NEURONAL CONTROL OF BIRD SONG PRODUCTION

Thesis by

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Long Abstract

Male songbirds sing to establish species and individual identities, to maintain territories, and to stimulate reproductive behavior in conspecific females. The ability to produce a stereotyped song is therefore necessary for reproduction. In many species the patterns of song are learned by young birds from adults in a process involving two stages—an auditory phase involving storage of a song model, and a sensorimotor phase in which the bird learns to reproduce the model by using auditory feedback. Lesion results (Nottebohm et al., 1976) demonstrated that at least three discrete nuclei—HVc (Hyperstriatum ventrale, pars caudale), RA (n. Robustus Archistriatalis), and nXIIts (n. Hypoglossus, pars tracheosyringealis)—are indispensable to normal song production. These findings opened the way to study of a discrete vertebrate neural system which, uniquely, mediates production of an acquired yet stereotyped behavior.

However, the functional specializations of these nuclei cannot be discerned through lesion studies. For this reason I developed techniques for examining directly the neural correlates of song production—by making neuronal recordings from the freely-behaving, singing bird. From my studies I can draw the following generalizations about the relative roles of vocal control nuclei: (1) the telencephalic nucleus NIf (Nucleus Interfacialis, of Nottebohm, 1980), which provides an input to HVc and is anatomically the "highest" nucleus in the descending motor pathway, is uniquely placed among vocal control nuclei to be a generator of timing cues for song; (2) consistent with the unidirectional and serial connections between nuclei of the descending tract, NIf, HVc, RA, and nXIIts are activated sequentially prior to sound onset; (3) single-unit recordings demonstrate that there are neurons specialized for production of specific song elements; (4) a re-examination of hemispheric dominance in song control shows that both hemispheres normally make similar contributions to

all song elements; and (5) at least two types of inhibitory interactions between auditory and motor activities can be observed in the behaving bird.

The source of timing cues for song. Previous lesion and neuroanatomical studies have implicated eight brain nuclei in the control of song. I recorded neural activity from seven of the eight nuclei, in order to assess the presence and patterning of song-related activity, and to localize the site or sites where timing cues for song are generated. I confirmed the suggestion from lesion studies that the serially connected nuclei HVc, RA, and nXIIts each produce song-correlated neural activity and showed quantitatively that these nuclei are activated sequentially when the bird vocalizes. I further demonstrated that many multiple-unit recordings from these areas show a clear modulation of activity pattern which corresponds to the temporal pattern of song. This finding implicates these nuclei in the genesis or transmission of timing cues for song. I then made recordings from Nucleus Interfacialis (NIf), which provides an input to HVc, and found a similar song-related pattern of activity. The pre-sound latency of recordings from NIf was longer than that of comparable recordings from HVc, suggesting that NIf provides timing information to HVc. To test this interpretation, I sectioned the pathway from NIf to HVc; postoperatively, these subjects were unable to produce stereotyped song. A sham operation involving the same amount of tissue damage but sparing NIf had no effect on song. HRP studies show only one input to NIf (with the possible exception of the auditory nucleus Field L), from nucleus Uva of the thalamus. Recordings from this nucleus showed no changes in activity during vocalization in the adult, and bilateral lesions of this nucleus had no effect on song. To test for other inputs to NIf which might not transport HRP, I made a series of recordings from eight sites in the vicinity of NIf. None of these recordings showed song-related activity. The combination of three findings—song-related patterning of activity in NIf, the necessity of NIf for normal song patterning, and the absence of song-related activity in inputs to NIf—imply that

NIf is a source of timing cues for song, and is the only song control nucleus to which this statement can be unequivocally applied. The fact that elimination of input to NIf has no effect on song suggests that NIf produces a learned central motor program for song.

Recordings made from two other putative song control nuclei, MAN (Magnocellular nucleus of the Anterior Neostriatum) and area X, showed no changes in activity during song or other vocalizations. Transection of the pathways linking these two nuclei to HVc had no effect on song. Thus my recording experiments demonstrate that lesion effects provide a better indication of the necessity of a nucleus for adult song <u>production</u> than do studies of anatomical connections. Whether Uva, MAN, or area X plays a crucial role in song <u>development</u> remains to be determined.

<u>Single units with specialized roles in song production</u>. Elucidation of the neural mechanisms of song control ultimately requires an analysis at the single-unit level: the clues to the interactions involved may be masked in multi-unit recordings. Accordingly, I have developed a new technique for recording from single neurons in song system nuclei of the freely-behaving, singing mockingbird. The method employs an X-Y microdrive which, when chronically implanted, is sufficiently stable so that units can be isolated and held for periods of up to several hours.

My recordings from mockingbird HVc revealed single units with several classes of specialized roles in song production. Many cells exhibit premotor activity for all song syllables, and do not respond to those same syllables presented as auditory stimuli. Some of these cells show long-latency (e.g., 500 msec) "anticipatory" activity at the initiation of song and between syllables of a long song bout, thus demonstrating a role for HVc beyond the purely motor aspects of sound production. A few cells show more selective premotor activity, producing highly stereotyped bursts of spikes for only a few syllables. The distinctions between sounds for which these cells do or do not show activity may be quite subtle, suggesting that HVc units may encode subtle variations in sound production. This interpretation is supported by the high degree of temporal specificity of unit firing with respect to timing of the syllable.

A re-examination of hemispheric dominance in song control. Series of studies by Nottebohm et al. demonstrated that left-side lesions in the song system of several species inflict much more severe damage to song than comparable right-side lesions. These studies led to the hypothesis that the left hemisphere plays a dominant role in song production. My neural recordings, however, led to a completely different interpretation of the lesion results. Recordings from the left and right hypoglossal nerves innervating the syrinx invariably showed very similar activity patterns for any given vocalization. These patterns consist of neural bursts and silent periods of different durations, with each song syllable represented by a unique neural homologue. Because the hypoglossal nerve represents the "final common pathway" for song control, the neural activity it transmits must convey coded commands for song production. To the extent that my recordings reveal these commands, it appears that both right and left nerves are transmitting the same messages to their respective syringeal halves. Section of right or left hypoglossal nerve in a subject from which nerve recordings were also made led to the classical behavioral deficit, including complete disappearance of certain syllables, but these behavioral deficits were uncorrelated with any consistent pattern differences in the two nerves. Recordings from right and left HVc of the same bird also showed similar activity patterns for a given song element. These results are incompatible with any all-or-nothing mechanism of lateralization in the song control system. However, they are consistent with the absence of hemispheric anatomical asymmetries in song control nuclei.

The critical feature of the qualitative dominance theory is the independent contribution of different sets of sounds by the two sound sources in the syrinx—the right and left internal tympaniform membranes. By blocking airflow through the right or left bronchus, I was able to eliminate the function of one membrane and observe the set of song syllables produced by the bird with only the other membrane. With this bronchus-plugging technique, I found that the right syringeal half alone is sufficient to produce easily recognizable counterparts to most syllables normally produced by both syringeal halves. This result contradicts the conclusion from nerve-section experiments that most sounds are contributed by the left side only, but is completely consistent with the nerve recordings which indicate bilateral participation in sound production. Taken together, these results indicate that both hemispheres and syringeal halves make similar contributions to the production of all song elements.

Interaction between auditory and motor activities in an avian song control nucleus. Intracellular recordings from anesthetized birds have shown that many neurons in HVc respond to auditory stimuli. I confirmed this result in multi-unit recordings from awake behaving birds, and further demonstrated 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. I showed that the song-correlated temporal pattern of neural activity persists even in the deaf bird. Furthermore, 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, I showed 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 time-locking of pre-motor activity in HVc to song elements survives the loss of auditory feedback by deafening even though auditory inputs to HVc produce clear responses to sounds heard by the normal quiescent bird. Normal songs are produced by deafened adults in some species. 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. Recordings from Field L neurons in the singing bird show auditory responses to song elements which persist during singing, suggesting that the sensorimotor interaction I observed occurs within HVc. If so, it is likely that the motor inhibition of auditory inputs to HVc plays a role in song development or song maintenance. If present throughout development of song, this inhibition would appear to serve the paradoxical function of rendering these inputs inaccessible for guidance of motor learning, unless such guidance does not occur within HVc or involves nonspiking interaction between or within cells. These considerations thus impose constraints on possible neural mechanisms of song control.

In single-unit recordings I have confirmed the expectation from multi-unit data that at least some auditorily responsive neurons are inhibited during singing, thus demonstrating a type of sensorimotor interaction at the single cell level. One cell in mockingbird HVc was inhibited by playback of all syllables of the bird's own song, and showed premotor activity for some syllables. Another cell which was inhibited by playback showed specific motor activity, firing for only one of two very similar song syllables. While the functional significance of these interactions is unknown, it will clearly be of great theoretical importance to know whether other types of sensorimotor interaction are exhibited by single cells in the vocal control system. Such cells would be likely candidates for specialized roles in song learning or song maintenance.

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General Introduction

For the student of behavioral development, the acquisition of song in birds represents a unique case in which an early sensory experience manifests itself as a motor pattern later in life. Birdsong exhibits some of the most interesting phenomena of behavioral development and learning such as the effects of sensory exposure, deprivation, and isolation, inborn perceptual preference, and critical impressionable period. A complex pattern of neuromuscular coordination underlies birdsong; this pattern is learned in many species. Thus, song offers a unique opportunity to study how complex motor programs are stored and executed by the brain.

The neural system for song control, a discrete chain of nuclei and fiber tracts, was discovered (Nottebohm, Stokes and Leonard, 1976) by means of a series of lesion experiments which demonstrated their necessity for normal song. This discovery opened the way to study of the acquisition and maintenance of a complex motor program by a neural system specifically devoted to these tasks. Until recently, the lesion experiments provided the only means of assessing the relative roles of various brain areas in song. Among the outstanding issues generated by these experiments are the theories concerning hemispheric dominance, the mechanisms of song production, and song learning. The studies described here were designed to address these issues directly, with neurophysiological methods, by making neuronal recordings from freely-behaving, singing birds.

In most species, song is the most complex of vocalizations. The component sounds of the song are called syllables, each of which consists of one or more notes or elements grouped together in time. The notes or elements are visualized as continuous markings on the time-frequency sound spectrogram. The sequence and timing in which different syllables occur and alternate with silent intervals constitutes the temporal pattern of song. Many birds use fixed temporal patterns in their songs, while others use variable patterns.

Adult song syllables are rendered very stereotypically in both spectral and temporal domains. However, behavioral studies (Thorpe, 1958; Marler and Tamura, 1964; Marler, 1970) have shown that in many species the timing and spectral characteristics of song elements are learned by males from adult birds, and that this learning process occurs in two phases which 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 (Konishi, 1965), suggesting that there should be a regulatory connection between the auditory and the vocal motor control systems. By contrast, if the deafening operation is performed after the song has been "crystallized" by the adult, the subject is able to maintain stereotyped song performance, and to redevelop song normally in succeeding years. The maintenance of song after deafening in the adult suggests that the song pattern is in fact acquired by the central nervous system during development, possibly by feedback-mediated entrainment of pattern generators in the vocal control system.

I have sought to characterize the roles of various stations in the vocal control pathway by recording the neural activity patterns produced during song. The logical first step in this study was to establish which station in the descending pathway generates the earliest activity time-locked to song elements, for it is here that some of the events of song learning must take place. But before the developmental events underlying song can be physiologically assessed, it is necessary to characterize the patterns of neural activity used by the adult to generate crystallized song. These considerations were the basis for the studies presented in Chapter 1. By assessing the presence and patterning of song-correlated activity in a given nucleus, and by performing lesion experiments designed to test predictions derived from neural recordings, I sought to make fundamental assertions about the origin and transmission of timing cues for song.

For the studies presented in Chapter 2, I made a series of single-unit and multi-unit recordings from various stations of the vocal control system. My primary goal was to find the site or sites in the system where neural cues for song are generated. Nottebohm et al. (1976) concluded from their lesion studies that HVc (Hyperstriatum ventrale, pars caudale) was the highest center for song control, and inferred from other evidence that song learning processes take place in this nucleus. I show that NIf (Nucleus Interfacialis), which provides an input tract to HVc and is thus "higher" in the descending motor tract, is uniquely placed among known song control nuclei to generate timing cues for song.

Birdsong and human speech are thought to possess a number of similar attributes, among which lateralization of song control has drawn much attention as the first example of a consistent right-left neural asymmetry in a vertebrate other than man (Nottebohm, 1980). The demonstration of a discrete neuronal system for song control has opened a new avenue for investigation of the mechanisms of lateralization. However, the hemispheric dominance theory is based on results of central and peripheral lesion experiments from which inferences were drawn concerning the normal function of song control nuclei.

Many species of songbirds can produce complex sounds containing overlapping, non-harmonically related frequencies. By analyzing many examples of this phenomenon, and by drawing inferences from comparative anatomical studies of the avian vocal organ, the syrinx, Greenewalt (1968) developed the two-voice theory of song production, which predicts that each half of the syrinx functions as a separate sound source. This independence of function was theoretically possible because of the bilateral symmetry of the syrinx which is reflected in paired muscles, nerve innervation, air supplies, and the left and right internal tympaniform membranes

(ITM) whose oscillations are thought to produce the sound components of song. Behavioral and neuroanatomical evidence for the idea of two sound sources in song production was provided by a series of studies performed by Nottebohm and colleagues (reviewed in Nottebohm, 1980). The first of these studies demonstrated that section of the left hypoglossal nerve branch innervating the syrinx caused much more severe deficits to song, in several species of songbird, than right nerve section. Subsequently, Nottebohm, Stokes, and Leonard (1976) demonstrated that left-side lesion of either of the telencephalic song-control nuclei (Fig. 1), HVc (Hyperstriatum ventrale, pars caudale) or RA (Nucleus Robustus archistriatalis) produced greater song deficits than corresponding right-side lesions.

In the interpretation of the hypoglossal transection studies, it has been assumed (Nottebohm, 1980, pp. 91-92) that affected portions of songs were controlled by the cut hypoglossus and unchanged portions by the intact hypoglossus. Occasional evidence for song elements controlled by the interaction of both hypoglossus nerves has been encountered (1980, p. 92). Nevertheless, the logical alternatives for lateralized sound production in the syrinx seem clear from anatomical considerations. Production of a typical one-voice element of song (a single fundamental and its harmonics) requires oscillation at those frequencies by one or both ITM's in the syrinx. If an element is "contributed" (strictly speaking) by the left side, the left membrane must oscillate and the right must be silent. This "all-or-nothing" model is therefore the most parsimonious explanation of dominance in song control.

The lesion experiments demonstrate that a functional asymmetry exists at some level in the vocal control system. However, they do not imply that the highest levels of the system are sites of dominance. If the site of functional dominance is below HVc, then lesion of HVc, because this nucleus controls or provides signals to lower stations in the unilateral descending pathway, will inevitably mimic lesion of a lower level. Thus the removal of a central component cannot be used to determine whether the site of functional dominance is central or peripheral.

As discussed in Chapter 3, I sought to examine the bilateral control of song production directly by recording neuronal activity at both central and peripheral levels of the vocal control system. My recordings showed very similar activity patterns in right and left hemispheres, at both central and peripheral levels, for any given vocalization. I then investigated directly the sound production roles of right and left syringeal halves by plugging the left or right bronchus of several birds so as to eliminate bilateral vocal contributions. I found that birds singing with only the "subordinate" right side were able to produce the vast majority of song syllables. My results indicate that both left and right hemispheres and syringeal halves make similar contributions to all song elements.

Katz and Gurney (1981) 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 (Kelley and Nottebohm, 1979). While the significance of auditory responses in HVc is 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. Having developed techniques for recording neuronal activity in singing birds, I was able to examine the roles of auditory and motor activity during song in vocal control nuclei; the results are reported in Chapter 4. I showed that the song-correlated temporal pattern of neural activity persists even in the deaf bird. Furthermore, I demonstrated that in the normal bird at least two types of auditory-motor interactions occur: motor inhibition of excitatory auditory responses, and auditory inhibition of spontaneous activity in some cells which show specific premotor or motor activity.

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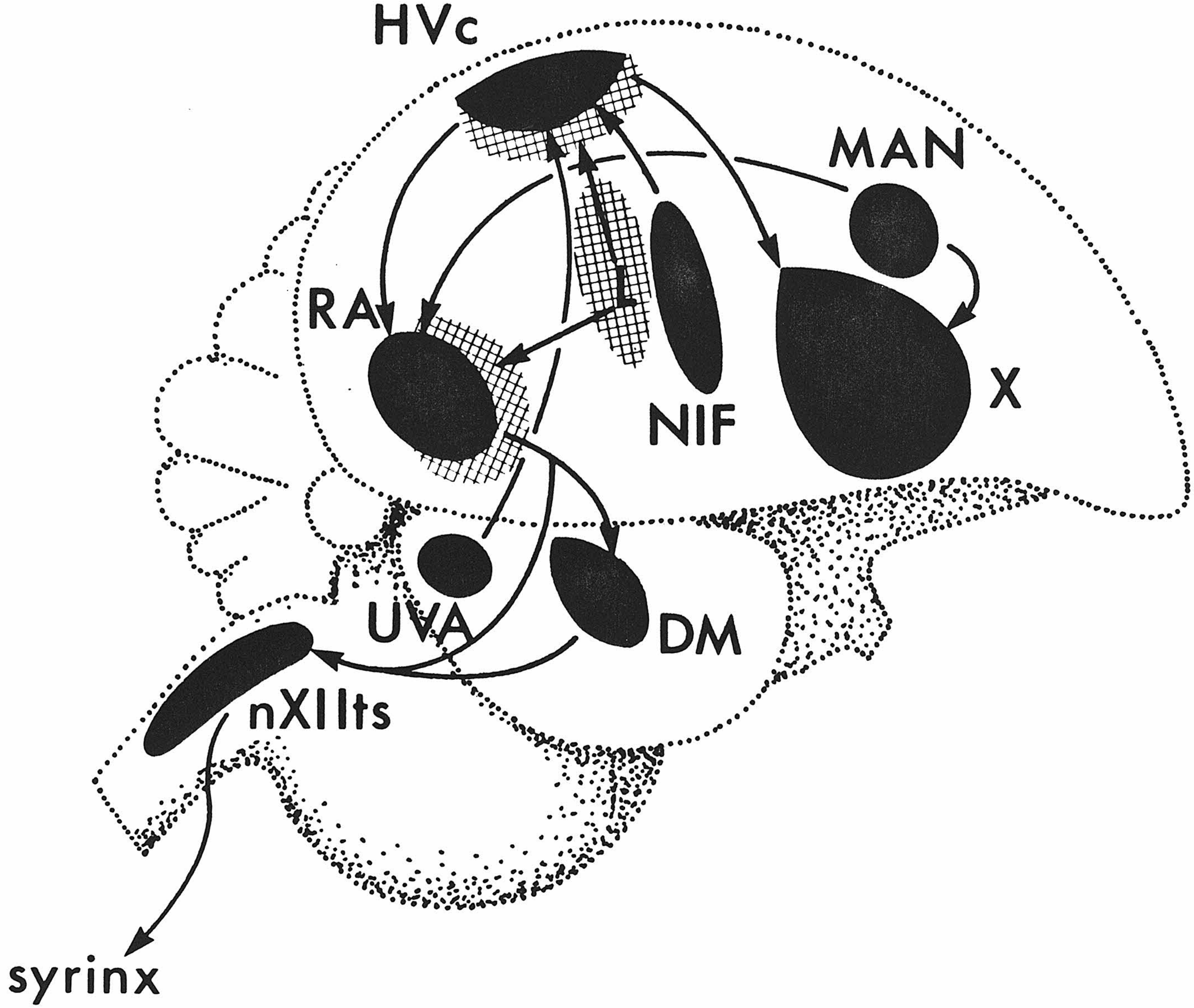
Figure 1. Schematic diagram of the vocal control system in songbirds. Arrows indicate anterograde connections between nuclei. Lesion studies have shown HVc (Hyperstriatum ventrale, pars caudale) and RA (n. Robustus Archistriatalis) are necessary for normal song production. Hatched areas represent auditory pathways. Other abbreviations: NIf (Nucleus Interfacialis); nXIIts (n. Hypoglossus, pars tracheosyringealis); MAN (Magnocellular nucleus of the anterior neostriatum); X (area X). (original by M. Gurney, slightly modified.)


HVc
MAN
RA
X
NIF
UVA
DM
nXIIts
syrinx

Chapter 1

MATERIALS AND METHODS

A total of 140 birds of four species (zebra finches, Poephila guttata; mockingbirds, Mimus polyglottos; white-crowned sparrows, Zonotrichia leucophrys; and canaries, Serinus canarius) were used for these experiments. I used Wasserschläger canaries bred in this laboratory from a stock donated by Dr. Peter Marler of the Rockefeller University. Other breeds of canaries and zebra finches which were used in some experiments were purchased from local breeders. Mockingbirds and white-crowned sparrows were raised by hand from the nestling stage. Some of these animals were used in the development of techniques; the statements made in this report are based on data collected from 65 individuals. The birds were individually housed in sound attentuation chambers (Industrial Acoustics, Bronx, NY).

Multiple-unit neural recordings. I found that neural recordings of multiple-unit activity were well suited to many of the purposes of this study, because they reliably show the activity of many neurons and the temporal pattern of their discharge. Though I have also developed a single-unit technique for use with the singing mockingbird (see below), the method is impractical at this time for smaller species of songbirds. Measurements of evoked potentials were also made, but these tend to be susceptible to head movements, which inevitably accompany singing.

In order to make neuronal recordings from the brains of singing birds, I had to overcome two major technical obstacles: 1) because birdsong plays a territorial and reproductive role in the natural behavior of the male songbird and is largely refractory to behavioral conditioning, I had to create conditions under which birds would sing while restrained by a recording cable or other devices; and 2) because song production entails massive involvement of musculature, I found that the movement artifacts associated with singing often plagued recordings obtained during song. The latter problem was particularly acute among loud singers such as the mockingbird and canary, but was virtually eliminated by employing a coaxial electrode of a design developed in this laboratory, and constructed as follows. A length of 33 or 34 gauge stainless steel tubing was electrically etched to a tapered tip in 50% sulfuric acid. An insulated NiCr wire, 62 μm 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 of 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 stereotaxically 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. Singing was induced in all experimental birds by subcutaneous implantation of testosterone proprionate (Schering Co.) in the amount of approximately 5 mg per bird. When necessary, birds were induced to sing by removal of their mates, or by placing another male nearby. Vocalizations and neural activity were recorded simultaneously on magnetic tape (Teac 334OS, 19 cm/sec). Songs produced by implanted subjects were compared with preoperative songs to ensure that no deficits had resulted from implantation procedures. Sound recordings were analyzed with a Kay Electric Co. Model 7029A Sound Spectrograph. Correlations between vocal and neural activities were examined by displaying them simultaneously on either a storage oscilloscope or on paper film made with a Grass Instrument Model C4L Kymograph camera. Electrolytic marking lesions in Nissl-stained sections were used to verify electrode locations.

