern of their discharge. Single-unit recordings, which would allow statistical sampling of active neurons, are impractical at this time in freely moving birds, and evoked potentials tend to be susceptible to head movements, which inevitably accompany singing. The best multiple-unit recordings were obtained with a coaxial electrode developed in this laboratory and constructed as follows. A length of 33 gauge stainless steel tubing was electrically etched to a tapered tip in 50% sulfuric acid. An insulated NiCr wire (62  $\mu\text{m}$  in diameter) was then inserted in the tubing, and this assembly was coated with Stoner Mudge lacquer (Mobil Oil). The wire was cut flush with the tapered end to the tube and beveled to a rounded tip. The wire and tube were connected to separate sockets of a miniature connector. The electrode assembly was then stereotactically implanted in a subject anesthetized with Equithesin. For recording sessions, flexible cables were used to connect the electrodes to a differential amplifier. Implanted subjects sang despite the restraining effects of the cables on their heads. Vocalizations and neural activity were recorded simultaneously on magnetic tape. Songs produced by implanted subjects were compared with preoperative songs to ensure that no deficits had resulted from implantation procedures. Electrolytic marking lesions in Nissl-stained sections were used to verify electrode locations.

Auditory stimulus presentations consisted of tape-recorded vocalizations played through an audio amplifier and loudspeaker. Playback sound amplitude was monitored with a VU meter and matched to the sound amplitude of the original song or varied according to need for each experiment. Sound propagation time from the speaker to the bird's head was never more than 2 msec. The small enclosed volume of the box (60  $\times$  50  $\times$  50 cm) ensured a relatively uniform sound field around the bird and minimized disparities in sound intensity at the bird's

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Abbreviations: HVC, hyperstriatum ventrale, pars caudale; RA, nucleus robustus archistriatalis.

own ear between playback stimuli and sound produced by the bird.

Histograms were prepared with the aid of a PDP11-40 computer; individual sweeps for these histograms were triggered by the output of a sound level detector.

## RESULTS

Representative song samples and correlated neuronal activity from HVC are shown in Fig. 1A. All recordings obtained from within the HVC boundary, in all three species tested, showed greatly increased neuronal activity time-locked with song elements, whereas control recordings from outside the HVC boundary showed no changes in activity time-locked with song. Song is a complex vocalization produced predominantly by territorial males. The complexity of song consists in the structure of its sound elements as well as in their sequencing and timing. Although we cannot as yet make predictions about the detailed relationships between HVC activity and the characteristics of the sounds produced, we have consistently seen a clear correspondence between the pattern of multiple-unit discharge in HVC and the timing of song elements. This finding, when taken in conjunction with the Nottebohm lesion results, implies that HVC either generates or relays learned temporal cues for song.

Recordings from the same electrode locations showed activity associated with production of calls (brief sounds produced by both sexes in a variety of circumstances) (Fig. 1B). Song is learned by males and requires auditory feedback for its normal development, whereas calls in most species studied do not re-

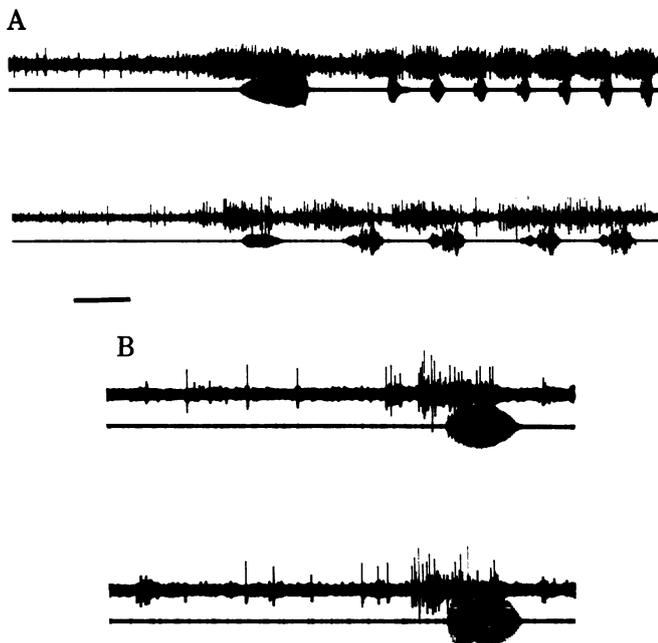


FIG. 1. Relationship between neural activity in HVC and song elements produced by the bird. In each record, the upper trace represents multiple-unit activity recorded from left HVC, and the lower trace is the amplitude envelope of the sound produced by the bird. (A *Upper*) Initial portion of a song produced by a Waterschläger canary, showing the time-locked correspondence of HVC activity and song elements; (*Lower*) portion of a zebra finch song, also showing a clear correlation of neural activity and song elements. (B) Two examples of male zebra finch calls, together with associated left HVC activity. Neurons recorded at the same site also showed prominent song-related activity. There was a relatively constant latency between the onset of the neural burst and the beginning of sound; this constancy characterized all HVC activity for both song and calls, in all species tested. Bars = 100 msec.

quire learning from other birds (8). Thus, cells recorded from the same site were involved in production of both the learned patterns of song and the unlearned patterns of calls.

To show that the activity patterns observed during singing were not simply due to auditory responses to song elements, we studied neuronal activity in HVC of two zebra finches before and after deafening by bilateral removal of the cochlea (9) (Fig. 2). We found that deafening in the adult zebra finch did not audibly affect song or the pattern of neuronal activity in HVC during song production. Although the possibility of proprioceptive input to HVC has not been ruled out, our data clearly showed time-locked neural activity leading sound production in the deafened bird. If we assume that proprioception does not contribute to these timing cues, then we may infer that a learned central motor program for song production is manifested in HVC.

To assess the functional significance of auditory inputs to HVC, we conducted preliminary studies of neuronal responses to tape playback of the subject's own song. These studies were done with two canaries and one white-crowned sparrow. Although a detailed investigation of the selectivity of auditory responses in HVC is beyond the scope of this report, we were able to draw several conclusions from our data. First, there were clear responses to many elements of the bird's own song, presented in the forward direction; these responses were much stronger than those to the same song elements played in reverse (Fig. 3). This result implies that these neurons are selective for specific temporal sequences of sound in the song. Second, neurons responded more vigorously to some elements of a complex song segment than to others. Finally, in agreement with Katz and Gurney who obtained responses to noise bursts, we showed that responses were not limited to the sound patterns of the bird's own song. In the case of the Waterschläger canary, there were clear responses to some elements of the song of another, nonsibling, Waterschläger individual. These responses were much more pronounced than those to song elements of a canary of a non-Waterschläger variety or to the songs of other species. Similarly, HVC neurons in the white-crowned sparrow showed clear responses to segments of conspecific song.

However, our most significant finding concerning auditory responses in HVC comes from the relationship between motor activity, as seen in the normal singing bird, and sensory activity as represented in playback responses to the same song elements during nonsinging periods. Careful comparison of the time courses of these two patterns of activity, in both the white-crowned sparrow and the canary, showed that many playback-correlated spikes occurred in time segments during which no comparable spikes were seen in the motor records (Fig. 4A). In other words, the peaks of auditory activity and motor activity were distinct in time, as can be seen in the comparison of auditory and motor histograms (Fig. 4B). Thus, there is manifested in HVC of the singing bird an interaction between motor and



FIG. 2. Persistence of song-related discharge in HVC of the deafened bird. (*Upper*) Baseline activity in left HVC of a nonvocalizing deafened zebra finch. (*Lower*) Song produced by a deafened zebra finch, together with left HVC activity. The complex sound patterns of song in this species were reflected in the relatively complex spike discharge patterns during song. Nevertheless, our data showed patterns of activity that were clearly correlated with production of song elements. Bar = 100 msec.