Hypoglossal nerve recordings. Because the trachea to which the hypoglossal nerves are attached moves during vocalization, recording from that portion of the nerve proved impractical. However, a careful examination of the route through which the nerve entered the skull revealed that it coursed within a bony tube across a part of the middle ear cavity, which is much enlarged in birds. Subjects were anesthetized and held in a special head-holder with ear bars and a beak clamp. The middle ear cavity was accessed through a hole made in the skull area directly caudal to the crossing of the posterior and external semicircular canals. A sharpened metal tip of the type used for microelectrodes was used to make a small (ca. 100 μ) hole in the bony tube, and a 25 μ enamel-coated nichrome wire, whose cut tip would serve as electrode, was inserted through the hole into the nerve. For differential recording two electrodes were implanted side by side or a few hundred micrometers apart whenever space permitted. The electrodes were fixed in place with dental cement (Grip cement, L. D. Caulk Co.), which filled a portion of the cavity between the skull and the bony structure housing the vestibular system. This procedure ensured very stable multi-unit recordings free of movement artifacts. In selected cases the placement of electrodes was confirmed by postmortem dissection and inspection.

Single-unit recordings. The multi-unit recordings obtained with the above techniques often showed spikes of very good (> 5:1) signal-to-noise ratios, giving rise to the hope that the activities of single neurons could be discriminated and analyzed. However, in practice such discrimination was very difficult and unreliable, even with a voltage-and-time-window discriminator. Accordingly, I enlisted the aid of our expert machinist Herb Adams in the design and construction of a new microdrive (Fig. 1), suitable for chronic mounting on the heads of large songbirds such as the mockingbird. This microdrive consisted of two stages: a tower of specified height which advanced the electrode, and an X-Y base which positioned the electrode for each new track. The tower stage consisted of a removable cap for changing electrodes, a top-mounted dial for advancing the electrode in controlled steps, and a core in which threads on the inner surface of an outer screw, which was rotated by the top dial, turned against the threads of an inner screw. As a result the inner screw could be advanced or retracted without itself rotating. The electrode shaft was housed within this inner screw, and rigidly fixed to it by a set screw which served as contact for recording purposes. A short, flexible lead wire, cemented to the set screw with silver epoxy (Amicon, Lexington, MA), connected the electrode to a contact assembly (Microtech, Boothwyn, PA). A gold pin served as a differential electrode for recording purposes. The X-Y base of the microdrive consisted of two sliding stages with dovetails cut at right angles to each other, and set screws to lock each into position. These stages allowed movement of the tower assembly in two dimensions, so that the electrode could be positioned over any point in a 4 x 4 mm area. The electrode was constructed using a 0.005 in. Pt-Ir rod, electrically etched in cyanide or chlorine bleach solution to a blunt tip (Evarts, pers. comm.), then thinly coated with the appropriate solder glass (Corning) (Wolbarsht). A tip of approximately 6-10 μ was exposed by passing negative current through it in a saline solution; tips were microscopically inspected with the aid of a fiber-optics light source (Dyonics, Woburn, MA).

Implantation of the microdrive involved first removing the entire skull surface over the area of interest, with care taken to avoid damage to the dura. The entire microdrive assembly including electrode was then positioned over the exposed brain area and cemented into place. The contact plug, preassembled with gold pin for differential contact, was then positioned anterior to the microdrive and cemented, with the gold pin contacting the dura, at an angle which corresponded to the vertical in the freely behaving bird. For recording sessions it was necessary to employ a commutator to prevent coiling of the recording cable between the subject and the electrode amplifier. Other details of recording sessions and data analysis are as described above.

Bronchus plugging. Subjects were anesthetized and their bodies were restrained with two strips of masking tape, the ventral side up, on a small operating platform whose orientation can be adjusted. The bronchi were accessed through the interclavicular air sac which surrounds the syrinx. This air sac could be easily located by following the trachea into the thoracic cavity. As soon as the thin membrane of the air sac was cut the syrinx became visible with appropriate illumination from a Zeiss operating microscope. A blunt glass probe was used to deflect the syrinx to one side so that the bronchus of the other side became visible under the syringeal musculature. One side of a sharpened forceps was used to make an incision between bronchial rings while the syrinx was being deflected. A level well below (0.5-1 mm) the lower edge of the internal tympaniform membrane was chosen as the site of the incision. The plug consisted of a 1 mm length of small-gauge (0.25 mm O. D.) polyethylene tubing packed tightly with cotton wool. One end of the plug was beveled so as to allow an easy entry through the bronchial incision. After the plug was positioned in the bronchus, tissue adhesive (Histoacryl, Braun Melsungen) was applied liberally around it, a procedure which not only held the plug firmly in place, but also rendered the cotton packing completely impenetrable to air. It was possible to visualize a meniscus of tissue adhesive above the proximal end of the plug, thus ensuring that the bronchus was completely sealed.

The same procedures of dissection were used to examine the state of the plug and the bronchial incision at the completion of each experiment. In all cases the plug remained at the site or original placement. In most instances a considerable amount of tissue growth occurred above and below the plug. The bronchial incision remained open in one case and closed in all other cases. Whenever birds did not have to be sacrificed, they were returned to the breeding stock after removal of the plugs.

Playback experiments. Auditory stimulus presentations for motor-auditory interaction studies consisted of tape-recorded vocalizations, typically the bird's own song, 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 cm by 50 cm by 50 cm) ensured a relatively uniform sound field around the bird, and minimized disparities in sound intensity at the bird's own ear between playback stimuli and sounds 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.

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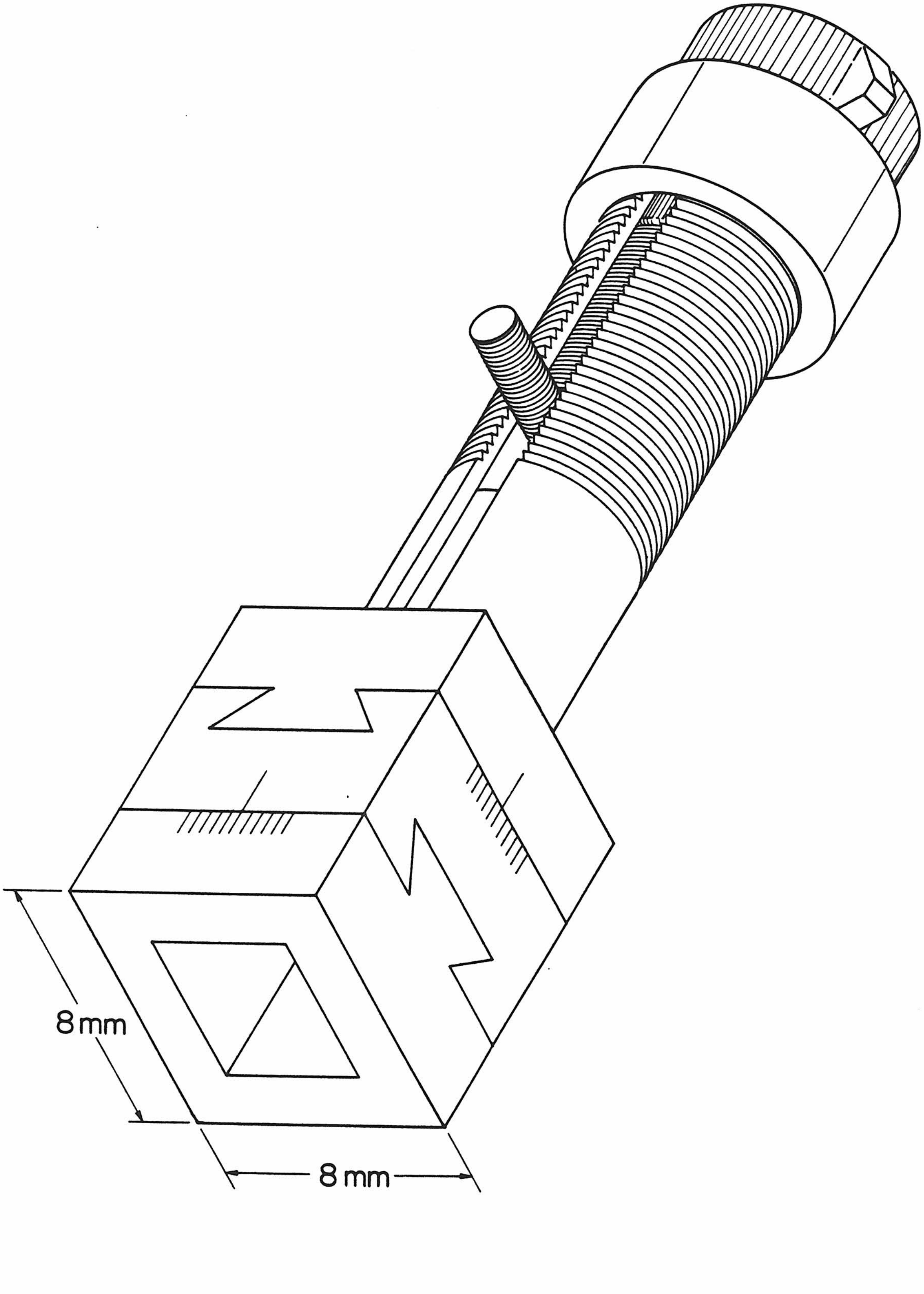
<u>Figure 1</u>. Line drawing of the X-Y microdrive developed by Mr. Herb Adams for recording single unit activity from the singing bird. The dovetail edges at bottom separate the two sliding stages, which allow positioning of the electrode anywhere within a 4 X 4 mm area. The plastic dial at top advances or retracts the electrode by means of the small threads above the setscrew (center), which serves as electrode contact for recording sessions.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Chapter 2

CEREBRAL SOURCE OF TIMING FOR BIRD SONG CONTROL

Introduction

Song acquisition in birds is a process which involves memorizing a model, then reproducing the model with the aid of auditory feedback. The necessity for auditory feedback in many species ends when the song reaches its final form, suggesting that the motor program for song has become "centralized". The uniqueness of the avian vocal control system (Nottebohm, Stokes, and Leonard, 1976) as a model for the generation of patterned motor output derives from these phenomena and their implications for the neural mechanisms of song production. No other known vertebrate neural system combines the advantages of discrete nuclei, generation of a stereotyped pattern which can be easily studied, and neural plasticity during a well-defined period in the subject's life. To best exploit these advantages, we must determine the site or sites at which acquired cues for song are generated, because these sites must carry out some of the events of song learning. The question of the origin of timing cues for song was preliminarily addressed by the lesion studies through which the vocal control system was discovered. Based on these studies, nucleus HVc (Hyperstriatum ventrale, pars caudale) has remained the highest known efferent center for song control. I undertook to study the neural mechanisms of song directly by recording neuronal activity from the freely behaving, singing bird. My studies lead to two main conclusions. First, the four nuclei of the descending motor tract are activated sequentially, in descending order, at the onset of sound production. Second, NIf (Nucleus Interfacialis), which provides an input to HVc and is thus "higher" in the descending motor pathway, is uniquely placed among song control nuclei to be a generator of timing cues for song.

Results

HVc multi-unit recordings. Representative song samples and correlated neuronal activity from HVc are shown in Fig. 1a. All neural recordings obtained from within the HVc boundary, in four species of songbirds, showed greatly increased neuronal activity temporally correlated with song elements, whereas control recordings from just outside HVc showed no such changes in activity. The song-related pattern of neural activity is the neural correlate of the temporal pattern of song: a burst of neural activity precedes each syllable and an inactive period precedes each silent interval. Just as the pattern of frequency and amplitude modulation within each syllable is unique, so is the pattern of neural burst for each syllable, so that a series of different neural bursts alternating with inactive periods of different durations constitutes the neural correlate of the temporal pattern of 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).

An examination of the role of NIf in song production. Because NIf provides an input to HVc, it is possible that HVc merely relays motor commands which are generated in NIf. Accordingly, I used the same multi-unit recording technique employed in HVc to record activity from NIf of the singing zebra finch. I found a pattern of activity similar to that seen in HVc, with time-locked neural bursts clearly leading the onset of sound (Fig. 2A). I examined the relationship between this NIf activity and HVc activity for homologous vocalizations (contact calls) from a second bird of the same species. When averaged over many such comparisons, these data show that the onset of NIf multi-unit activity in the zebra finch leads that in HVc by approximately 10 msec (Figs. 2B,C, Fig. 18). This observation coincides with the demonstration from proline autoradiography studies that no reciprocal connections exist from HVc to NIf (E. Akutagawa, personal communication), and is consistent with the concept of a hierarchical relationship between NIf and HVc. Accordingly, I set out to examine the hypothesis that NIf is a "higher" song control station than HVc.

To investigate the necessity of NIf for normal song production, I performed a pathway section experiment eliminating connections between NIf and HVc in two zebra finches. The behavioral results of this experiment were quite dramatic. Neither of the subjects ever again produced normal song. Over a period of many months each produced instead a variety of song-like vocalizations (Fig. 3), with more complexity and variety than simple call notes strung together, but without any stereotyped phrase structure from one rendition to the next. To the ear these "songs" were unlike any produced by normal zebra finches. Several features of the song were highly variable, including the form and duration of individual syllables, the inclusion and ordering of selected syllables in the song phrase, and the duration of song. Preoperatively these birds had sung quite normally in that they consistently produced a stereotyped song phrase. None of the characteristic features of these phrases was preserved following the operation.

To determine whether the behavioral effect of the NIf pathway section experiment was due to surgical effects alone, I performed a comparable sham surgery on another zebra finch. I followed the same procedure as in previous pathway section experiments, but made the incision anterior to NIf so that the descending motor tract was spared. This bird sang normally on the first day after surgery (Fig. 4). He maintained normal song until sacrificed for histology two months later. Thus my surgical procedures, though quite radical, did not in themselves cause any disruption to song. This fact is of interest when one considers that the anterior pathway section experiment also eliminated the tract from MAN to HVc, and from HVc to Area X.

Having preliminarily examined the role of NIf in song production, I sought to record the activities of nuclei supplying inputs to NIf. A recent study by Nottebohm, Kelley, and Paton (1982) demonstrated with HRP uptake that nucleus Uva of the thalamus provides the major input to NIf, as well as one of the inputs to HVc. For this reason, I made multi-unit recordings from this nucleus during both song and calls, and saw no changes in Uva activity related to vocalization, either before or after sound (Fig. 5). Nor did I observe activity variations phase-locked to the respiratory rhythm, a possibility suggested by Nottebohm et al. (1982). As an independent test of the role of Uva in song, I made bilateral lesions which eliminated approximately 95% of Uva bilaterally as well as neighboring nuclei such as nucleus spiriformis. These lesions, though rather massive, did not affect the normal delivery of song (Fig. 6) and calls, whereas comparable lesions of HVc or RA invariably have drastic effects on song (Nottebohm et al., 1976).

To assess the possibility of input fibers to NIf which do not take up HRP, I made multi-unit recordings from sites in the vicinity of NIf. Eight such locations were recorded during both song and calls in the zebra finch, and four such sites in the canary. In all cases I failed to observe any premotor activity for song, or any other vocalization-related activity. Since my multi-unit electrode must scan a large area (ca. 300 μm), and electrode placements were widely scattered around NIf, I feel justified in concluding that there are no input fibers to NIf, detectable with currently available methods, which convey timing cues for song in the adult.

Single-unit recordings from HVc. The questions of pattern generation in the song system may ultimately require an analysis at the single-unit level, since the clues to these questions may be masked in multi-unit recordings. Single-unit recordings also uniquely provide a means for assessing the mechanisms of sensorimotor interactions such as the motor inhibition of auditory inputs that I have observed in HVc multi-unit recordings (Chapter 4). Motivated by these considerations, I have developed a new technique for recording from single neurons in song system nuclei of the freely-behaving, singing mockingbird. The method employs an X-Y microdrive which allows repeated electrode penetrations over a 4 x 4 mm area, and is sufficiently stable so that units can be isolated and held for periods up to several hours.

To date my experiments with this technique have been focused on HVc, because the superficial position of this nucleus makes it a more easily accessible target for electrode penetrations than the deeper NIf cells. My data show several classes of specialized roles in song production for units in mockingbird HVc. Many cells exhibit premotor activity for all mockingbird song syllables (Fig. 7), and do not respond to those same syllables presented as auditory stimuli. Some of these cells have relatively constant pre-sound latencies for the various syllables, while others show very long-latency (ca. 500 msec) "anticipatory" activity at the initiation of song (Fig. 8). In twenty cases of song initiation following silent periods of 10 seconds or more, the mean pre-sound latencies of activity onset (375 msec) were significantly longer than comparable latencies following silent periods of 1-5 seconds (mean = 121 msec) ($\underline{t}$ test, df = 19, $\underline{t}$ = 8.56, $P<0.01$). In general, these units produced relatively sporadic activity for successive repetitions of the same syllable. However, careful examination revealed consistent pattern differences corresponding to different syllables (Fig. 7). In many cases these differentiated firing patterns were evident in premotor activity, some tens of milliseconds before sound onset.

A few cells in HVc show more selective premotor activity, producing highly stereotyped bursts of spikes for only a few syllables (Figs. 9-12). The distinctions between sounds for which these cells do or do not show activity may be quite subtle. For example, both sets of repeated syllables in Fig. 9 are noticeably similar to zebra finch song syllables to which this mockingbird was repeatedly exposed during its first year. Yet the HVc unit is active only for the first set of syllables, suggesting that HVc cells may encode subtle variations in sound production. This interpretation is supported by the high degree of temporal specificity of unit firing with respect to timing of the syllable, which can be seen most clearly in Fig. 10. This unit fired regularly for only one of two trill segments, both of which were very similar to canary trills heard often by this mockingbird. There was a constant phase relationship between bursts of unit activity and individual trill elements. Neither of these cells showed an excitatory auditory response to playback of the same syllables for which it produced motor activity when the bird sang.

The motor-specific cells described above were generally not active before the onset of sound, raising the possibility that their selective activity patterns could be due to proprioceptive or auditory feedback. However, I recorded from a class of HVc neurons for which this explanation can be ruled out (Figs. 11, 12). These units consistently fired well in advance of the earliest activity in the hypoglossal motor nerve (see discussion below) for certain syllables, and were consistently silent for other syllables. As indicated in the bottom record of Fig. 11, their activity for certain elements was not restricted to pre-sound periods. Nevertheless, the fact that they discriminated between syllables, in advance of the earliest motor neuron activity, is strong evidence for premotor specificity in these cells.

Recordings from RA. Nucleus RA (n. Robustus Archistriatalis) receives a strong descending projection from HVc, and in turn projects to the motor neurons in nXIIts. Lesions of this nucleus severely disrupt song (Nottebohm et al., 1976). Correspondingly, multi-unit recordings from RA during song show patterned activity corresponding to the song pattern. RA recordings (Fig. 13) can be distinguished from HVc recordings on two grounds. First, the rate of spontaneous activity is much higher in RA than in HVc, giving the effect of lower signal-to-noise ratios in the song related activity as compared to baseline firing. Second, at song termination there is an obvious cessation of neural activity; this repression decays gradually over a period of 2-4 seconds. Such repression may well be common in HVc (see Chapter 4), but is at least much less noticeable because of the large difference in background activity. In other respects the song-correlated patterns of multi-unit activity in HVc and RA, recorded simultaneously from the same zebra finch, are qualitatively similar.

Quantitative comparison of these data reveals that the activity onset in HVc leads that in RA by 5-10 msec (Fig. 18).

Recordings from the hypoglossal motor nerve. In Chapter 3 I present data from the canary showing activity patterns in the hypoglossal motor nerve innervating the syrinx, and show the presence of simultaneous bilateral activity in the right and left nerves for any given vocalization. Fig. 14 illustrates hypoglossal nerve activity in the singing mockingbird. The pre-sound latencies of this activity, in three species of songbirds, ranged from 20 to 30 msec for most elements, though in the mockingbird some latencies as long as 50 msec were encountered. Longer latencies were generally associated with higher amplitude, sharp-onset song syllables. Because it is almost certain that the hypoglossal nerve provides the sole innervation to the syrinx, these latencies provide an upper limit to the time at which proprioceptive feedback from the syrinx can be generated.

Multi-unit recordings from MAN and Area X. Two other telencephalic nuclei, MAN (Magnocellular nucleus of the Anterior Neostriatum) and Area X, have been included in the vocal control system by virtue of their anatomical connections to HVc and by other criteria (Konishi and Akutagawa, 1981; Ryan and Arnold, 1981). For this reason I made multi-unit recordings from these nuclei in the singing zebra finch. As expected from the magnocellular constituency of MAN, recordings from this nucleus tended to show good signal-to-noise ratios among the larger spikes (Fig. 15). By the same token, the small-cell population of Area X was reflected in the relatively undifferentiated recordings from within its borders (Figs. 16, 17). However, both nuclei behaved as if they played no role in song production: I could detect no consistent changes in baseline firing rates correlated with either song or calls, in either Area X or MAN, even in the period of some seconds between introduction of a female into the home cage of the male and the resultant initiation of song. These results are in keeping with indications from my pathway section experiments in which disruption of connections between MAN and HVc, and Area X and HVc, produced no apparent effect on song or the frequency of its delivery.

Discussion

Activity-onset relationships in the descending motor tract. The four nuclei which produce song-related motor activity are arranged in a linear sequence by virtue of their unidirectional anatomical connections (Nottebohm et al., 1976; Nottebohm et al., 1982). This suggests that neural commands from higher stations in the pathway may activate lower stations. My multi-unit recordings support this hypothesis by demonstrating that the four nuclei in the descending motor tract—NIf, HVc, RA, and nXIIts—are activated in sequence prior to sound onset (Fig. 18). Thus the physiological and anatomical observations are consistent with a hierarchical organization of the motor system for song control.

Assessment of the role of HVc in song production. 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 I cannot as yet make predictions about the detailed relationships between HVc activity and the characteristics of the sounds produced, I 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 lesion results (Nottebohm et al., 1976), implies that HVc either generates or relays learned temporal cues for song.

Multi-unit recordings from the same electrode locations showed activity associated with production of calls (brief sounds produced by both sexes in a variety of circumstances). Song is learned by males and requires auditory feedback for its normal development, whereas calls in most species studied do not require learning from other birds (Lanyon, 1960). Thus, cells recorded from the same site were involved in production of both the learned patterns of song and the unlearned patterns of calls.

Several lines of evidence show a correlation between anatomical features of HVc and behavioral indices of song learning. The volume of HVc fluctuates seasonally (Nottebohm, 1981), corresponding to the presence and absence of singing behavior, and is dramatically larger in males than in females which do not sing (Nottebohm and Arnold, 1976; Gurney and Konishi, 1980). Furthermore, there is a correlation between the volume of HVc and the size of the song repertoire (Nottebohm, Kasparian, and Pandazis, 1981). However, the classical lesion studies which demonstrated the necessity of HVc for normal song were also subject to a classical limitation of lesion studies: any input tract to HVc would be effectively eliminated by the same lesion. Logically, one cannot assess the role a given nucleus plays in song learning without directly analyzing its contribution to normal song; simply removing a component from the circuit is insufficient. Furthermore, any contribution to motor activity for song can be attributed to either of two sources—intrinsic pattern generation within the nucleus in question, or transmission of activity which originates in one or more inputs to that nucleus. Because of this ambiguity, statements concerning song learning processes can only be applied unequivocally to a vocal control nucleus which generates song-correlated patterns of activity. This requirement is not necessarily met by HVc.

The origin of timing cues for song control. What requirements must be met before a nucleus can be considered a source of timing cues for song? First, the pattern of neural activity it produces must be correlated with the temporal pattern of song. My multi-unit recordings show such time-locking for several nuclei in the zebra finch, the "highest" of which (on anatomical grounds) is NIf. Second, the pre-sound latency of increased discharge in the candidate nucleus must be longer than that in other nuclei. NIf activity in the zebra finch satisfies this requirement by leading that in HVc by several milliseconds. Third, removal of the nucleus from the song-production circuit should produce song deficits. I demonstrated that section of the pathway from NIf to HVc leads to complete disappearance of normal or stable song patterning, while comparable sham surgery had no effect. Finally, the sources of input fibers to the nucleus should not produce song-related activity or be necessary for song production. I have performed three experiments to satisfy these conditions for thalamic nucleus Uva, and for any other inputs to which conventional HRP methods might be insensitive. Multi-unit recordings from Uva in the zebra finch showed no activity changes during song or other vocalizations, and bilateral lesions of Uva left song unaffected. Multi-unit recordings from widely-scattered locations in the vicinity of NIf also consistently failed to show song-related activity.

It is not known as yet whether NIf cells receive input from adjacent Field L, thought to represent the avian homologue of auditory cortex. My NIf multi-unit recordings in the zebra finch showed clear responses to clicks and other stimuli, but it is possible that the activities of nearby Field L neurons were represented. While I have recorded song-related activity in Field L neurons of the singing canary (unpublished observations), I did not see any instances of premotor activity in this nucleus. It seems safe to assume that any input to NIf from Field L would constitute auditory feedback and could only be generated after the initiations of sound output. Thus it could not account for the pre-sound onset of activity in NIf and other nuclei. In addition, it is well established that deafening does not affect song patterning, at least in some species, if performed in the adult after song crystallization. We have previously shown that HVc in a bird so treated produces normal song-related activity patterns, and in view of my other results it seems safe to assume that NIf does as well. Thus I conclude that NIf is the first site in the descending motor pathway where

I can observe a pattern of neural activity corresponding to the song pattern. As such NIf must function as a source of timing cues for song.

It is of interest that three of the anatomically-implicated song control nuclei—Uva, MAN, and Area X—showed no changes in neural activity in my recordings during song and other vocalizations. Lesions of these three nuclei or the pathways linking them to the descending motor tract, performed in our laboratory as well as by Nottebohm et al. (1976), had no effect on song. Thus my recording experiments demonstrate that lesion effects provide a better indication of the necessity of a nucleus for adult song production than do studies of anatomical connections. Whether Uva, MAN, or area X plays a role in song development remains to be determined.

Central vs. peripheral control of birdsong. When deprived of auditory feedback during the sensorimotor phase of song development, all species tested so far are unable to crystallize normal song patterns. If the same auditory deprivation is imposed after the song is perfected, the song can be maintained for many months and even redeveloped in succeeding years. Although it has been claimed (Nottebohm et al., 1976) that canaries deafened as adults undergo a progressive degeneration in song quality and syllable stereotypy, I have observed normal, stereotyped syllable production more than one year postoperatively (and following a normal seasonal molting cycle) in a Wasserschläger male canary deafened as an adult (unpublished observations). Song system nuclei must therefore develop a mechanism for generating song patterns in the absence of auditory feedback. This conclusion simplifies the issue of central vs. peripheral control of song patterning, because it implies that one of two states is attained in the song system of the normal adult. Either the song pattern must be generated independently from any sensory feedback, or proprioceptive feedback of an unknown nature is somehow responsible for maintenance of the pattern. The possibility that proprioception can contribute to the maintenance of crystallized song cannot be excluded. Nevertheless, the present results shed new light on this issue, particularly as it pertains to the initiation of song and the overall timing and selection of song syllables. I will examine this issue first from a logical perspective, and second from the point of view afforded by the present results.

The logic of proprioception in song maintenance. Let us suppose that there exists in some form within the song control system a learned, proprioceptively mediated program such that each repeated syllable is shaped solely by proprioceptive cues from the preceding syllable. In this scheme the role of system nuclei is simply to initiate song, and thereafter to compare incoming proprioceptive cues with the acquired tape, making the necessary corrections to maintain the desired song quality. However, the scope of such a mechanism is sharply limited by two considerations. First, the intersyllable interval for repeated syllables must be invariant, since the arrival time of feedback cues would be time-locked to the preceding syllable. This requirement is not satisfied by the mockingbird, which can vary intersyllable intervals over a considerable range (unpublished observations). Second, the tonal quality of the first rendition of a repeated syllable cannot be as good as that of succeeding repetitions, because there are no feedback cues to guide the shaping of the first syllable. This requirement is also violated by mockingbird song, in which the first syllable in a train is typically identical to the rest. There cannot, therefore, be any major contribution to song selection or shaping from this type of mechanism.

Alternatively, the contribution of proprioceptive cues to syllable shaping could be largely intrasyllabic, so that cues from early in the syllable are used to shape the remainder of the syllable. In its most extreme form, this mechanism would require only a set of initiation commands from central sources, with all subsequent activity generated in response to peripheral events—a reflex arc between the syrinx and the brain. The use of such a mechanism requires that there be systematic differences in the feedback derived from very similar onsets of syllables, because of the very large repertoires of some species such as the mockingbird and the inevitable similarities of many syllables. Such differences in feedback could be generated by the use of different syringeal muscles to create very similar sounds; the different feedback patterns from these muscles could cause divergence in later portions of syllables. The major limitation of this mechanism lies in the length of the reflex arc and the time required to traverse it; we can reasonably assume a minimum of 50 msec between a given command in HVc or NIf and the arrival of feedback resulting from the command. In fact, commands may be generated continuously so that feedback is continuously available; but if this is so the system is essentially functioning under central control, and not according to the peripheral control mechanism under consideration here. If commands are generated only at the initiation of a syllable, and thereafter at a rate set by the proprioceptive delay, then the temporal modulations in song syllables cannot be more rapid than the total time required to complete the circuit from NIf or HVc to the syrinx and back again. Since one can easily find examples, in the songs of mockingbirds and many other species (Greenewalt, 1968), of very rapid and reproducible modulations in song syllables, this mechanism cannot be in general use in the control of song. Of course it is possible that a less extreme form of this mechanism is used, and that relatively subtle modulations of syllables are the result. But the 50 msec constraint would apply in any case, so that all rapid modulations in song must be centrally controlled.

A physiological examination of the role of proprioceptive feedback. Section of either the left or right hypoglossus nerve innervating the syrinx produces song deficits; bilateral nerve section eliminates song completely. These results implicate the syrinx as the major organ of sound production. Long-term unilateral denervation results in a marked atrophy of the ipsilateral syringeal musculature, strongly suggesting that the hypoglossus constitutes the exclusive source of syringeal innervation. Thus any proprioceptive feedback which originates in the syrinx must be conveyed to the brain via the hypoglossus. It is difficult to imagine how feedback from any other source, such as the intercostal muscles involved in respiratory control, could play a crucial role in maintaining song patterns in the absence of auditory feedback, since this maintenance would inevitably involve very subtle control of neural commands to the syrinx.

Assuming that the syrinx is the only important source of feedback cues for song control, and the hypoglossal nerve its only innervation, we may conclude that any song-related activity in the brain which precedes the onset of activity in nXIIts must be central in origin. The maximum presound latency observed in mockingbird hypoglossal nerve was 50 msec; such long latencies were usually associated with high-amplitude, sharp-onset song syllables. Typical latencies were 20-30 msec; though some variation in these latencies was seen for successive repetitions of the same elements, the range of latencies was nevertheless characteristic for a given syllable or class of similar elements. The same general statement can be applied to the activities of HVc single units; onset latencies and especially firing patterns varied systematically from syllable to syllable, even for neurons which showed activity for every syllable. Thus both hypoglossal nerve and HVc units show activity patterns containing cues for the selection of song syllables.

The crucial point lies in a latency comparison between these "selective" song cues in HVc and nXIIts. The clearest evidence for central control of song patterning comes from the existence of specialized neurons in HVc, firing specifically for certain song elements, and producing this specialized activity well in advance of the earliest activity in nXIIts. Whether or not this specific activity originates in HVc cannot be addressed at present because I have not yet recorded from single units in NIf. Nevertheless, the property of pre-motor specificity exhibited by some HVc units implicates these cells in the selection or shaping of song elements, or both, and strongly suggests that these processes occur centrally, in HVc or at a higher level.

In some premotor-specific units the timing of activity is closely matched to the timing of the associated syllable, even though the intersyllable interval may vary. Lanyon (1976) has shown that several aspects of mockingbird song, including mean intersyllable interval, are modifiable by experience in the adult mockingbird. Thus the most parsimonious explanation of the correspondence between unit firing and syllable timing is that the units acquire their stereotyped relationships to selected syllables.

The necessity of NIf for normal song production in the zebra finch, the longer-latency multi-unit activity in zebra finch NIf as compared with efferent activity in other nuclei of the same species, and the absence of reciprocal connections between NIf and HVc, strongly suggest that the earliest timing cues for song are generated by NIf. To the extent that these timing cues are learned, NIf must be a locus of song learning processes during a well-defined period of development. Thus it appears that NIf, unique among known neural substrates for centrally produced and patterned behaviors, manifests characteristics which are acquired during development. I cannot over-emphasize the value of a discrete neuronal system which mediates the acquisition and production of a stereotyped motor program.

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Figure 1A. Relationship between neural activity in HVc and song elements produced by the bird. In each record the upper trace represents multi-unit activity recorded from left HVc; the lower trace is the amplitude envelope of the sound produced by the bird. Upper record: initial portion of a song produced by a Wasserschläger 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. Time calibration = 100 msec.

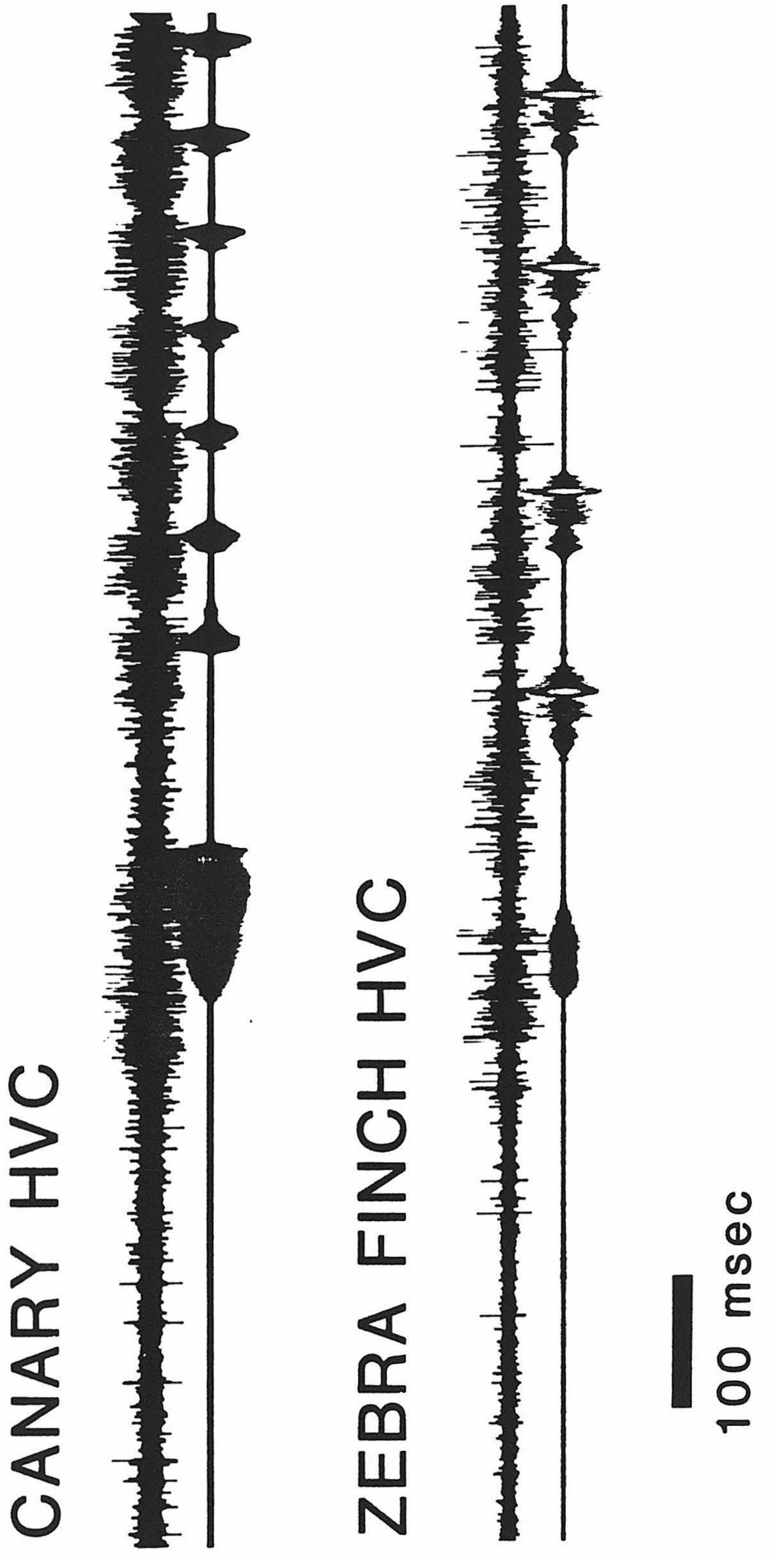

CANARY HVC
ZEBRA FINCH HVC
100 msec

Figure 1B. Relationship between HVc neural activity and call elements produced by the bird. Two examples of male zebra finch calls are shown, 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. Time calibration = 100 msec.

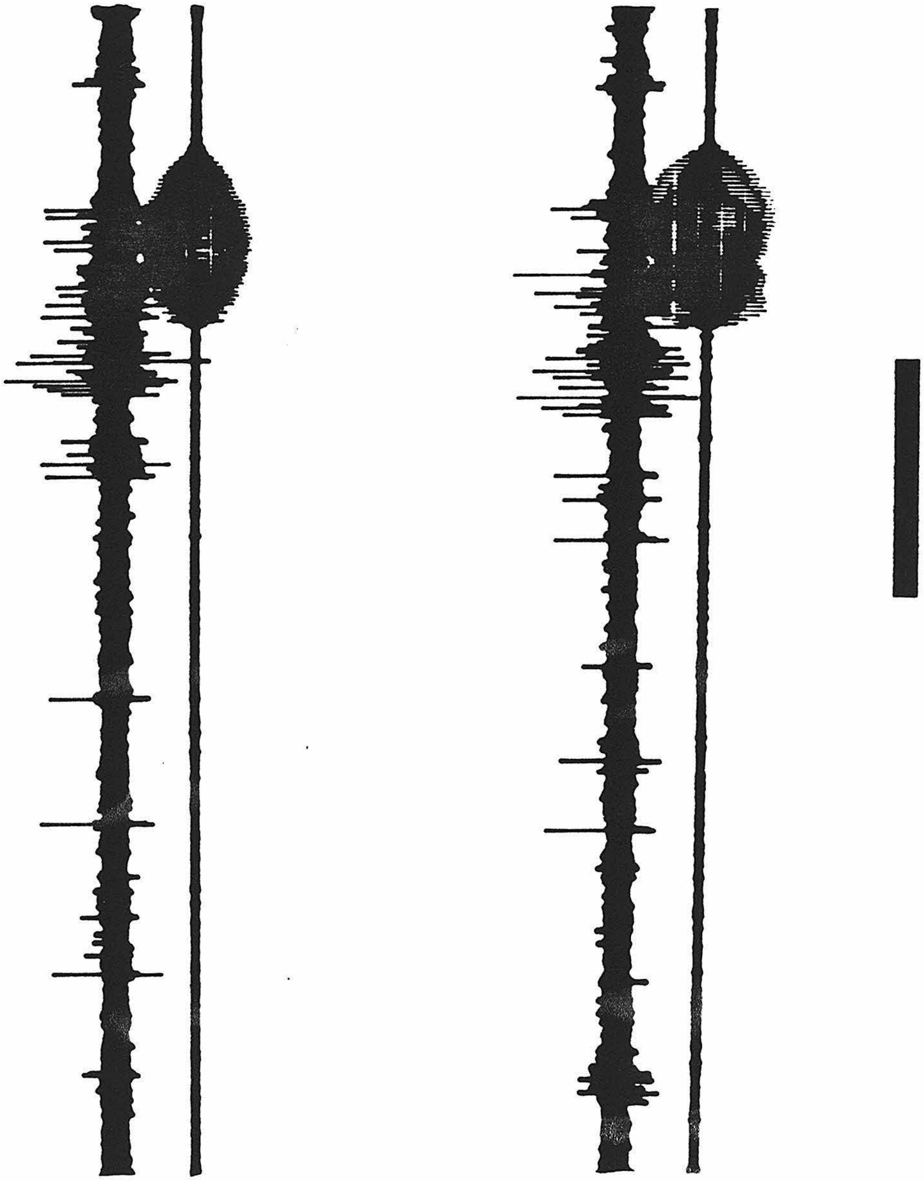


Figure 2A. Multiple-unit neural recordings from NIf (Nucleus Interfacialis) of the singing zebra finch. Upper traces: neural activity recorded from NIf. Lower traces: song syllables produced by the bird. (Lower record is baseline during a non-singing period. There was a time-locked correspondence of NIf activity and song elements which was qualitatively similar to that seen in HVc. However, NIf activity onset preceded that in HVc by several milliseconds [see Fig. 2B].)

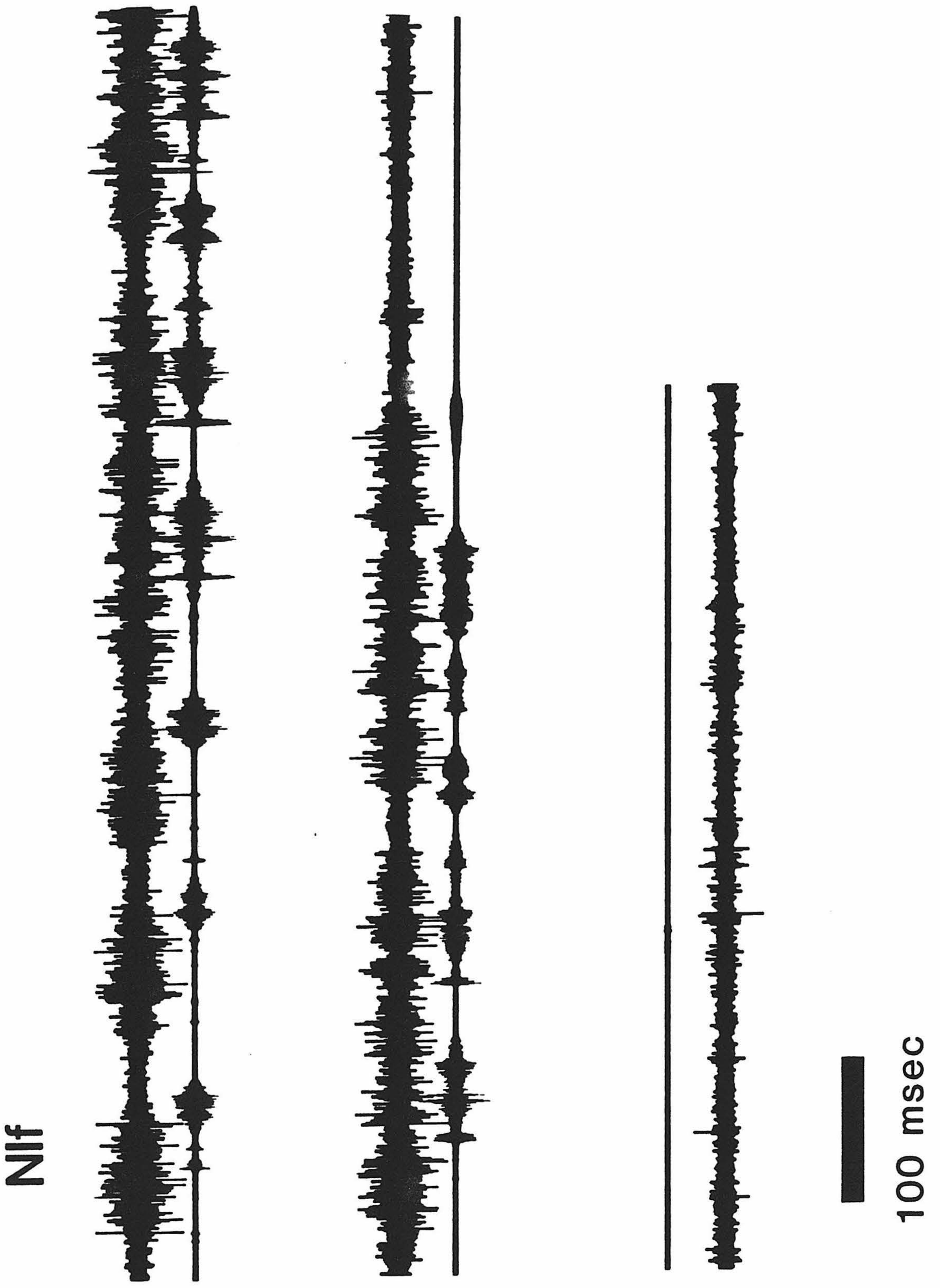

Nlf
100 msec

Figure 2B. Relationship between multiple-unit activity in NIf and HVc for homologous vocalizations (contact cells). Upper record: representative example of multi-unit activity recorded from left NIf (upper trace) and a contact call produced by a zebra finch. Lower record: representative case of multi-unit activity recorded from left HVc of a second bird during production of a homologous contact call. Presound latency for NIf was approximately 60 msec; that for HVc was about 50 msec. For a histogram comparison of these data, see Fig. 2C, and Fig. 18.