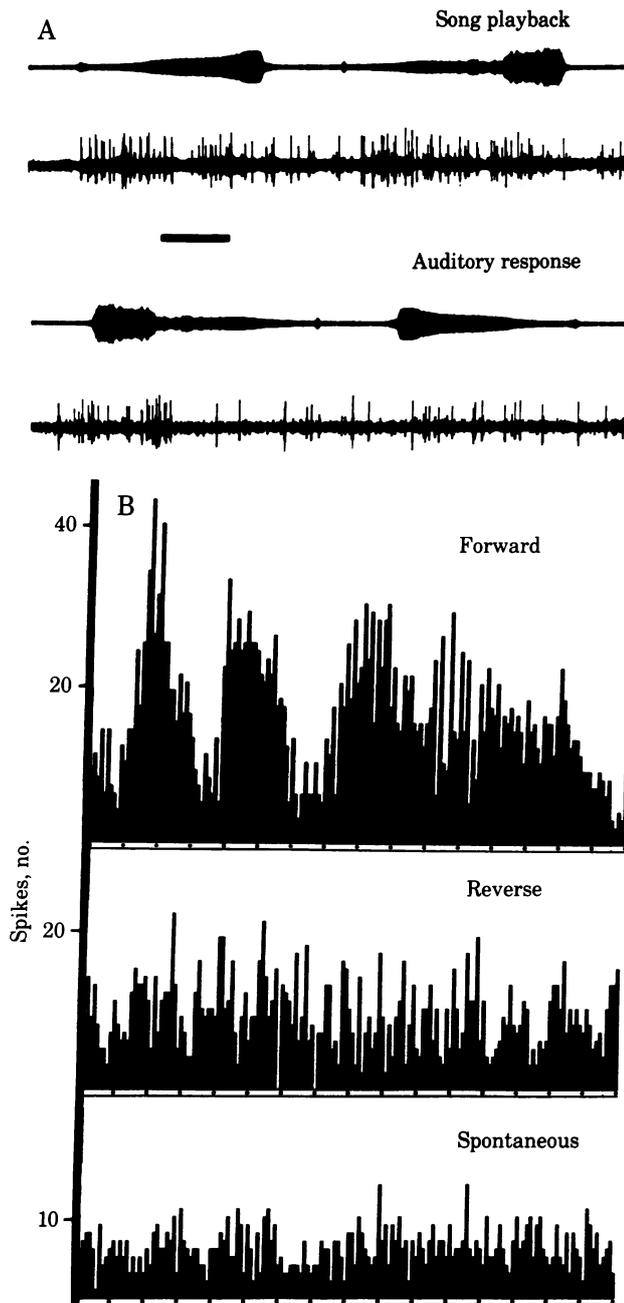


FIG. 3. (A) Auditory responses to normal and reverse playback of a bird's own song. (Upper) Portion of song played back to a quiescent nonvocalizing canary and the neural responses of left HVC neurons to these sounds; (Lower) the same song elements played in reverse, during the same recording session, and the associated response from the same electrode. The response to forward playback was stronger than that to reverse playback. The large burst of spikes in the reverse playback trace began before the sound and was probably spontaneous. Bar = 100 msec. (B) Histograms of responses to normal and reverse song playback. (Upper) Amplitude-window discriminator output representing summed responses to 20 presentations of a canary's own song elements. The two prominent peaks at left represent the two elements shown in A. (Middle) Summed responses to 20 repetitions of the same elements played in reverse. The histogram is reversed to allow direct comparison with peaks seen in the normal case. (Lower) Summed activity from 20 sweeps of baseline activity from the same session, showing fewer spikes overall than in either of the two auditory response cases. Duration of individual histogram sweeps was 2.4 sec.

auditory activity, in that the expected auditory response is absent when the bird sings. To investigate the nature of this mo-