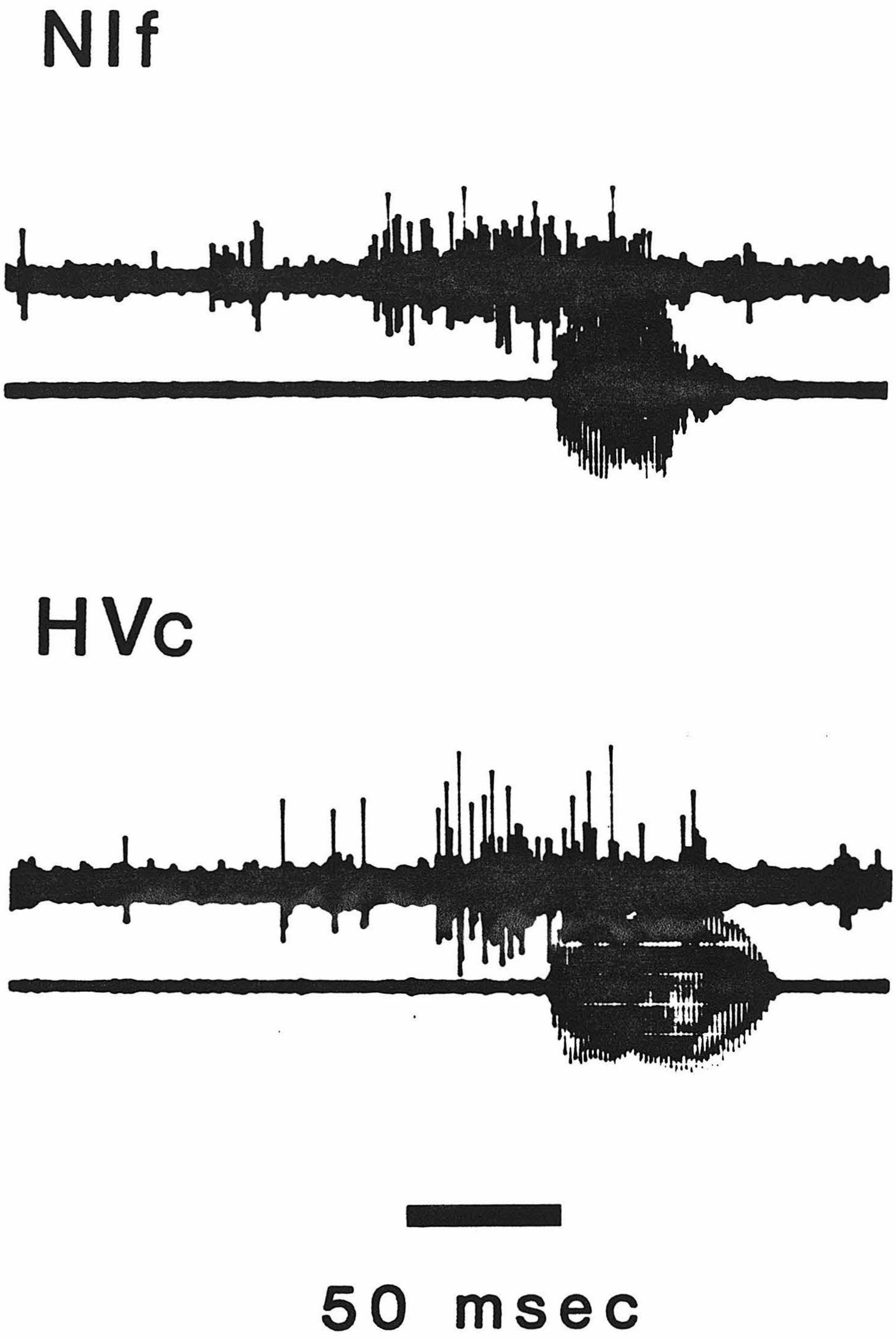
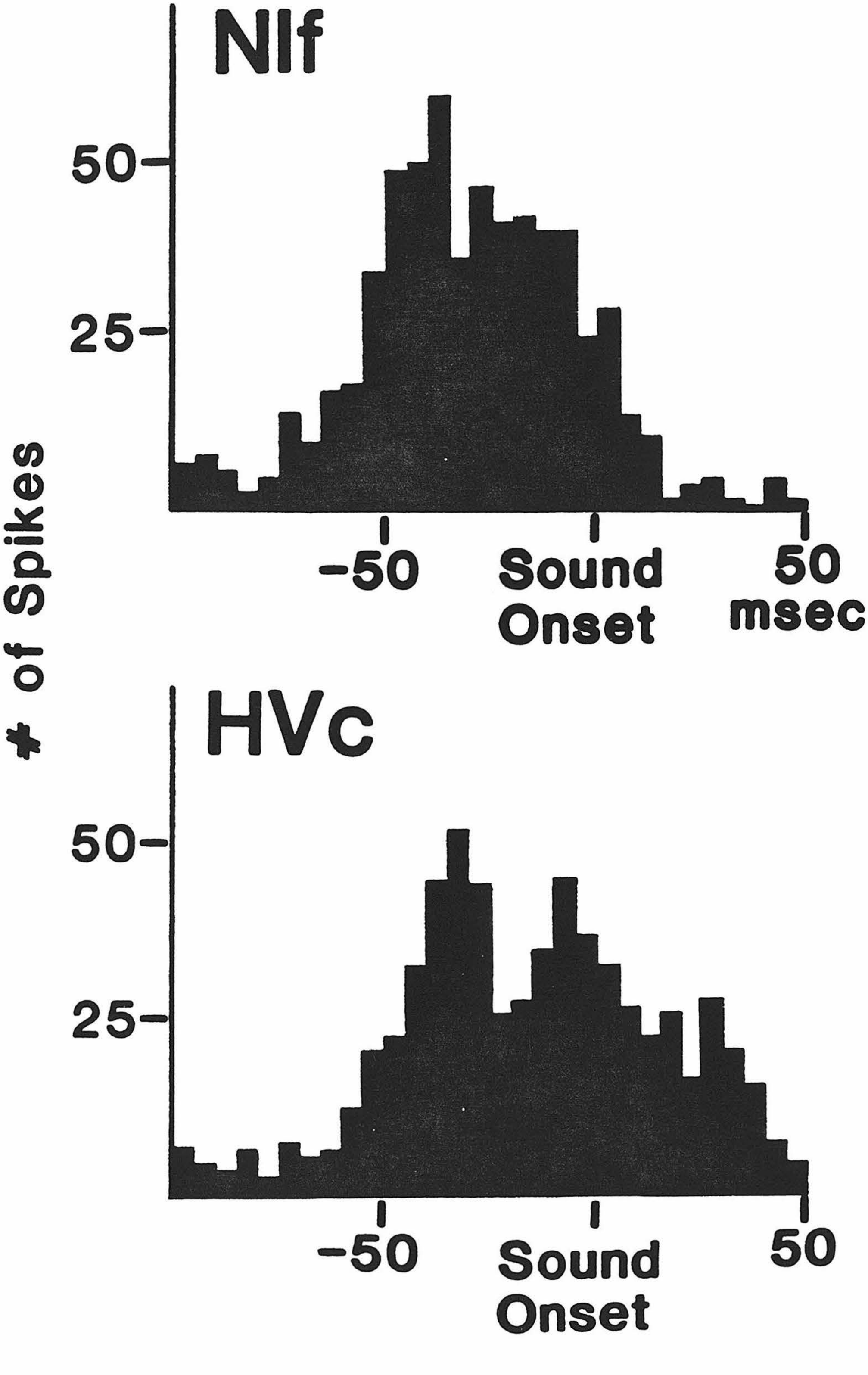

NIf
HVc
50 msec

Figure 2C. Histograms showing the relationship between multiple-unit activities in NIf and HVc. Each histogram represents summed activity from 25 cases of the type illustrated in Fig. 2B. Each bin represents 5 msec. Both the onset and the peak of increased activity in NIf led that in HVc by approximately 10 msec. See Fig. 19 for comparison of these NIf data with a second set of HVc recordings, from another bird.



# of Spikes
NIf
50
25
-50
Sound
Onset
50
msec
HVc
50
25
-50
Sound
Onset
50

Figure 3. The effect on song of sectioning the pathway from NIf to HVc. (Upper record) Sonagram of normal, preoperatively-produced song of a zebra finch. (Lower three records) Sonagrams of representative "songs" produced by the same zebra finch after transection of the pathway from NIf to HVc (and the pathway from Uva to HVc). These songs carried no stereotyped phrase structure from one rendition to the next, and their component syllables bore no consistent resemblance to those of the preoperative song.

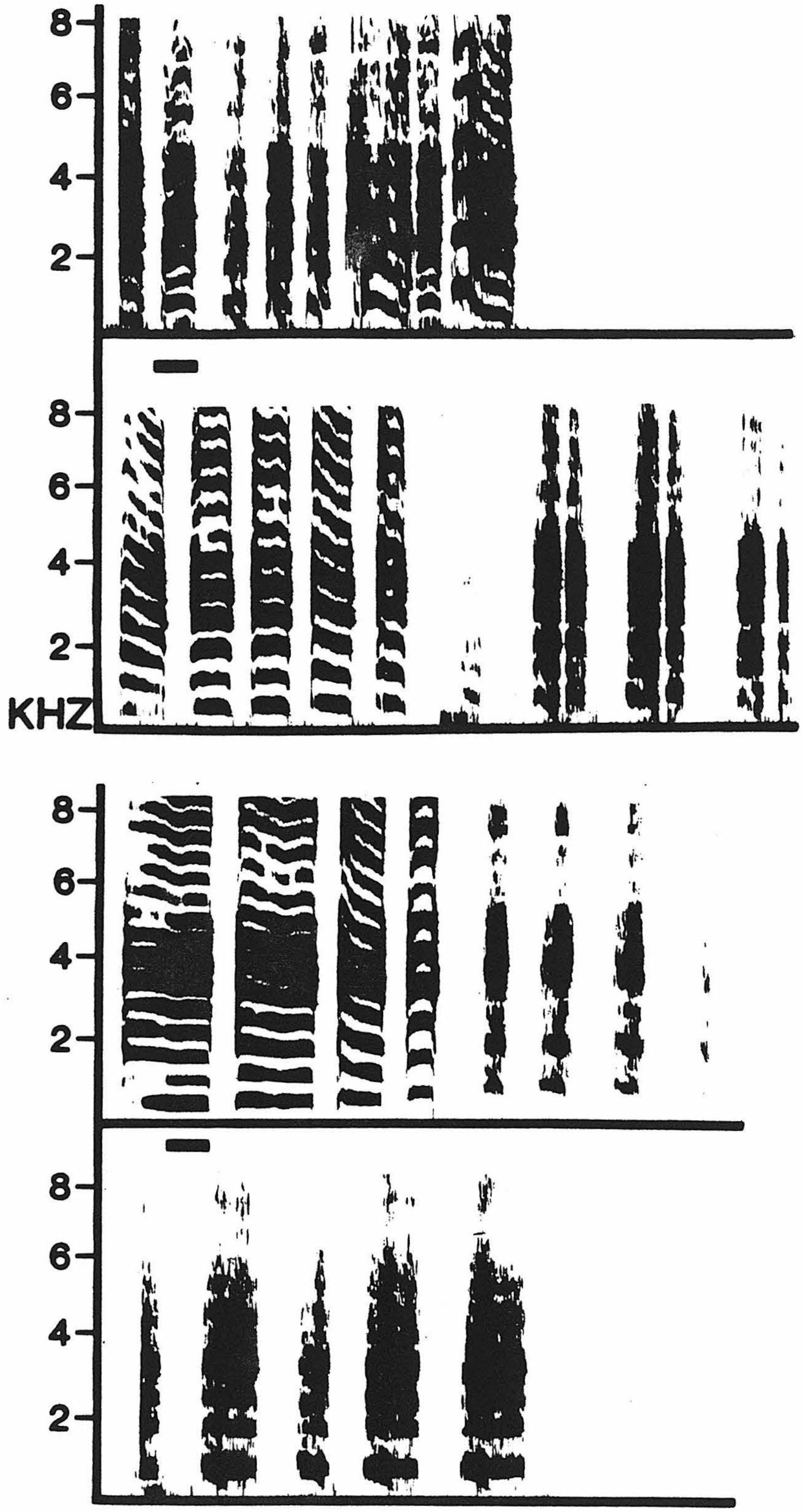

8
6
4
2
8
6
4
2
KHZ
8
6
4
2
8
6
4
2

Figure 4. The effect on song of a sham section comparable to that illustrated in Fig. 3. The incision incidentally eliminated the tract from MAN to HVc, and from HVc to area X. Nevertheless, the postoperative song phrase (lower sonagram) was unaffected by this surgical procedure. All preoperative syllables (upper sonagram) were preserved in morphology and timing. Time calibration = 100 msec.

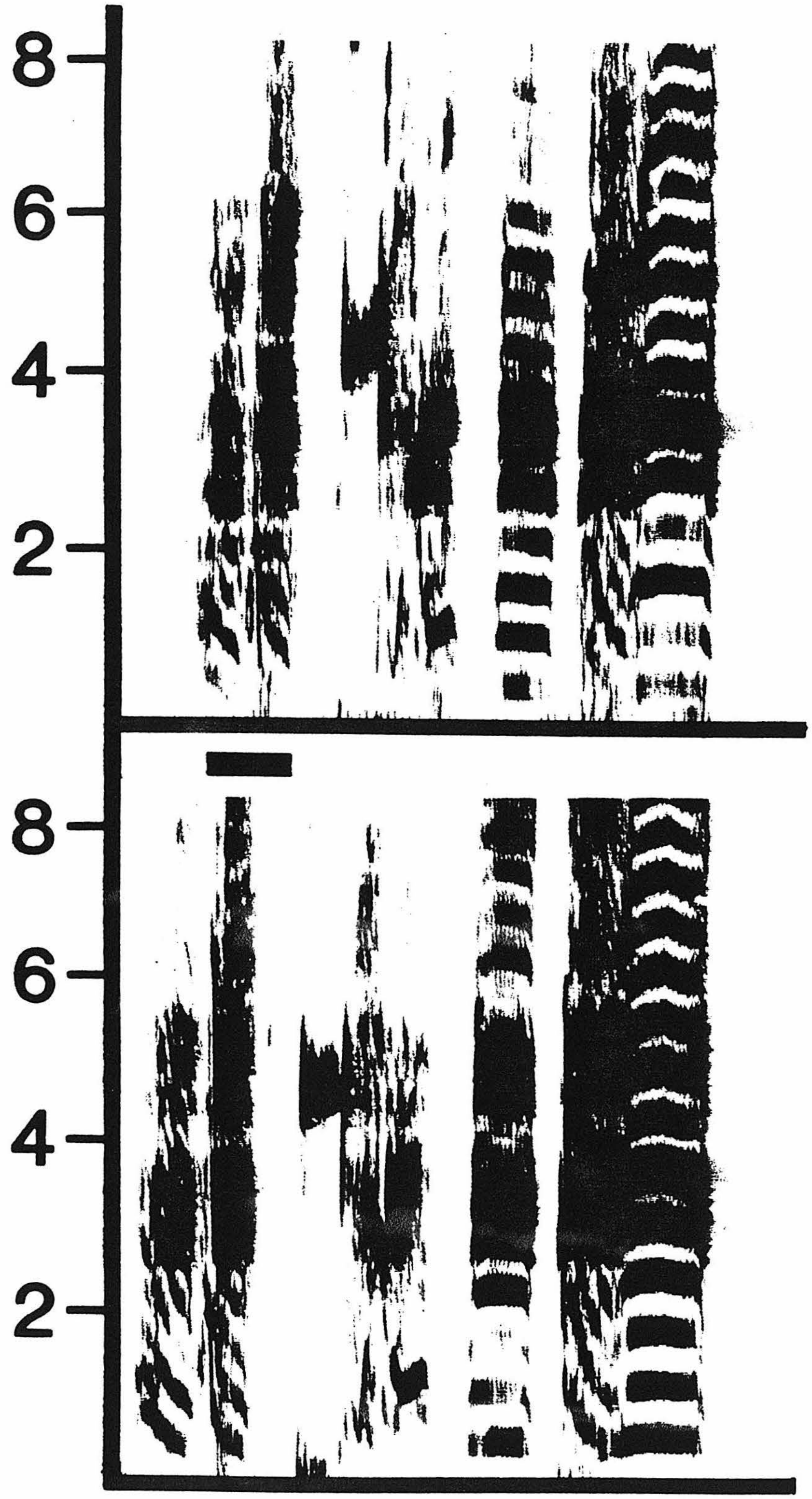

8
6
4
2
8
6
4
2

Figure 5. Multiple-unit recordings from thalamic nucleus Uva of the singing zebra finch. Uva projects anatomically to NIf and HVc, and could therefore transmit song cues to either of these nuclei. However, recordings from right Uva in the zebra finch showed no changes in neural activity correlated with song (the apparent slight increase of activity midway in the second record is due to an acoustic artifact induced by the sound of the song).

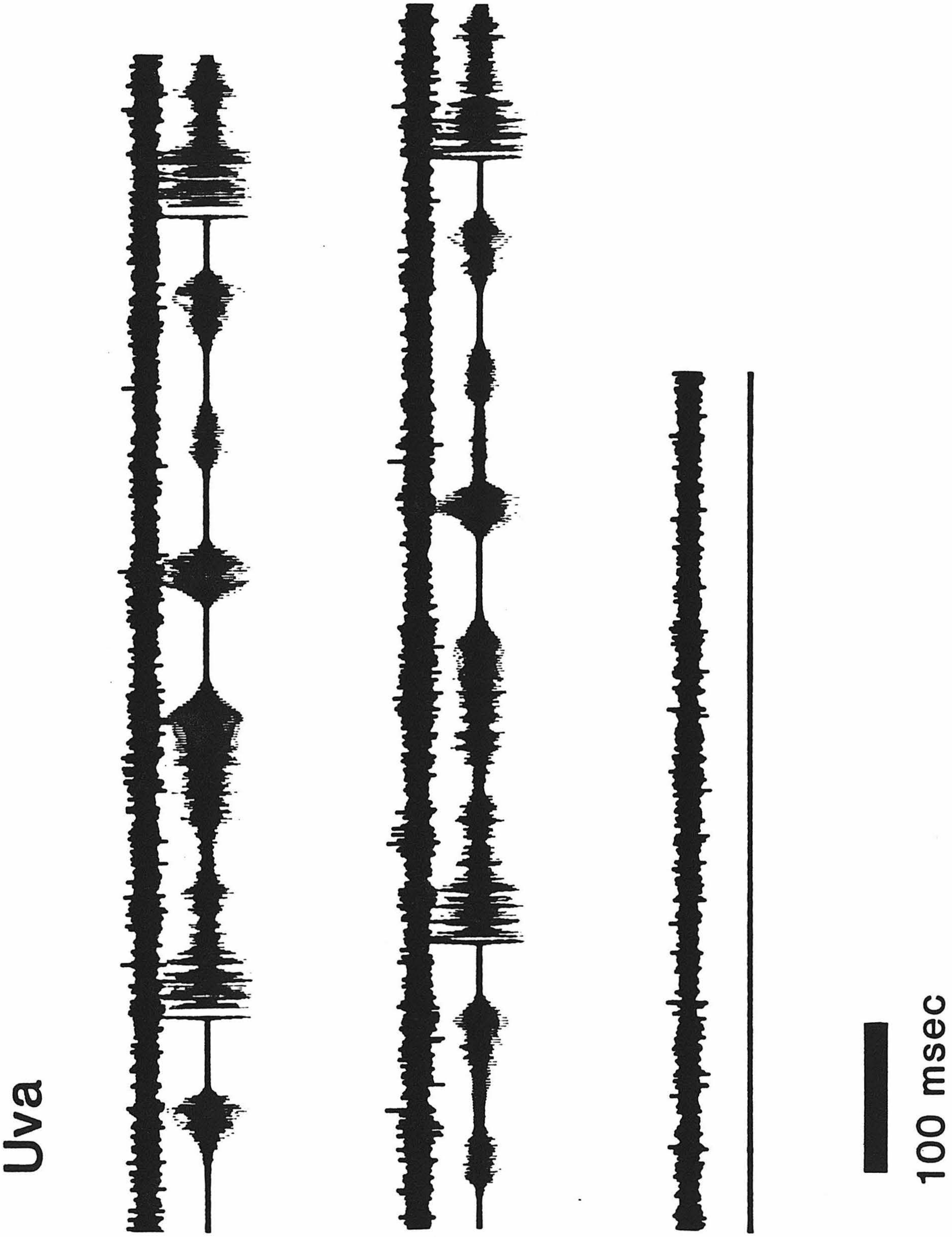

Uva
100 msec

Figure 6. The effect on song of bilateral lesions of thalamic nucleus Uva. (Upper sonagram) preoperative song of a normal zebra finch. (Lower sonagram) song produced after bilateral lesions which eliminated approximately 95% of right and left Uva. Song was unaffected by these lesions, whereas comparable damage to NIf, HVc, or RA has drastic effects on song. Time calibration = 100 msec.

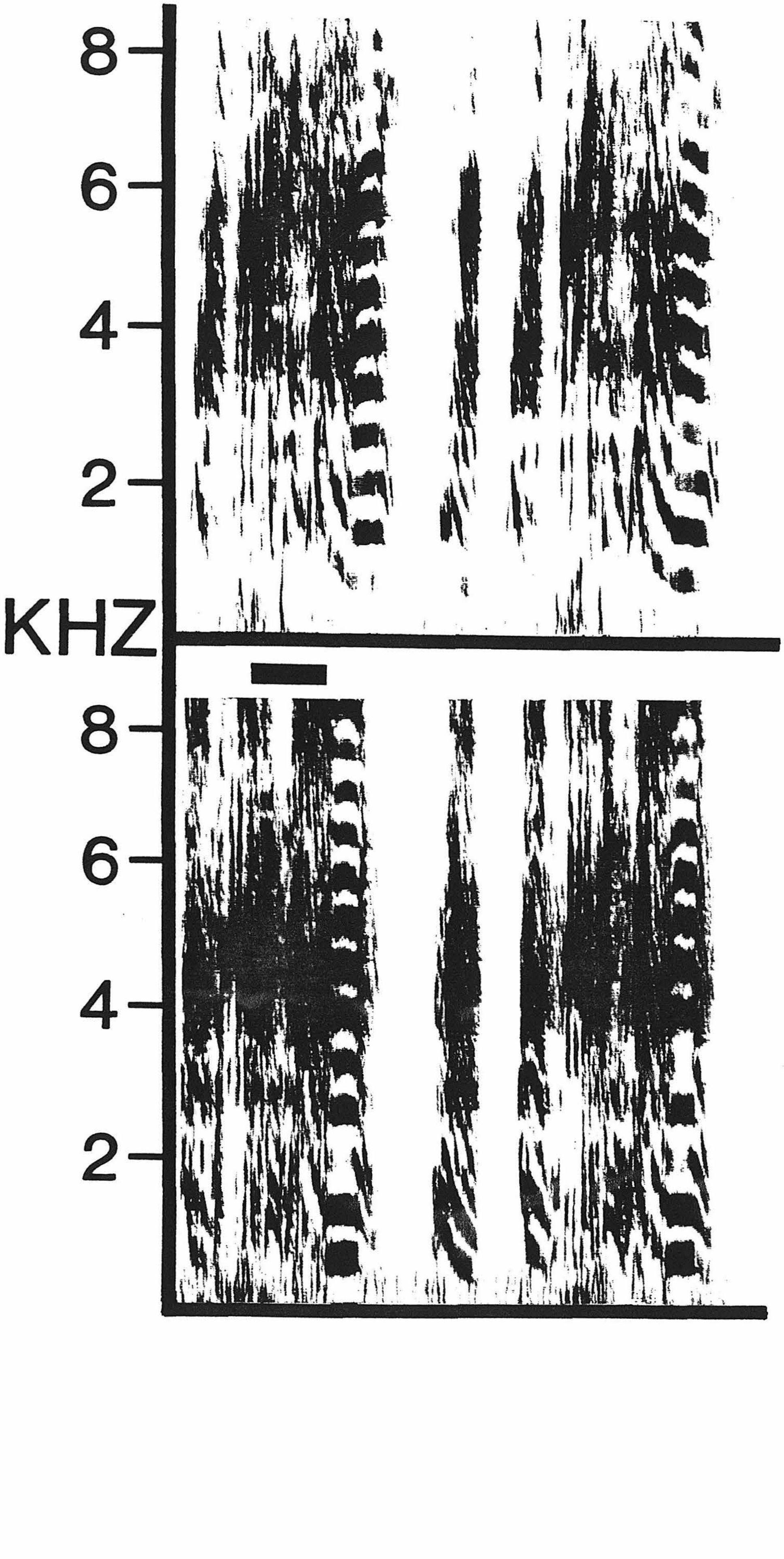

8
6
4
2
KHZ
8
6
4
2

Figure 7. A single-unit recording from HVc of the singing mockingbird which shows pre-motor activity for all song syllables. This unit did not respond to the same song syllables presented as auditory stimuli. Lower record shows some clustering of activity for trill elements; for comparison with a more specific patterns see Figs. 9 and 10.

MOTOR- GENERAL

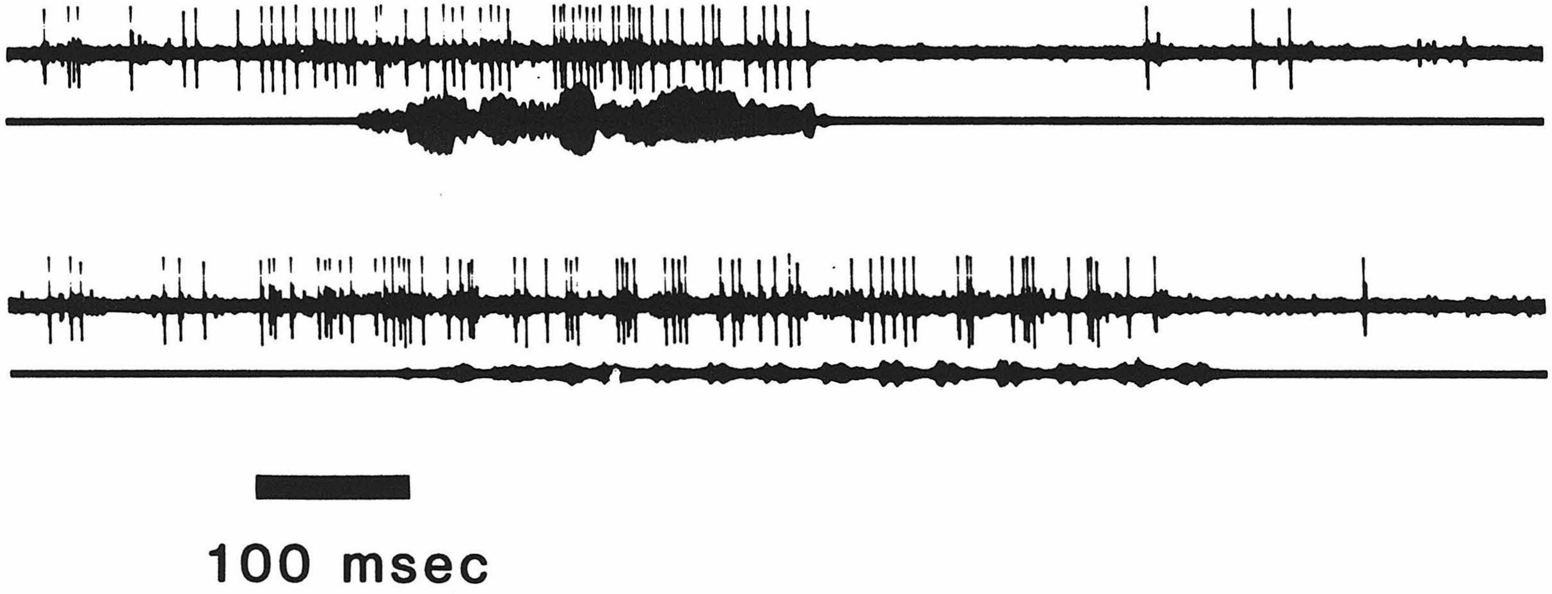


Figure 8. "Anticipatory" single-unit activity at the initiation of song. (Upper) recording from the same single unit shown in Fig. 7. (Lower) sonagram of mockingbird song syllable produced after a 15-second silent interval. Note the steady increase in unit activity leading up to sound onset.

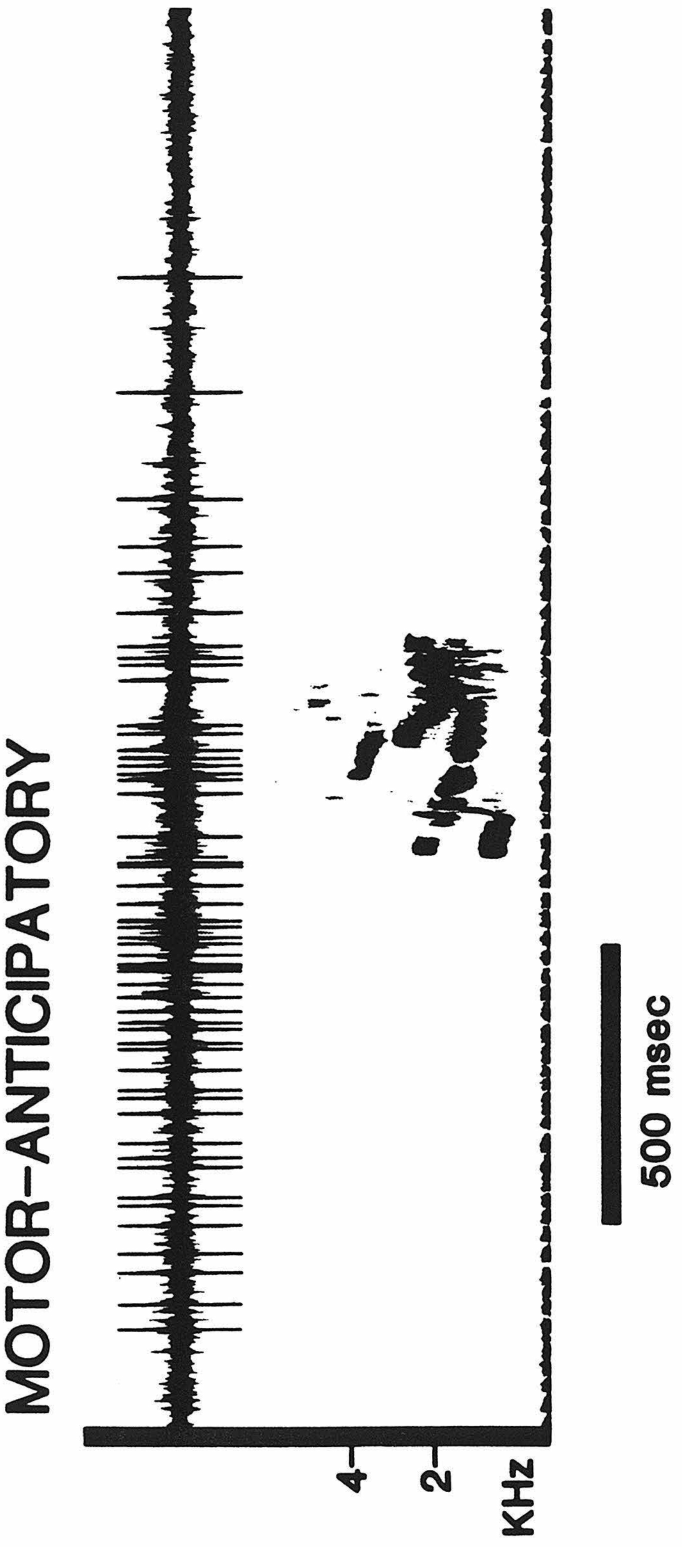
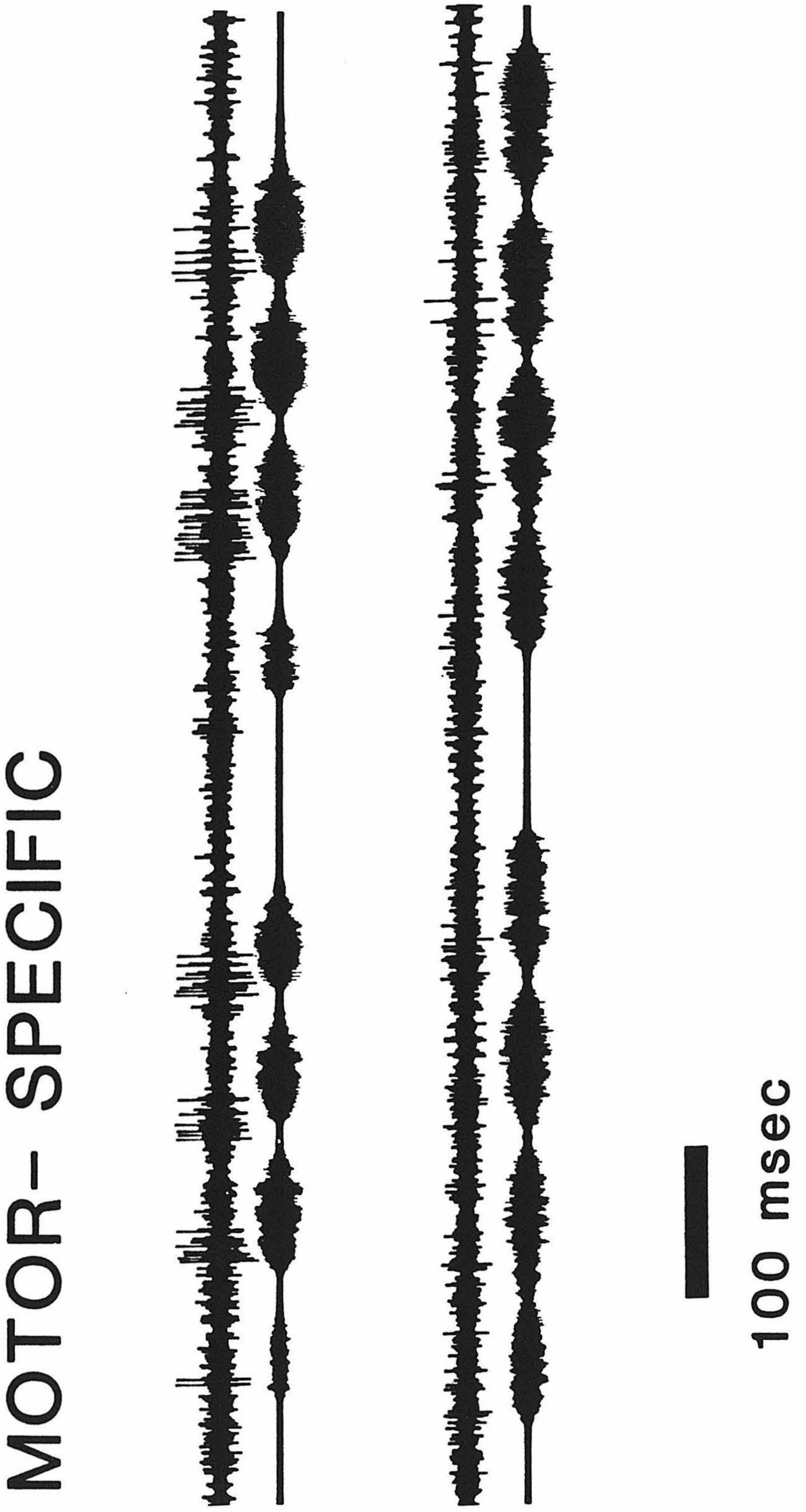

MOTOR-ANTICIPATORY
4
2
KHz
500 msec

Figure 9. A single-unit recording from mockingbird HVc which shows more selective motor activity during song than the unit illustrated in Figs. 7 and 8. This unit also fails to respond to those sounds for which it fires when the bird sings. Sonagrams of the repeated syllables (following page) in upper and lower records show that both are similar to zebra finch song syllables heard often by this mockingbird. Yet this unit was always active when the bird produced the upper syllable, and only sporadically active for the lower syllable.


MOTOR- SPECIFIC
100 msec

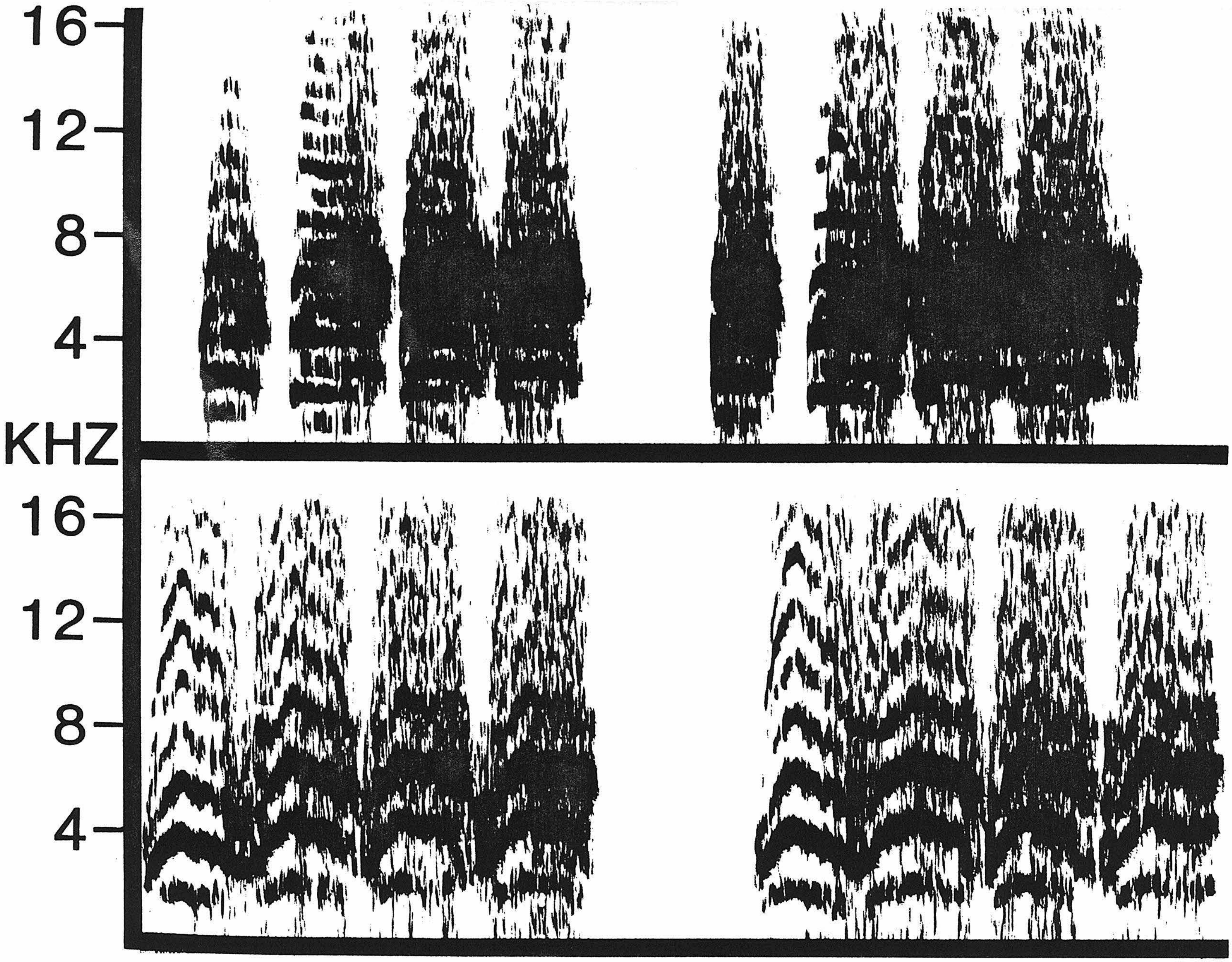
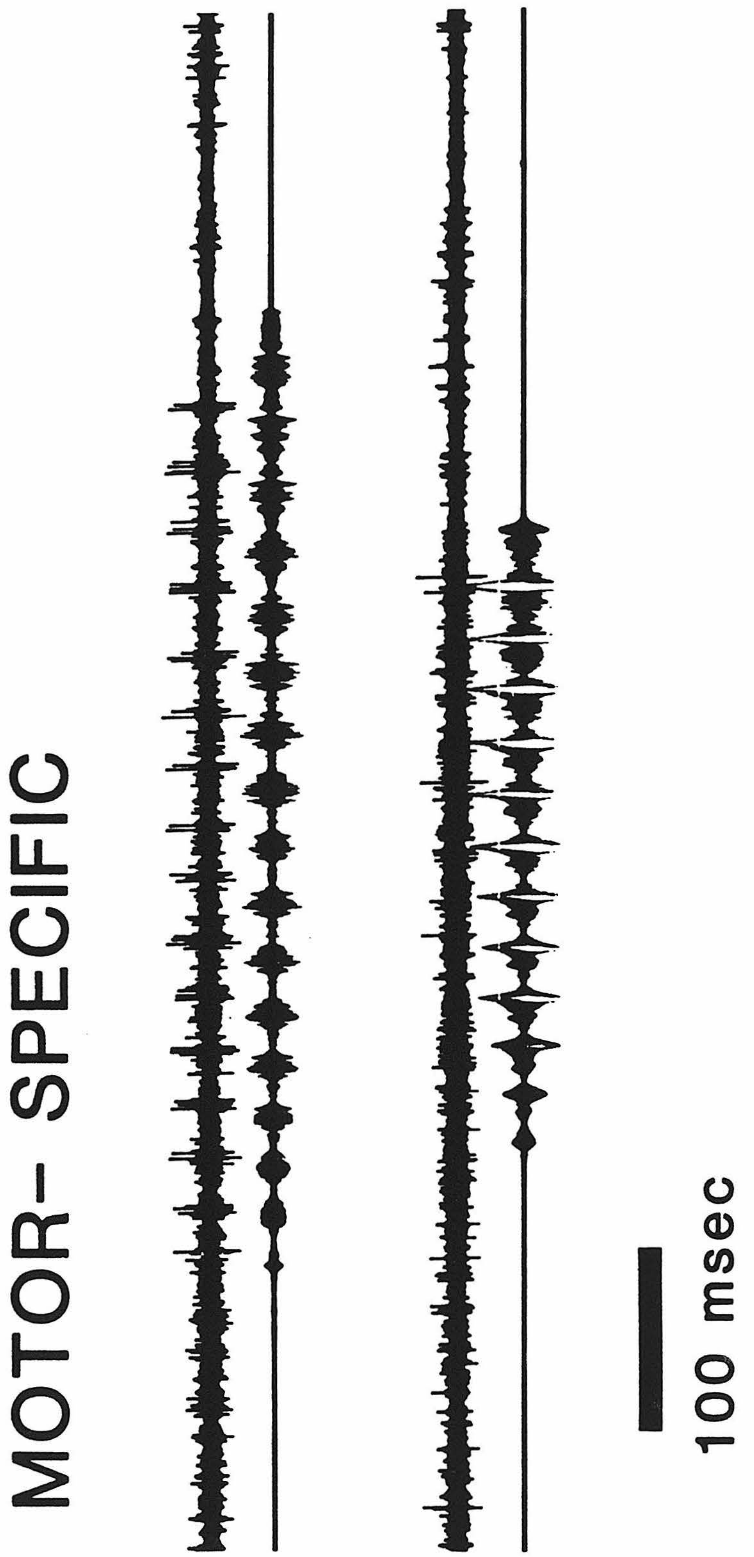

16
12
8
4
KHZ
16
12
8
4

Figure 10. Another example of a selective motor unit which did not show auditory responses to the same sounds. Both upper and lower records depict repeated syllables (sonagrams on following page) which are very similar to canary trills, to which this mockingbird was repeatedly exposed. The upper syllables were slightly higher in fundamental frequency. The unit fired a burst for each trill element (except the first and last, as shown) in the upper record, while firing only occasionally for elements in the lower record.


MOTOR- SPECIFIC
100 msec

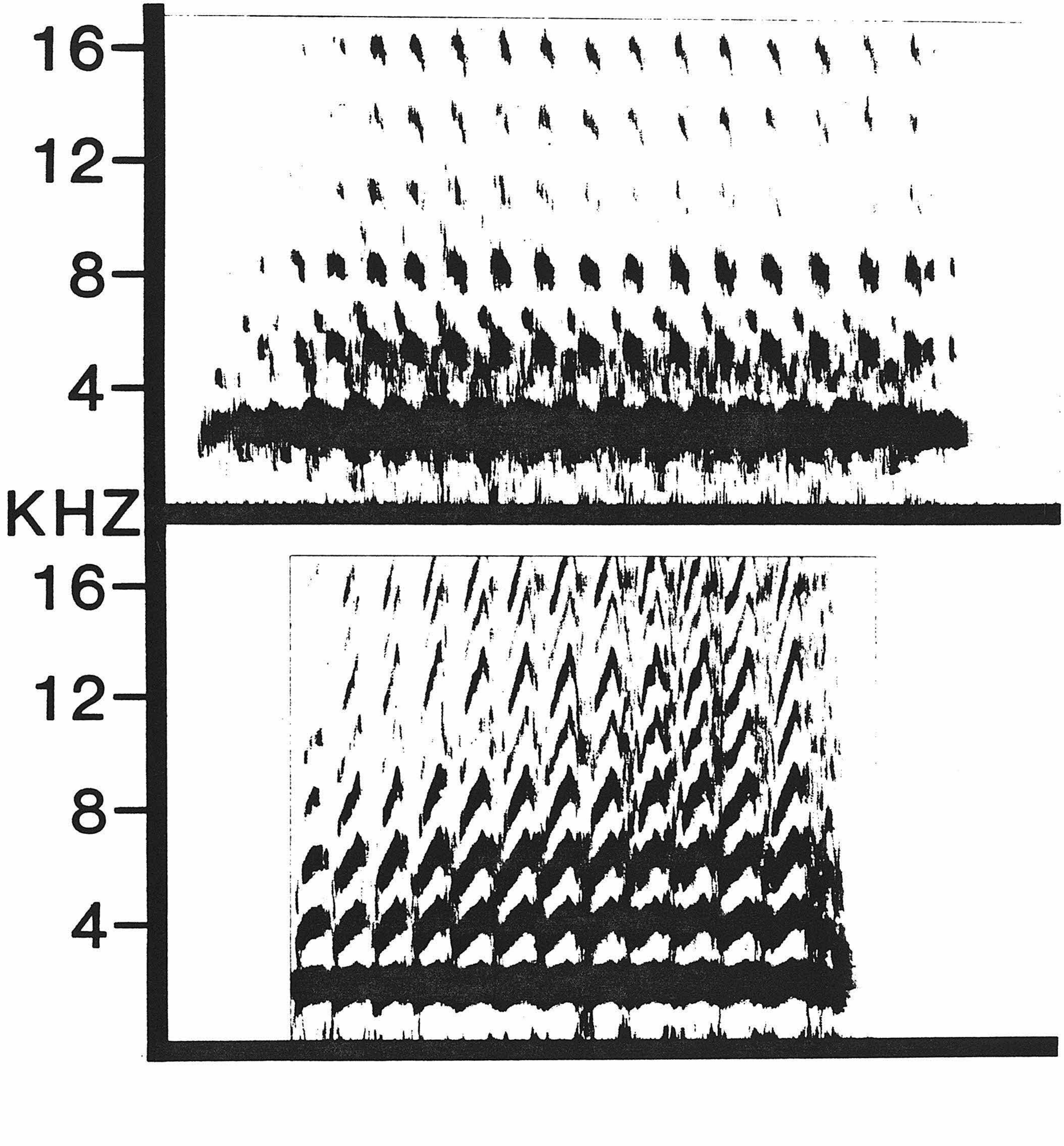

16
12
8
4
KHZ
16
12
8
4

Figure 11. A single unit recording from mockingbird HVc showing specific premotor activity. (Upper record) a repeated syllable for which the unit consistently fired well in advance of the earliest activity in the hypoglossal motor nerve. (Middle record) a syllable for which the unit was consistently silent. (Lower record) a repeated syllable for which the unit was consistently active after sound onset, firing with a constant phase relationship to the trill elements.