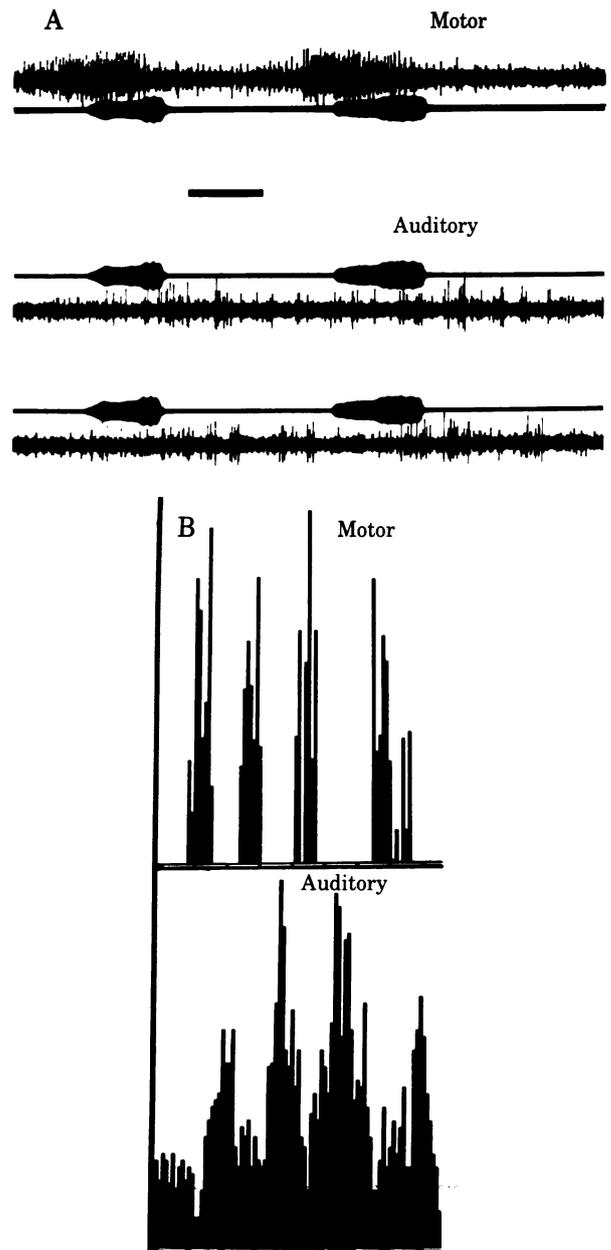


FIG. 4. (A) Comparison of motor activity and auditory responses to the same sound elements. (Upper) Two song elements produced by a male canary and the corresponding motor activity in left HVC; (Middle and Lower) representative examples of same-day responses to these song elements, recorded from the same electrode. In the vocalizing bird, a prominent motor burst led the sound elements and terminated before the end of sound. By contrast, in the lower records, the auditory response-correlated spikes occurred predominantly at the end of sound and after the song element. No spikes comparable in amplitude to the auditory response spikes could be seen at the same latencies in the motor records. Bar = 100 msec. (B) Histograms of motor and auditory activity. (Upper) Amplitude-window discriminator output from HVC neuronal activity correlated with production of four canary song elements, including the two shown in A (second and third peaks in the histogram); (Lower) summed responses to 30 repetitions of the four elements used as auditory stimuli. The two histograms were scaled to equalize the highest peaks. There was a clear distinction in time between motor and auditory peaks. Duration of individual histogram sweeps was  $\approx 1.7$  sec.

tor-auditory interaction more fully, we carried out an interference experiment consisting of presentation of various sound stimuli while the bird is singing and during the period after song

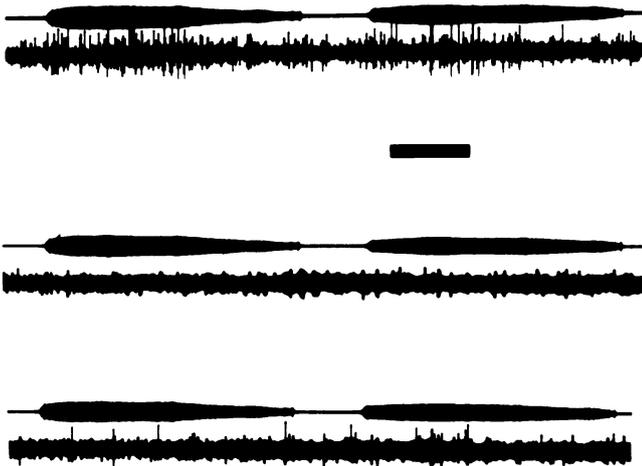


FIG. 5. Inhibition of auditory responses after song production. (Upper) Responses in left HVC of a canary to two conspecific song elements played back in a nonsinging period. (Middle) Responses to the same elements presented 1 sec after termination of a song of normal length. (Lower) Responses 14 sec after the same song, showing partial release from inhibition. Bar = 100 msec.

termination. Interference may be defined as a change in song-correlated activity that is attributable to the auditory stimulus. We reasoned that if the motor-auditory interaction is due to inhibition of all auditory inputs by motor activity, then no interference effects should be seen from varying the time of playback presentation; i.e., the pattern of activity should be the same as that seen in the normal case, without playback.

The result of this experiment, performed in a canary, revealed an interesting phenomenon. So far as we could discern, the normal pattern of motor activity in the singing bird was completely unaffected by playback sounds; i.e., there was no interference from auditory input. Furthermore, after a normal song of 10–20 sec, no recognizable auditory responses could be elicited to any sound tested, even to song elements that produced very strong responses at other times. This general inhibition of responses decayed slowly, over a period of seconds (Fig. 5). The same inhibition was not seen during prolonged presentation of loud auditory stimuli such as the bird's own song nor was it seen immediately after calls produced just before song. An intermediate effect was seen in several cases of playback after short songs of 2–6 sec.