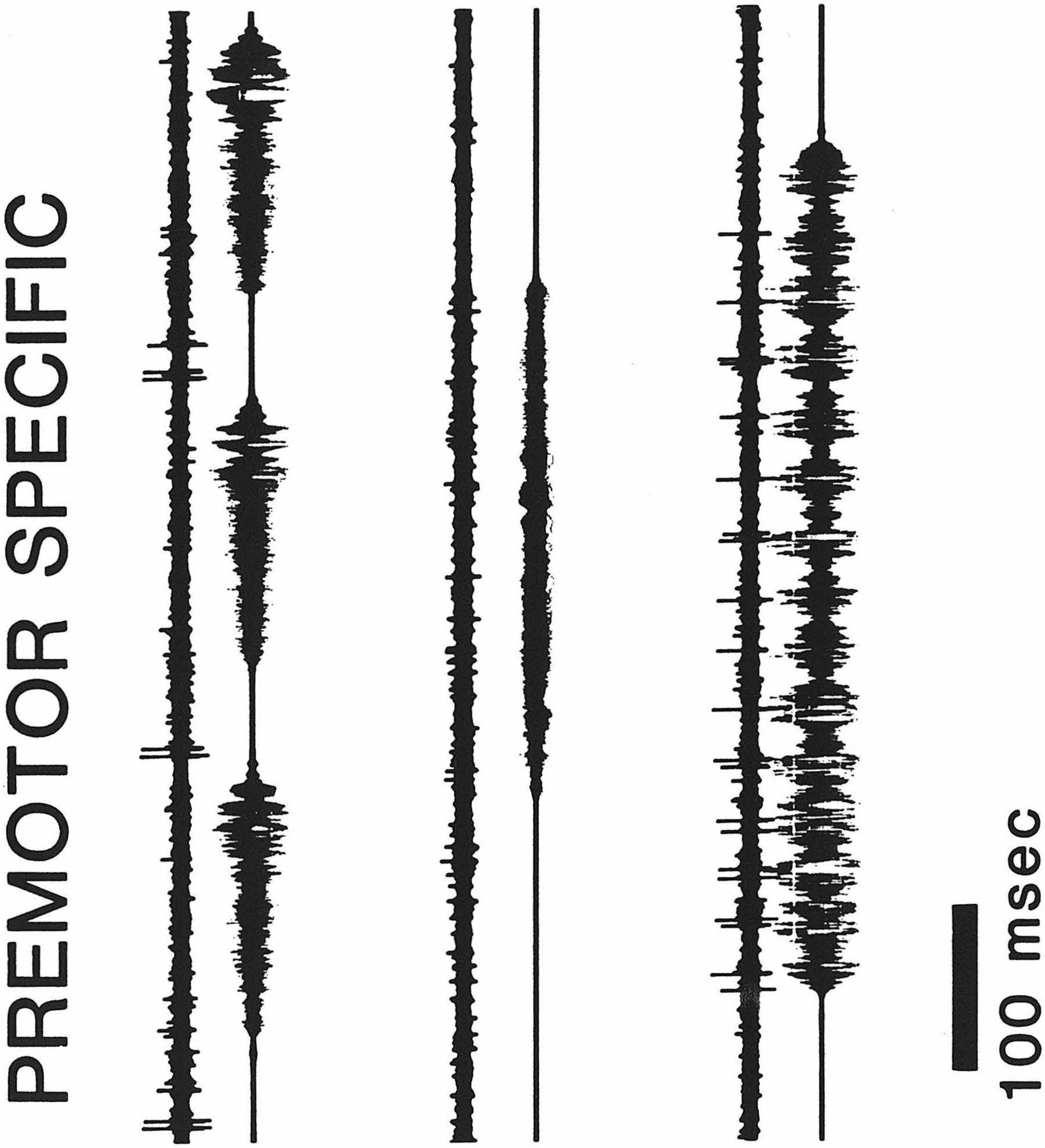

PREMOTOR SPECIFIC
100 msec

Figure 12. Another example of a unit showing specific premotor activity. This cell consistently fired well in advance of the syllable illustrated in the upper record, and was silent for most other syllables including the one shown in the bottom record.

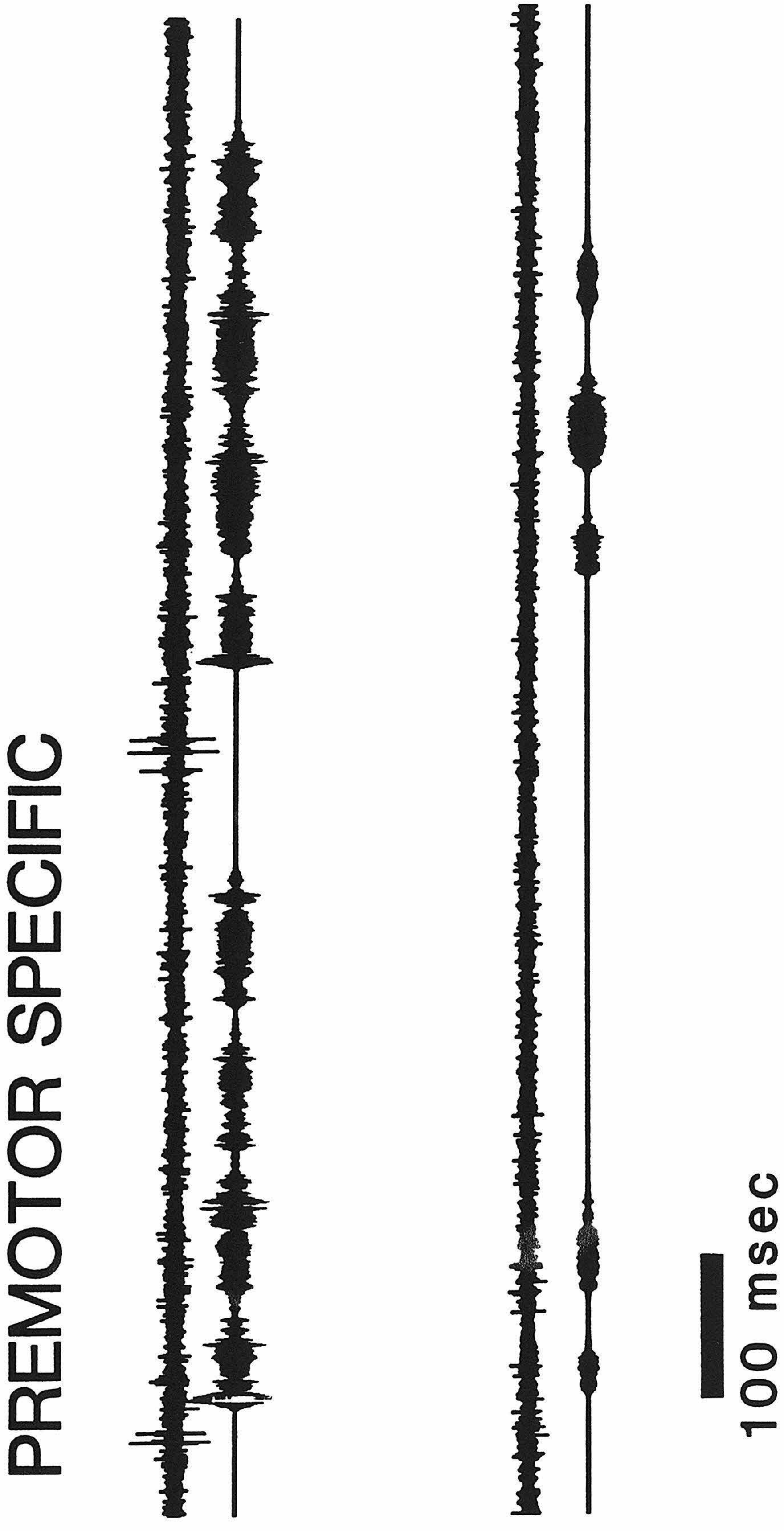

PREMOTOR SPECIFIC
100 msec

Figure 13. Multiple-unit recordings from RA and HVc, obtained simultaneously from a singing zebra finch. The three records shown are arranged as a continuous temporal sequence, top to bottom. Careful examination of the sound-related activity peaks (in upper record, for example) showed HVc activity onset and termination leading that in RA by several milliseconds, consistent with the anatomical connections between these nuclei. At the termination of each song rendition, there was a marked repression of activity in RA which decayed gradually over 2-4 seconds. During singing periods this repression obscured the large difference in spontaneous activity between HVc and RA (see last half of lower record).

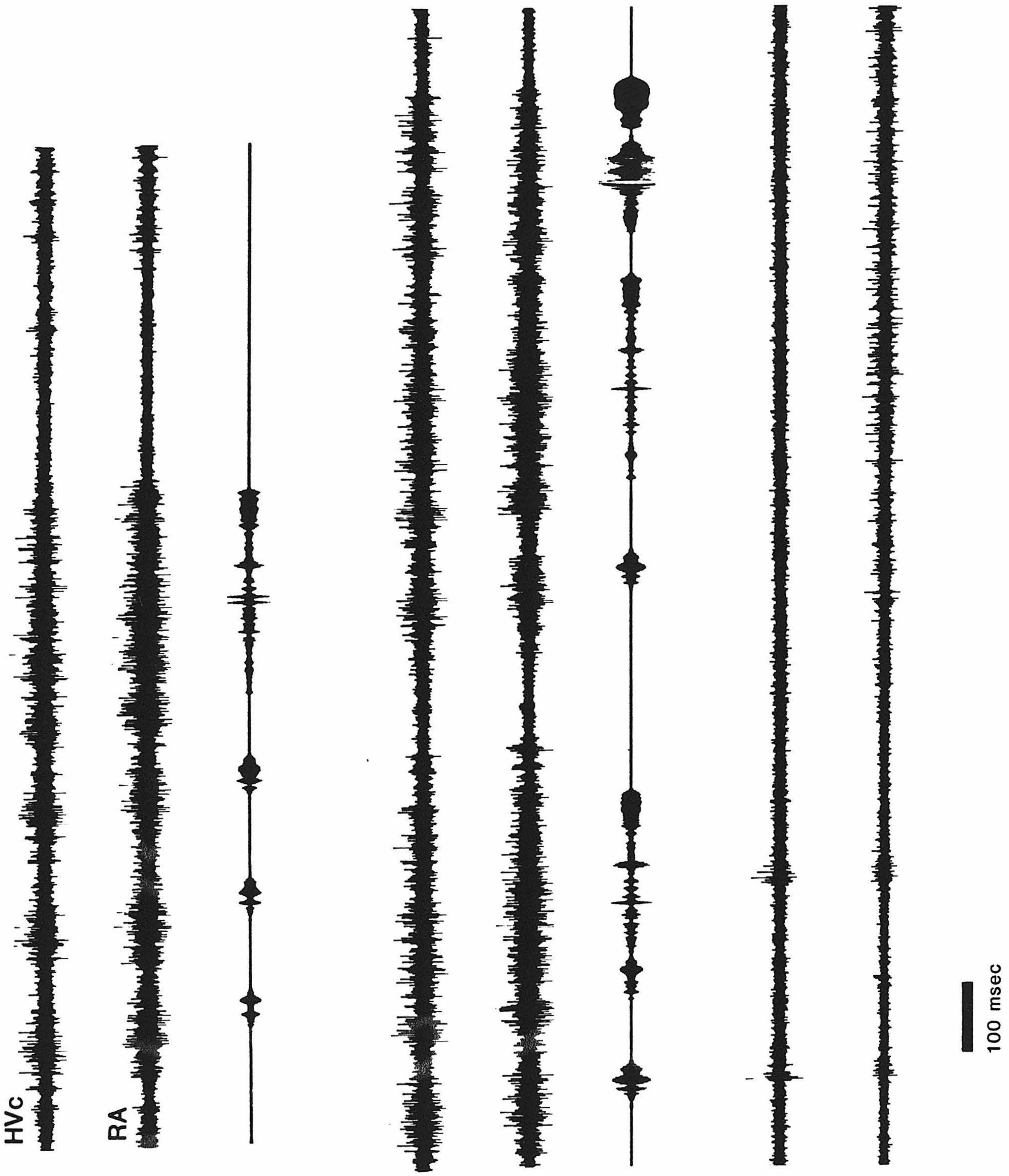

HVc
RA
100 msec

Figure 14. Multiple-unit activity from the left hypoglossal nerve of the singing mockingbird. A wide range of pre-sound activity latencies (20-50 msec) is illustrated; in the canary hypoglossus (see Fig. 2 in Chapter 3), no latencies longer than 30 msec were observed. Nevertheless, nerve recordings from both species shared several features: very low spontaneous activity rates, abrupt onset of activity preceding sound production, and a more gradual cessation of activity before sound termination.

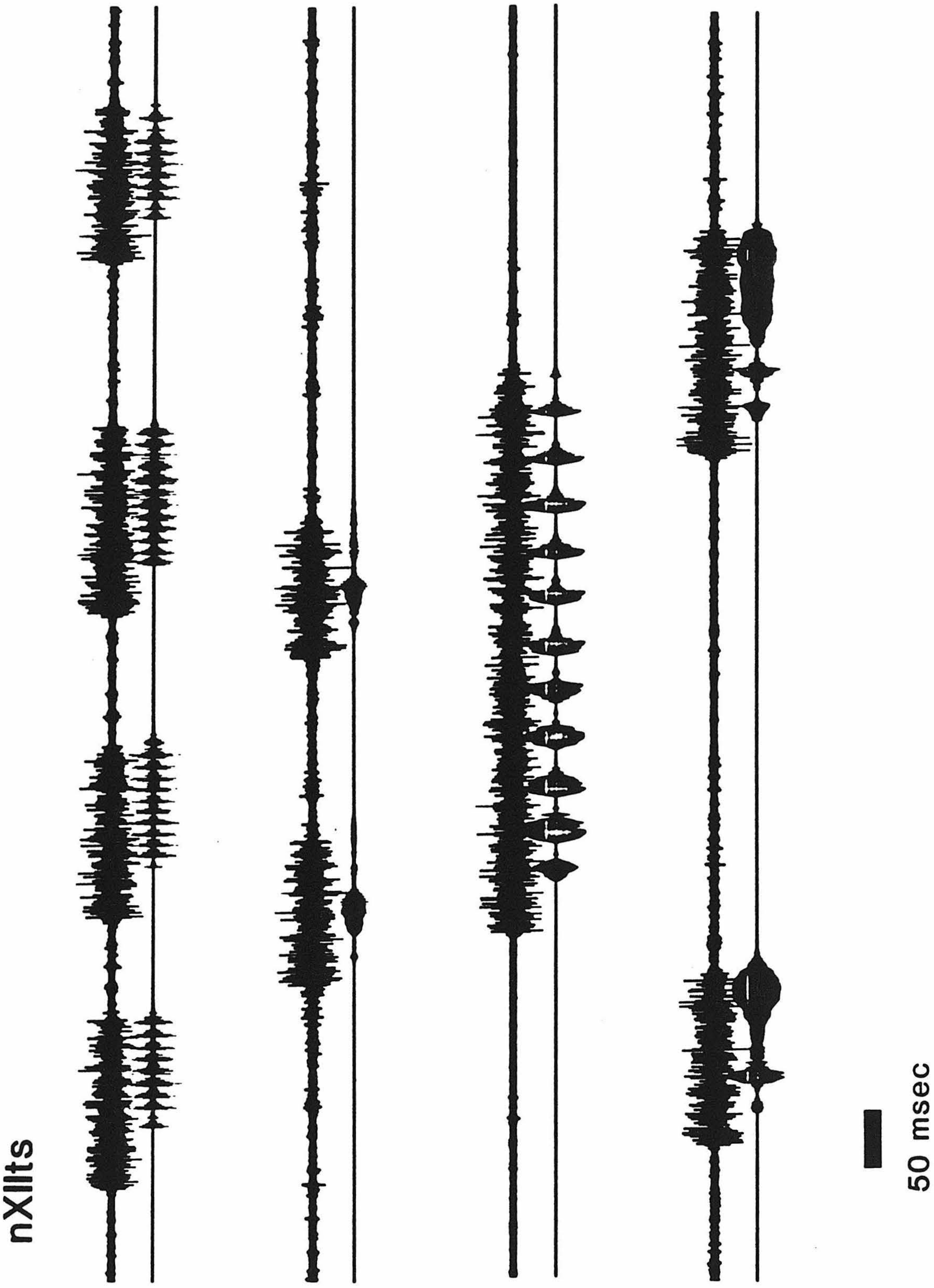

nXIIts
50 msec

Figure 15. Multiple-unit records of activity in MAN (Magnocellular nucleus of the Anterior Neostriatum) of the zebra finch during singing and non-singing periods. No detectable changes in activity accompanied song. Compared to recordings from Area X, those from MAN showed higher signal-to-noise ratios among the larger spikes.

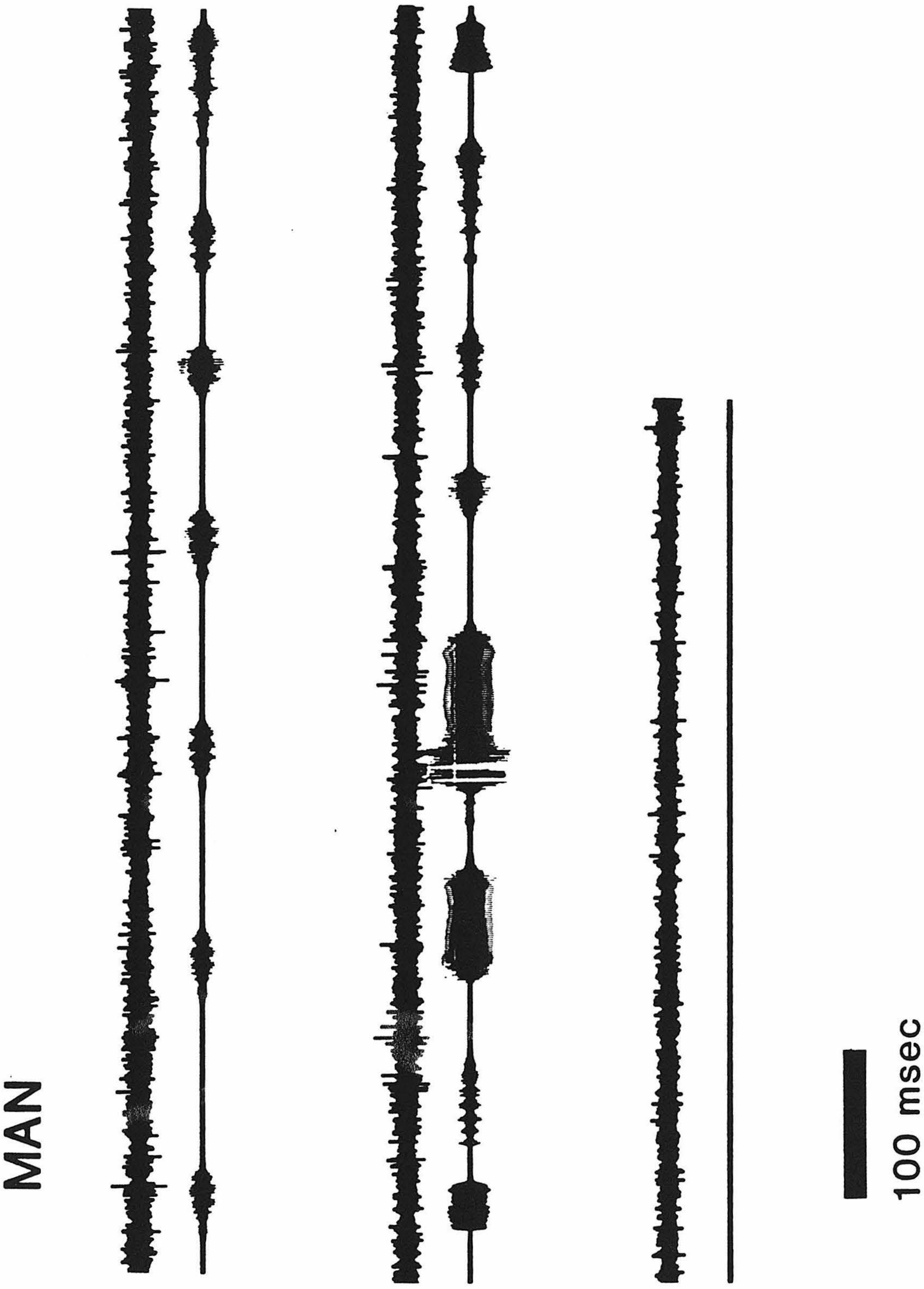

MAN
100 msec

Figure 16. Multiple-unit records of activity in area X of the zebra finch during singing and non-singing periods. The baseline rate of activity (bottom record) was maintained during song (top two records).

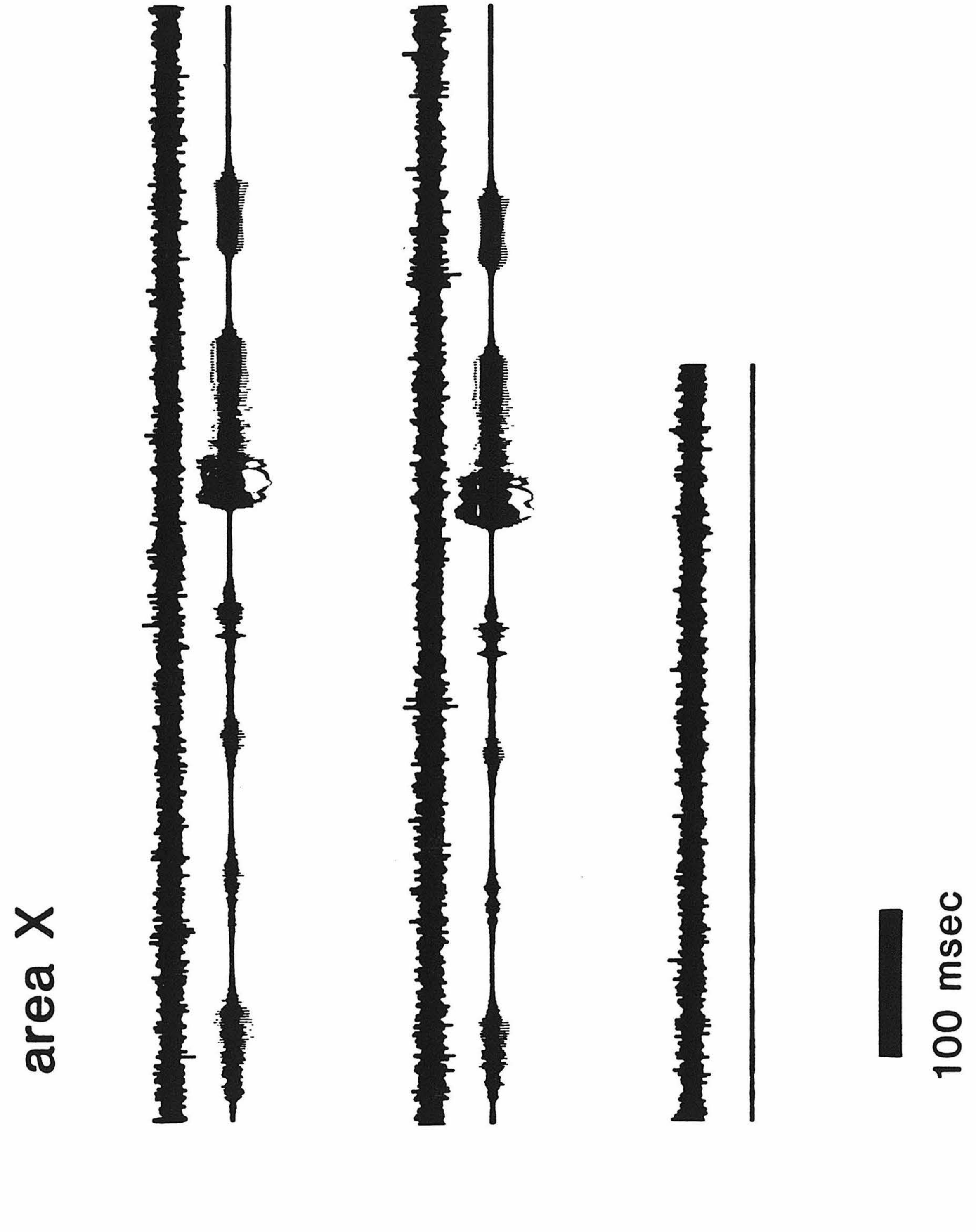

area X
100 msec

Figure 17. Multiple-unit records of activity in area X of a deafened canary taken during song. As in the normal zebra finch, there was no change in the baseline firing rate when the bird sang.

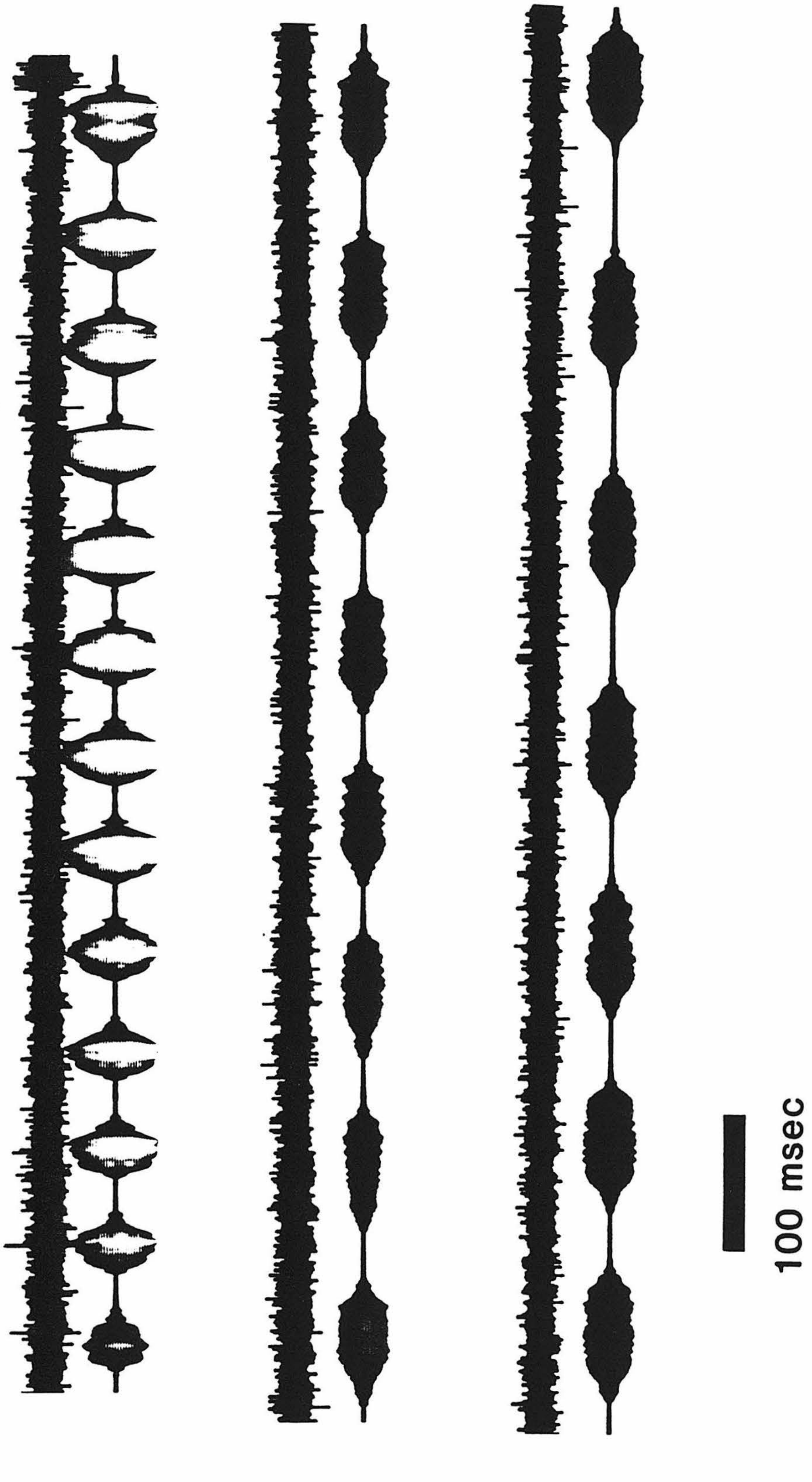
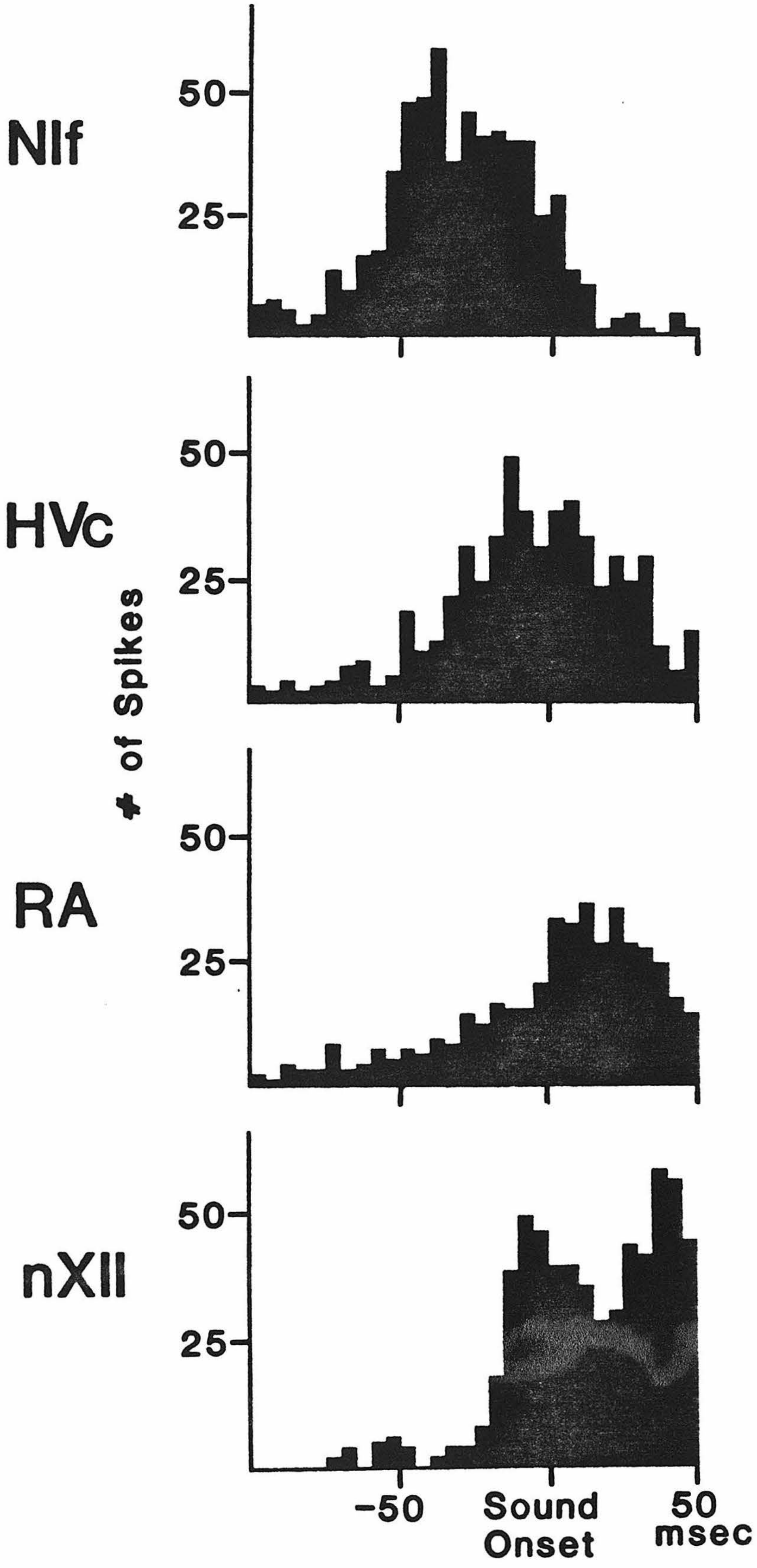

100 msec

Figure 18. Histograms showing the relationship between multiple-unit activities in NIf, HVc, RA, and nXIIts. Each histogram represents summed activity from 25 trials. The NIf histogram is the same as that in Fig. 2C (representing the zebra finch). HVc and RA histograms were obtained from the zebra finch recordings illustrated in Fig. 13, while the hypoglossal nerve data were taken from canary "aggression" trills somewhat similar in morphology to zebra finch vocalizations. There was a sequential activation of the four nuclei in advance of sound production, as expected from careful examination of the raw data records, and consistent with the unidirectional and serial connections between these nuclei.


NIf
HVc
RA
nXII
50
25
# of Spikes
-50
Sound Onset
50 msec

Chapter 3

A RE-EXAMINATION OF HEMISPHERIC DOMINANCE IN THE CONTROL OF BIRD SONG PRODUCTION

Introduction

The concept of hemispheric dominance in bird song control can be traced to the finding that section of the left hypoglossal motor nerve had much greater effects on song than corresponding section of the right nerve (Nottebohm, 1966, 1971). The dominance hypothesis was consistent with the acoustical analysis of Greenewalt, which demonstrated that many songbirds can produce notes containing two fundamental frequencies—the so-called two-voice phenomenon. Classical anatomical studies of the syrinx (Häcker, 1900; Rüppell, 1933; Setterwall, 1901) provided support for the view that separate sound sources in each syringeal half could function independently. The discovery of the vocal control system (Nottebohm, Stokes, and Leonard, 1976) led to the demonstration of asymmetric effects of unilateral brain lesion; these effects were interpreted as support for a central dominance hypothesis.

Having developed a technique for recording neuronal activity in the singing bird, I sought to examine directly the functional correlates of dominance in left and right HVc. Instead of physiological evidence for the dominance hypothesis, I found very similar patterns of activity in the two nuclei for any given vocalization. Bilateral recordings from the peripheral extreme of the vocal control system (the hypoglossal nerve) also showed very similar patterns of activity, suggesting that both hemispheres are similarly involved in the production of all song elements. This hypothesis was supported by results obtained from birds whose left or right bronchus was plugged, leaving only the contralateral syringeal half in operation. Under these conditions, subjects were able to produce clearly recognizable counterparts to almost all of their preoperative syllables.

Results

Hypoglossal nerve recordings. Any mechanism of neural lateralization of song control must be expressed in the activities of left and right hypoglossal nerves, the "final common path" to the muscles of the syrinx. Accordingly, I examined the temporal patterning of bilateral nerve activity in singing birds of two species—canaries, highly lateralized as judged by lesion results, and mockingbirds, in which the asymmetric effects of unilateral nerve section are somewhat less pronounced (unpublished observations). Qualitatively, the results were the same for all sets of recordings from either nerve in both species tested. As can be seen in Figs. 1 and 2, each syllable of song corresponds to a unique pattern of multi-unit activity in the nerve. The pattern for any given element was noticeably similar for different electrodes widely spaced within the same nerve, as well as for electrodes in the right and left nerves. As shown in Chapter 2, the song-related pattern of neural activity corresponds to the temporal pattern of song: a burst of neural activity precedes each syllable and an inactive period precedes each silent interval. I shall refer to this temporal correspondence between sound and neural activity as "time-locking." Just as the pattern of frequency and amplitude modulation within each syllable is unique, so is the pattern of neural burst for each syllable, so that a series of different neural bursts alternating with inactive periods of different duration constitutes the neural equivalent of the temporal pattern of song. In the highly "lateralized" canary, as well as in the mockingbird, the correspondence between bursts of neural activity and the patterning of song syllables was obvious in both right and left motor nerves.

To assess the relative contributions of right and left syringeal halves by classical criteria, I sectioned either the right or left nXIIts in two canaries and one mockingbird from which I also made nerve recordings. The behavioral results were consistent with earlier reports (Nottebohm, 1980). In both canaries and mockingbirds, left nerve section caused much more dramatic song deficits than right nerve section, and in the canaries some elements disappeared completely. However, these operative effects were uncorrelated with comparable indications of dominance in multi-unit discharge patterns. For example, multi-unit recordings from the portion of the nerve proximal to the cut end showed that the patterns of activity were closely matched in the two nerves for all song elements in Fig. 1B, including the whistle element which disappeared as a result of right nerve section.

HVc recordings. The general characteristics of HVc recordings were described in Chapter 1. Briefly, all recordings obtained within HVc showed increased neuronal activity time-locked with song syllables, whether those recordings were obtained from the right or left hemisphere. In general, multi-unit recordings from right and left HVc of the same bird showed, despite some variability, a striking correspondence in onset time and duration of increased neuronal activity for any given vocalization.

In many canary HVc recordings, the patterns of activity resembled those from the hypoglossal nerves in that each syllable of song was uniquely associated with a particular pattern. In other recordings from different electrode sites, no obvious modulations of pattern could be discerned; this contrast of pattern clarity in different recordings suggests some form of functional topography within HVc. However, the presence of obvious patterning was just as likely in right HVc recordings as in those from the left. Furthermore, in cases where obvious patterns could be detected in right and left HVc of the same bird, those patterns appeared to be indistinguishable (Fig. 3). This finding was obtained in the canary, a highly-lateralized species as judged by nerve-section results, as well as in the zebra finch which is not highly lateralized by the same criteria.

Song of bronchus-plugged birds. As an independent means of assessing unilateral syringeal function in the canary, I obstructed airflow through the right (one bird) or left (four birds) bronchus (Fig. 4) after recording the normal songs of the subjects, so that subsequent songs could be produced only by the contralateral syringeal half. In all cases, the results were qualitatively similar (Fig. 5). Following a postoperative recovery period ranging from four days to four weeks, all subjects produced easily recognized facsimiles of a large majority of pre-song syllables (22 of 26 syllables for the left-blocked bird shown in Fig. 5A; 22 of 27 for the right-blocked subject in Fig. 5B). Songs produced by the right-blocked bird, singing with only the left syringeal half, sounded quite normal to the ear except for their reduced amplitude, and close examination of sonagrams revealed only minor distortions in many syllables. By contrast, songs produced by left-blocked birds consisted of noisy and more distorted syllables, even lower in amplitude than those produced by the right-blocked bird. Nevertheless, these syllables could be easily matched with preoperative syllables by noting their temporal patterning and position within the song (Guttinger, 1979, 1981). The overall repertoire size in all cases remained essentially constant, with some variability in this measure being introduced by rarely-voiced syllables which may have been missed in one sample or the other. Songs produced exclusively by the left or right syrinx were of normal length with syllables spaced at normal intervals. Significantly, these birds were able to produce syllables showing evidence of simultaneous contributions of two non-harmonically related sounds (see G[G] and I[I] in Fig. 5A).

Discussion

Hemispheric dominance. The simplest neural correlate of hemispheric lateralization would be the presence of activity during vocalization in the dominant side and absence of activity in the other side; this may be called the "all-or-nothing" basis of dominance. In multi-unit recordings, whether obtained from right or left HVc or hypoglossal nerve, I never saw any instance of absence of activity during vocalization. I did see a close correspondence in premotor onset times and durations of song-related activity, and particularly in the nerve recordings I saw an obvious similarity in the temporal patterns of right and left discharge for any given vocalization. To the extent that pattern features revealed encoded commands for production of song syllables, it appeared from my data that both right and left nerves were transmitting the same messages to their respective syringeal halves. These results are incompatible with any all-or-nothing mechanism of lateralization in the song control system. However, they are consistent with the absence of neuroanatomical correlates of hemispheric asymmetry in the canary (DeVoogd and Nottebohm, 1981; Nottebohm, Kasparian and Pandazis, 1981).

A subtle form of neural asymmetry may involve different numbers of neurons performing similar roles in the neuromuscular control of the syrinx. In practice, this possibility can never be eliminated. Indeed, single unit recordings from HVc of a singing mockingbird revealed units possessing different patterns of discharge with reference to the temporal pattern of song (Chapter 2); some neurons fired prior to all vocalizations, while others fired only for specific song syllables. The discharge patterns of these specialized neurons would have been completely masked in multi-unit recordings from HVc. In addition, the hypoglossal nerve contains many neurons that discharge in phase with the respiratory rhythm (Manogue and Paton, 1982; McCasland and Konishi, unpublished observations). Although they are often recognizable during electrode implantation in either left or right hypoglossus, their activity soon disappears from my recordings. This indicates that multi-unit recordings may fail to demonstrate some of the functional properties of the whole nerve. Nevertheless, the absence of hemispheric asymmetry in the neuronal recordings led me to predict that both syringeal halves were normally involved in the production of all song syllables. The bronchus-plugging experiments served as a direct test of this prediction.

Direct assessment of unilateral syringeal function. From anatomical and acoustical considerations alone, we can sharply define the logical alternatives for lateralized sound production by the syrinx. The left-right pairing of the putative sound source in the syrinx, the internal tympaniform membranes, reflects the general bilateral symmetry of this organ. Thus we can be quite confident that the syringeal oscillator is a paired structure. Given the simple fundamental-plus-harmonics structure of the vast majority of normal canary song elements, there are only three possible relationships, at any given moment, between the two sound sources in the normal syrinx. (1) One source can be oscillating while the other is silent. (2) Both sources can oscillate to produce exactly the same sound. (3) Both sources can produce the same fundamental and harmonics but at different amplitudes. Case 1 represents qualitative dominance of one side over the other and the interpretation most easily and most often applied to Nottebohm's nerve-section results. The critical feature of this qualitative dominance mechanism is the independent contribution of different syllables or voices by right and left oscillators in the syrinx and therefore by right and left hemispheres of the brain. The theory implicitly requires that any one-voice element be contributed by one syringeal half or the other. Given this requirement, it is significant that a common result of unilateral denervation experiments is the production of anomalous sounds—noisy or poorly modulated syllables not present in the preoperative song. The qualitative dominance theory can provide only one explanation for the origin of these sounds: they must be produced by the denervated side as a consequence of the operation, presumably because of changes in the airflow pattern during song. I tested this explanation directly by eliminating the possibility of bilateral contributions to song. By blocking the left bronchus of highly "lateralized" canaries, I ensured that any vocalizations would be contributed only by the "subordinate" right side. This experiment shows what unilateral denervation cannot: that the right syrinx of the canary, working alone, produces a train of syllables the timing of which is identical to normal song, and the majority of which bear a striking, element-by-element resemblance to those in the preoperative song. All of the qualitative features of song syllables—duration, interval, frequency modulation, complex elements, two voices—can therefore be produced independently by either half of the syrinx. Unless this postoperative fidelity of song characteristics occurs only because the left bronchus is blocked, i.e. as an epiphenomenon of the surgery, it must reflect the role normally played in song production by the right side. We may conclude that both hemispheres are normally involved in the production of all qualitative features of song. This conclusion reinforces the suggestion from nerve and HVc recordings that both hemispheres contribute similar sets of commands to their respective syringeal halves. It is worth noting that even an "epiphenomenon" explanation of the bronchus-plug results cannot account for the reproduction of two-voice syllables by one syringeal half (see G[G] and I[I] in Fig. 5a). Taken alone, this result indicates that the Greenewalt theory of two-voice production by the syrinx is incorrect and suggests that a single internal tympaniform membrane in the syrinx can produce two unrelated sounds.

A comparison of the bronchus-plug and nerve-section results is illuminating in several respects. The goal of both experiments is to assess unilateral syringeal function. The fact that in both cases the left syrinx produces a larger number of completely normal syllables than the right strongly suggests that a functional asymmetry does exist somewhere in the system. However, the bronchus-plug result makes it clear that this asymmetry does not speak directly to the normal sound production role of right hemisphere song control nuclei. The reason for this is that the noises and other abnormalities in syllables produced by the bronchus-plugged bird are logically a product of the experimental conditions; otherwise these features would be evident in the preoperative song.

What is the basis for the higher distortion levels observed in songs produced only by the right side? I suggest two possibilities, neither of which is consistent with lateralization theory in its present form. First, it is possible that subtle differences in centrally generated motor patterns might result in a tendency for the right side to produce noisy syllables; this tendency would necessarily be corrected by the left side in the normal bird. But the consequence of this model is that the two sides must work together in shaping all song syllables—no syllable, or even a single voice of a two-voice element, can be solely contributed by either the left or right hemisphere. Alternatively, the asymmetry in operative effects may be viewed simply as a reflection of a peripheral asymmetry in syringeal anatomy (Nottebohm and Nottebohm, 1976), implying nothing about the central generation of song commands.

Nottebohm's nerve-section birds did not show recovery of preoperative syllables over the same time course for which my bronchus-plugged birds were recorded (Nottebohm and Nottebohm, 1976). In another study, birds whose left hypoglossi were sectioned at one year of age and who were recorded a year later showed recovery of very few preoperative elements, even in modified form (Nottebohm, Manning, and Nottebohm, 1979). Thus, it is unlikely that the left-plugged birds were actively modifying the motor program for song so as to produce more normal syllables with the subordinate right side.

An alternative interpretation of the nerve-section results. Suthers and Hector (1982) showed that both tracheal pressure and rate of tracheal airflow increase dramatically at the onset of click production in the swiftlet, an echolocating bird which lacks intrinsic syringeal musculature. Gaunt et al. (1973) showed in songbirds as well as in other species that tracheal pressure is typically well below bronchial pressure during vocalization, implying vocalization-related syringeal resistance to airflow. Bilateral denervation of the syrinx of canaries and other species renders the bird virtually aphonic, i.e. unable to engage the syrinx in sound production. If we assume that this failure is due to the loss of syringeal resistance, then it is almost certain that unilateral denervation would affect this resistance as well. It follows that any left-right asymmetry in airflow through the denervated side would be reflected in differential effects of left or right nerve section on song control. However, the overall timing aspects of song—intersyllable interval, ordering of syllables, and duration of song—should be unaffected. This result is invariably obtained in both nerve-section and bronchus-plug experiments.

Available results are consistent with the view that both right and left syringeal halves normally contribute the same sound components, but at different power levels. If this view is correct, the "elimination" of certain song elements following unilateral denervation may be explained as a threshold phenomenon, wherein a partial loss of power has a differential effect on the production of "high-power" and "low-power" syllables. Because the left side contributes more power, section of the left nerve should affect many syllables, including both high- and moderate-power cases. By contrast, after denervation of the relatively low-power right side, only the high-power syllables should disappear. This explanation generates an interesting prediction which for technical reasons is as yet untested. If, as in Fig. 4B, a syllable disappears following right nerve section, then we can predict that the same syllable would disappear if the left nerve were cut instead—a result which would be completely incompatible with the qualitative dominance hypothesis.