We also observed a tendency, on many occasions, for the canary to terminate his song abruptly as the playback trial was initiated. In 20 consecutive renditions, the song ended an average of 1.7 sec after the beginning of playback sound; the mean duration of these songs (12.2 sec) was significantly shorter (two-tailed  $t$  test;  $df = 38$ ,  $t = 2.80$ ,  $P < 0.01$ ) than the mean duration of 20 normal songs (17.0 sec) uninterrupted by playback. This observation suggests that a singing bird is able to respond behaviorally to sounds, even though auditory activity in HVC is inhibited.

## DISCUSSION

In this report, we demonstrate that HVC in the singing bird produces activity time-locked with the learned timing of song elements. By combining this finding with lesion results showing that HVC is necessary for normal song production, we conclude that HVC generates or relays learned cues for song. We show that this time-locking of activity survives the loss of auditory feedback, even though auditory inputs to HVC produce clear responses to sounds heard by the quiescent bird. The interfer-

ence experiment demonstrates that these responses are inhibited while the bird is singing and for a period of seconds after song termination. These findings suggest the possibility of a learned central motor program for song, functioning independently of sensory input in the adult. Because the bird must make use of auditory feedback to develop normal song, the autonomy of the motor program would have to be acquired during ontogeny. Furthermore, the motor inhibition of auditory inputs to HVC, if present throughout development of song, would appear to serve the paradoxical function of rendering these inputs inaccessible for guidance of motor learning, unless such guidance does not occur in HVC or involves nonspiking interaction between cells. Thus, it is tempting to speculate that the motor-auditory interaction we describe will show developmental plasticity and be involved in some way in the motor phase of song learning.

The existence of long-lasting inhibition of auditory input after song termination suggests that the function of the motor-auditory interaction is not simply to protect the motor program from the consequences of auditory feedback, because there should be no need for such protection after the termination of motor activity in HVC. In this respect, the interaction described here differs significantly from other well-known cases of motor-sensory interaction, such as saccade-related inhibition of visual inputs in the cortex (10) and vocalization-related suppression of auditory inputs in the bat (11). In visual cortex, saccade-related visual inhibition is an adaptive mechanism for avoiding incoherent visual input expected from a shift in eye position; this inhibition is restricted to the duration of the eye movement. In the bat, the central and peripheral attenuation of auditory responses to outgoing echolocation pulses also serves to protect the sensory system from overloading with intense stimulation; this attenuation presumably terminates before arrival of the echo a few milliseconds later. Inhibition of auditory cortical neurons during phonation in the squirrel monkey, as described in a recent report (12), is also confined to roughly the period of stimulated vocalization. We know of no previously documented case in which prolonged inhibition of central sensory activity can be attributed to normal performance of a motor task.

Canary males tend to sing during intervals when other males are silent. This tendency probably accounts for our observation in the canary of premature termination of song when playback stimuli are presented. The ability of the canary to respond to sounds while he is singing implies that some central auditory neurons must be functioning normally during vocalization. According to Katz and Gurney (6), HVC neurons that respond to auditory stimuli project to a nucleus (area X) of unknown function whereas those that do not respond project to RA, the next nucleus in the descending motor pathway. The coexistence of auditory and motor neurons within HVC suggests that the inhibitory interaction may take place within this nucleus. It will therefore be of interest to record activity patterns during song in auditory nuclei such as field L, in which neural responses to species-specific vocalizations have been characterized (13, 14); this approach should enable us to localize the source of inhibition.

Auditory responses in HVC very probably play a role in the development or maintenance (or both) of the crystallized song pattern. Nothing is known as yet about the nature of these responses in a young bird that has not developed an auditory template or has not crystallized the motor program for song. A comparison of auditory response properties and motor-auditory interactions in juvenile songbirds with those seen in the adult should indicate whether some aspect of the inhibition of auditory inputs is developmentally labile; any difference between

juvenile and adult cases would implicate this interaction in the processes of song development.

We believe that this preparation offers a unique opportunity to examine several important issues implicated in birdsong. Included among these issues are the neuronal correlates of hemispheric dominance in song control, the topographic organization of song control nuclei, the origin and distribution of timing cues for song, and the neuronal correlates of song learning.

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