Reversal of hypoglossal dominance: a re-interpretation. Nottebohm et al. performed a series of unilateral denervation studies to examine developmental aspects of hypoglossal dominance (Nottebohm, Manning and Nottebohm, 1979). When the left hypoglossal nerve was sectioned during the first two weeks after hatching, the ensuing song was found to be under sole right hypoglossal control, as judged from further nerve-section experiments performed in adulthood. The same experiment done in the third or fourth week resulted in song developed under "shared" right and left hypoglossal control. In both cases, the quality of song approached that of intact birds of the same age. Later hypoglossal section, performed when song development was well under way, had more drastic effects on adult song. From these observations, it was concluded that: 1) hypoglossal dominance is not a necessary condition for the production of normal canary song; 2) soon after hatching either hypoglossus is equipotential for dominance in song development; 3) the right hypoglossus loses its ability to produce normal song as development proceeds under left hypoglossal control. Reversal of dominance was therefore presented as a model system for the study of neuronal changes related to the learning of new motor tasks.

In all cases in which the syringeal muscles were examined following reversal of dominance, there was a clearly visible atrophy of the muscles on the denervated side, constituting a reversal of the normal periperal asymmetry. Comparable measurements of the volumes of left and right song control nuclei following denervation were not made. However, it is known that in the normal canary there are no systematic laterality differences in the volumes or other cytoarchitectonic features of these nuclei (DeVoogd and Nottebohm, 1981; Nottebohm, Kasparian, and Pandazis, 1981). These observations and the present results lead to an alternative interpretation of dominance reversal. All of the reversal data can be explained as easily by peripheral as by central mechanisms. One can think of these experiments as a rough assay of the right-left distribution of muscle mass in the syrinx. Presumably this distribution in the adult is differentially affected by hypoglossal nerve section done at various developmental stages, as one would expect by analogy with other developing neuromuscular systems (Landmesser, 1980.)

Other considerations. Because the effect of lesioning left or right HVc can only be measured by assessing deficits in vocal output, a functional dominance at any level in the vocal control system would be invoked in any such measurement. This implies that lesion of HVc cannot be used to pinpoint the site of functional dominance. Similarly, the destruction of the left hypoglossal nerve does not allow one to distinguish between lateralization of activity in the two nerves, and lateralization of the strengths of muscular contractions (which may be identically timed and patterned in the normal case) in the syrinx.

In the species of songbirds which have thus far been examined, the degree of lateralization observed is qualitatively correlated with the degree of asymmetry in the syringeal musculature, and is not correlated with volumetric differences in song control nuclei (Nottebohm, 1980). Hypoglossal dominance is exhibited by canaries which had no access to auditory feedback (Nottebohm and Nottebohm, 1976), necessary for normal song development (Konishi, 1965). The degree of dominance is unaffected by unilateral cochlear removal before song development (Nottebohm and Nottebohm, 1976); thus there is no evidence of asymmetry in the underlying processing of auditory inputs. Vocal repertoire size is essentially normal in canaries which have had early left hypoglossal section and have developed song under right hypoglossal control. My observations on several species of songbirds suggest that the extent of dominance is uncorrelated with the extent of song learning, size of vocal repertoire, duration of critical period, or extent of auditory experience.

Concluding remarks. The following hypothesis accounts for all of my results. Individual song syllables are produced by the combined actions of the right and left vocal control systems. Although each syringeal half can work in the absence of the other half, the two sides are normally coordinated to produce qualitatively the same sounds. The left-right asymmetry in the effects of bronchus plugging can be accounted for by assuming that the left bronchus transmits a greater driving force to the membrane than the right one, resulting in oscillations (sounds) of greater amplitude in the left membrane than in the right. Thus, in my view, the lateralization of song control is quantitative rather than qualitative (contrasting this system with human speech control in which fundamental qualitative differences are present between the two brain hemispheres). I conclude that it is premature, if not incorrect, to regard birdsong as a model system for the study of neural lateralization.

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Figure 1A. Relationship between activities in the left and right hypoglossal nerves of a singing canary. (Upper) Multi-unit activity recorded from the left hypoglossal motor nerve during production of fast trills by a canary. (Middle) Simultaneously recorded multi-unit activity from the right hypoglossal nerve of the same canary. (Lower) Oscillograph of sounds produced by the bird. The patterns of activity were very similar in the two nerves, for this and other song syllables. Bar = 100 msec.

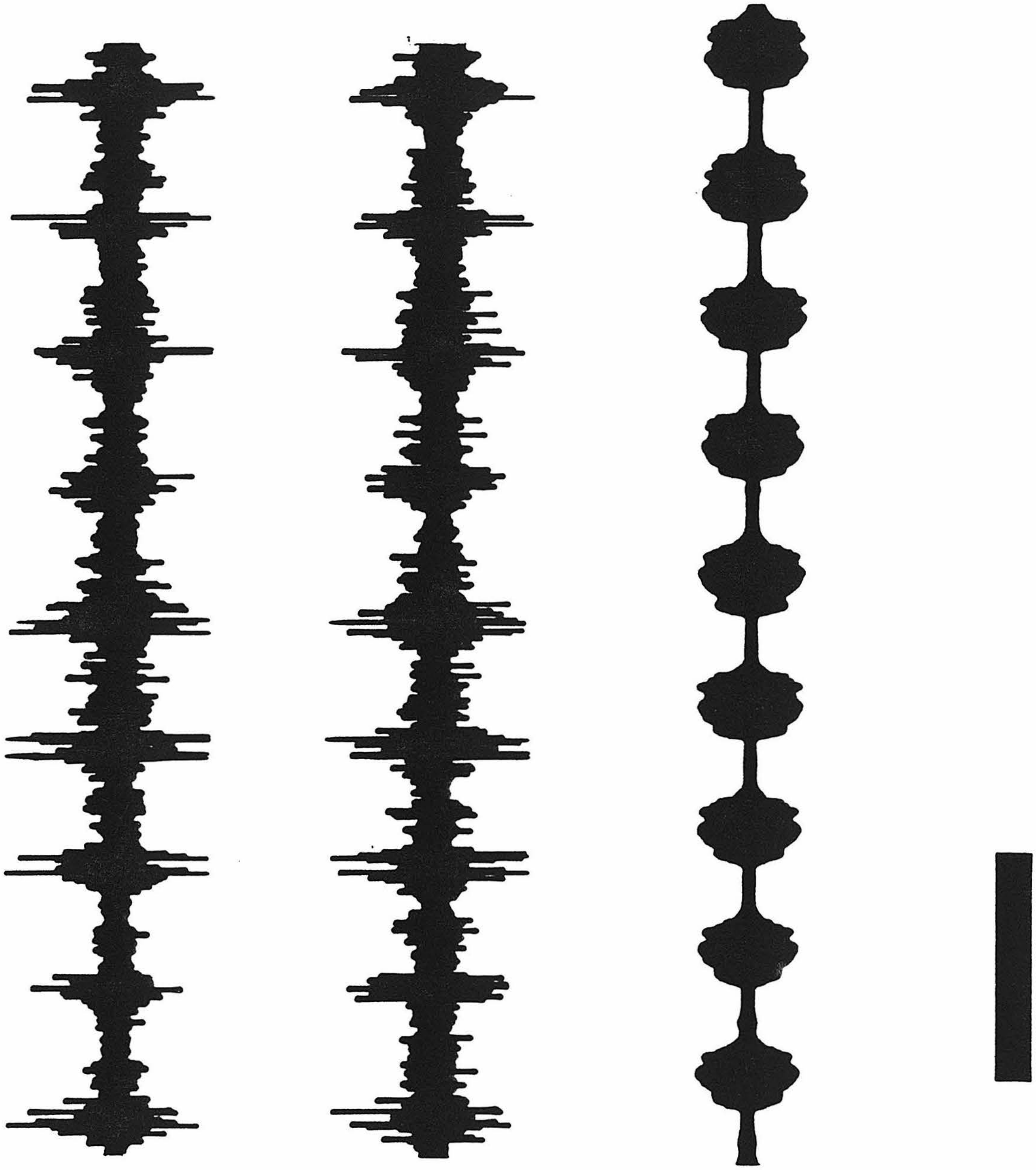


Figure 1B. Lack of correlation between bilateral nerve activity and the effect of nerve section on song. Simultaneous bilateral nerve recording from a singing canary (as in Fig. 2A) showing similar patterns in the two nerves for all elements (see Fig. 2C for the effect of nerve section on this song segment). Bar = 100 msec.

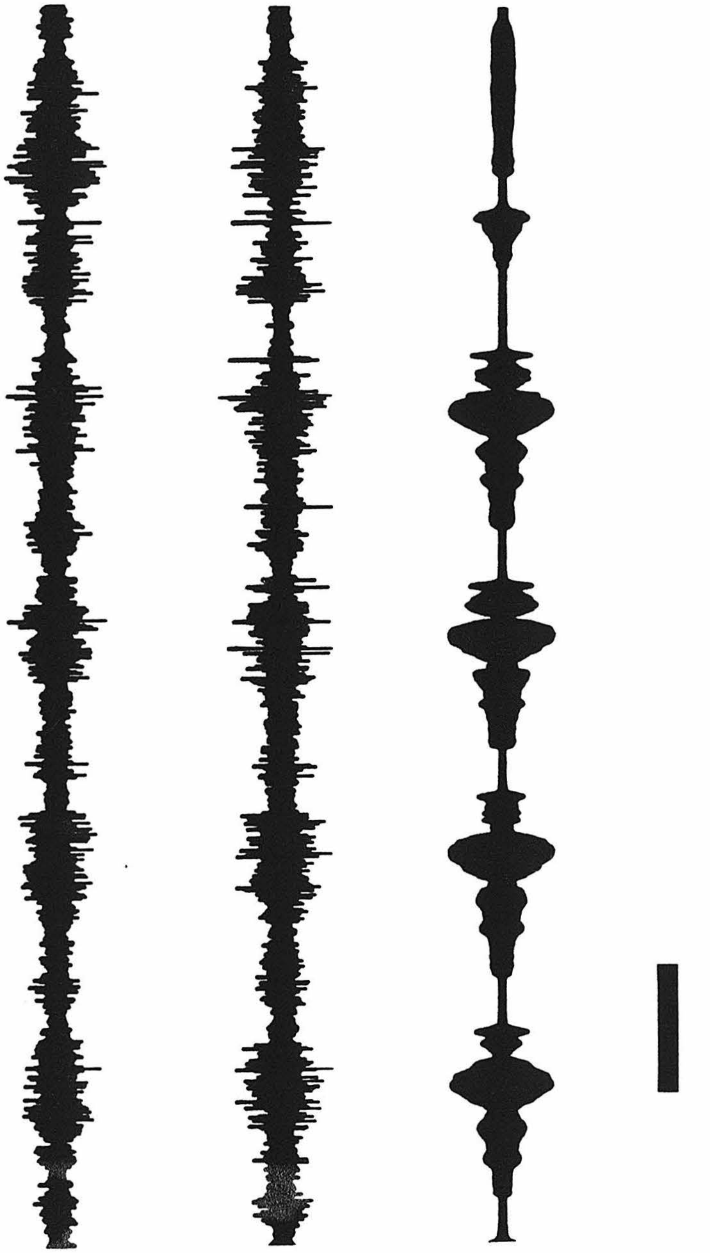
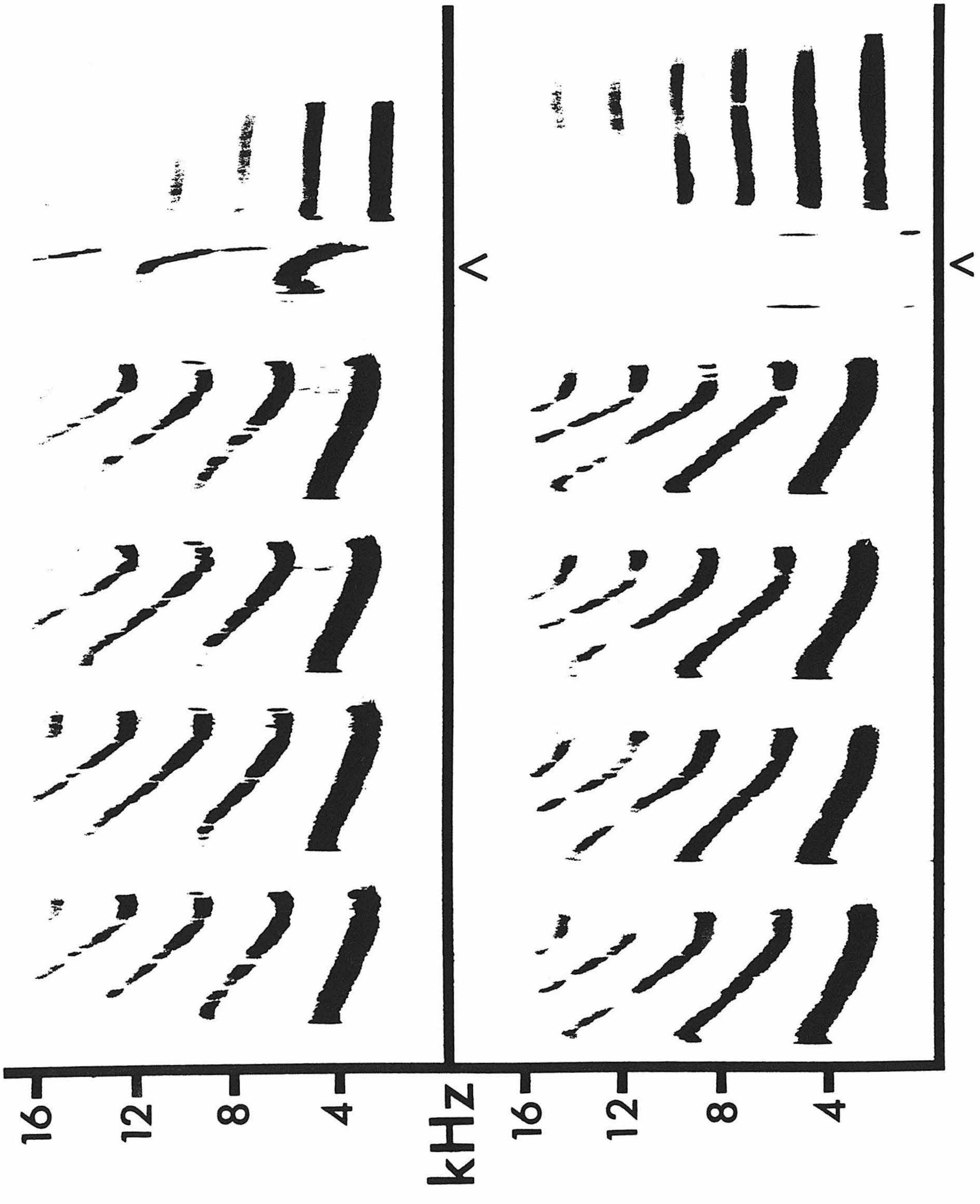


Figure 1C. Sonagraphs of the same song segment shown in Fig. 2B before and after section of the right hypoglossal nerve, showing a resultant disappearance of the indicated element. By the hemispheric dominance hypothesis, this element must have been produced under right-side control, but nerve recordings showed similar activities for this sound in right and left nerves before and after nerve section.


16
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8
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kHz
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12
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4

Figure 2. Relationship between activities in the left and right hypoglossal nerves of a singing mockingbird. (Upper traces) Left hypoglossal nerve activity for a variety of mockingbird song syllables (lower traces). Simultaneously recorded right hypoglossal nerve activity is represented in the middle traces. Each syllable was associated with a unique temporal pattern of discharge, most easily characterized by the onset time and duration of increased activity. Both nerves showed obviously similar patterns of activity for any given syllable.

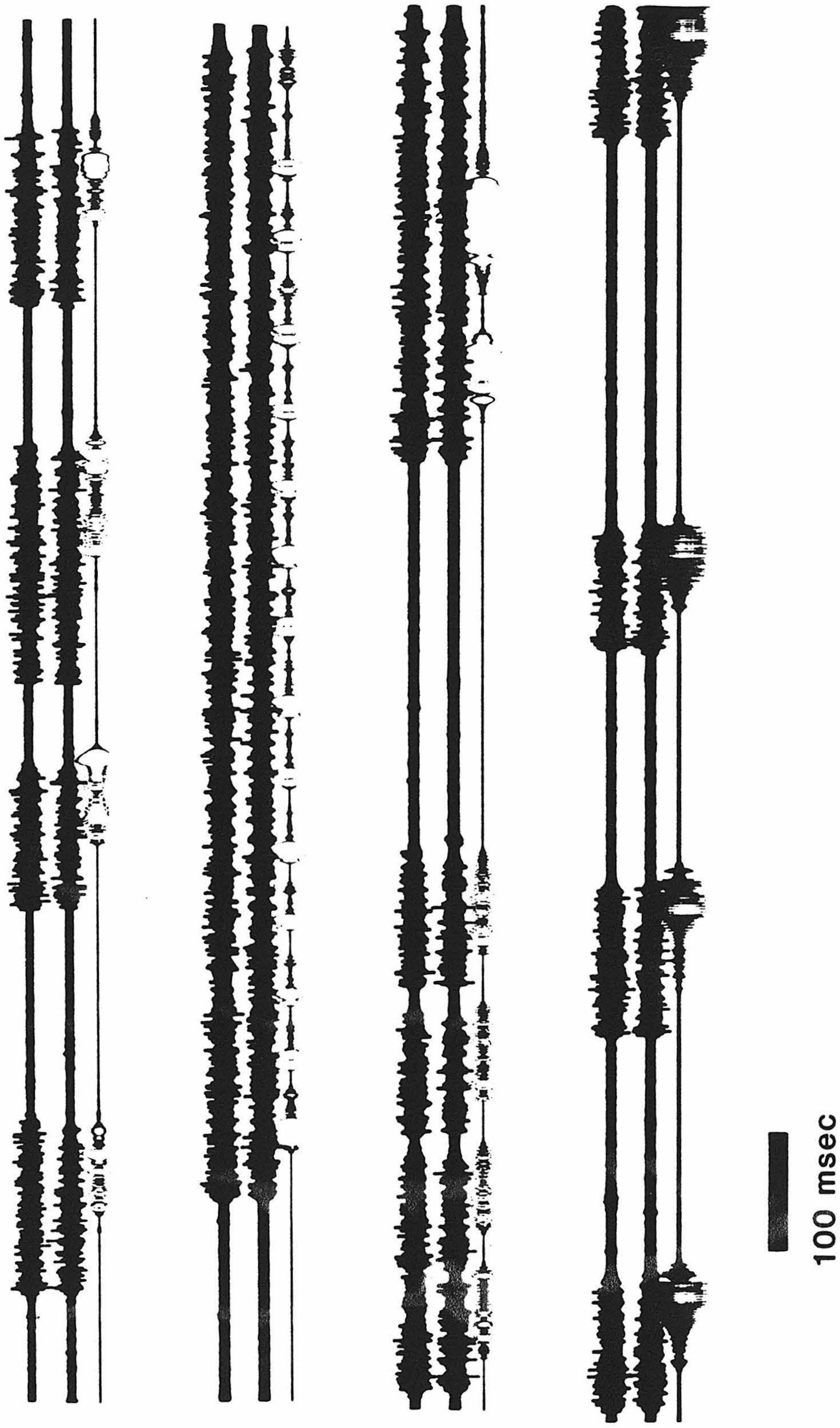
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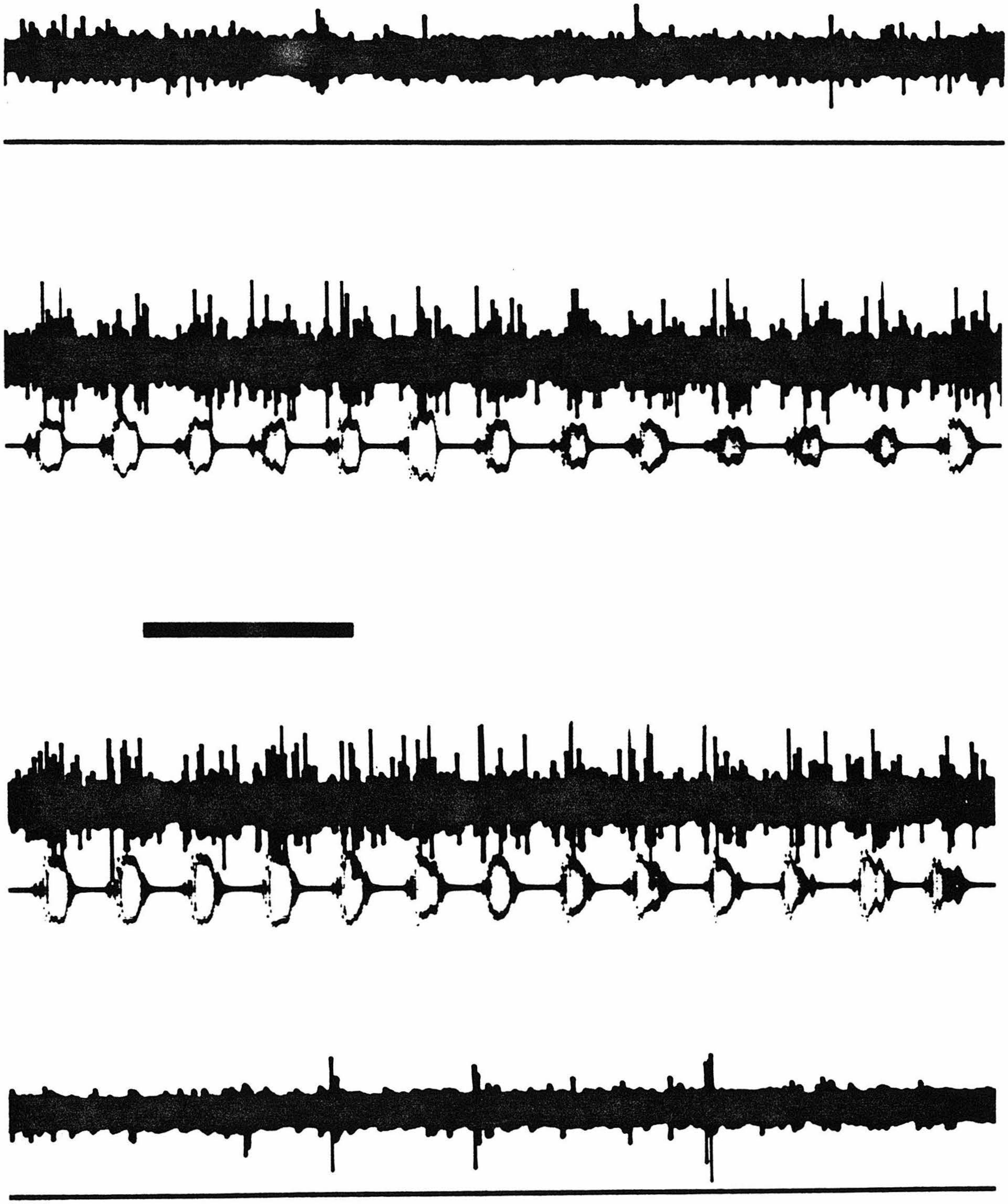
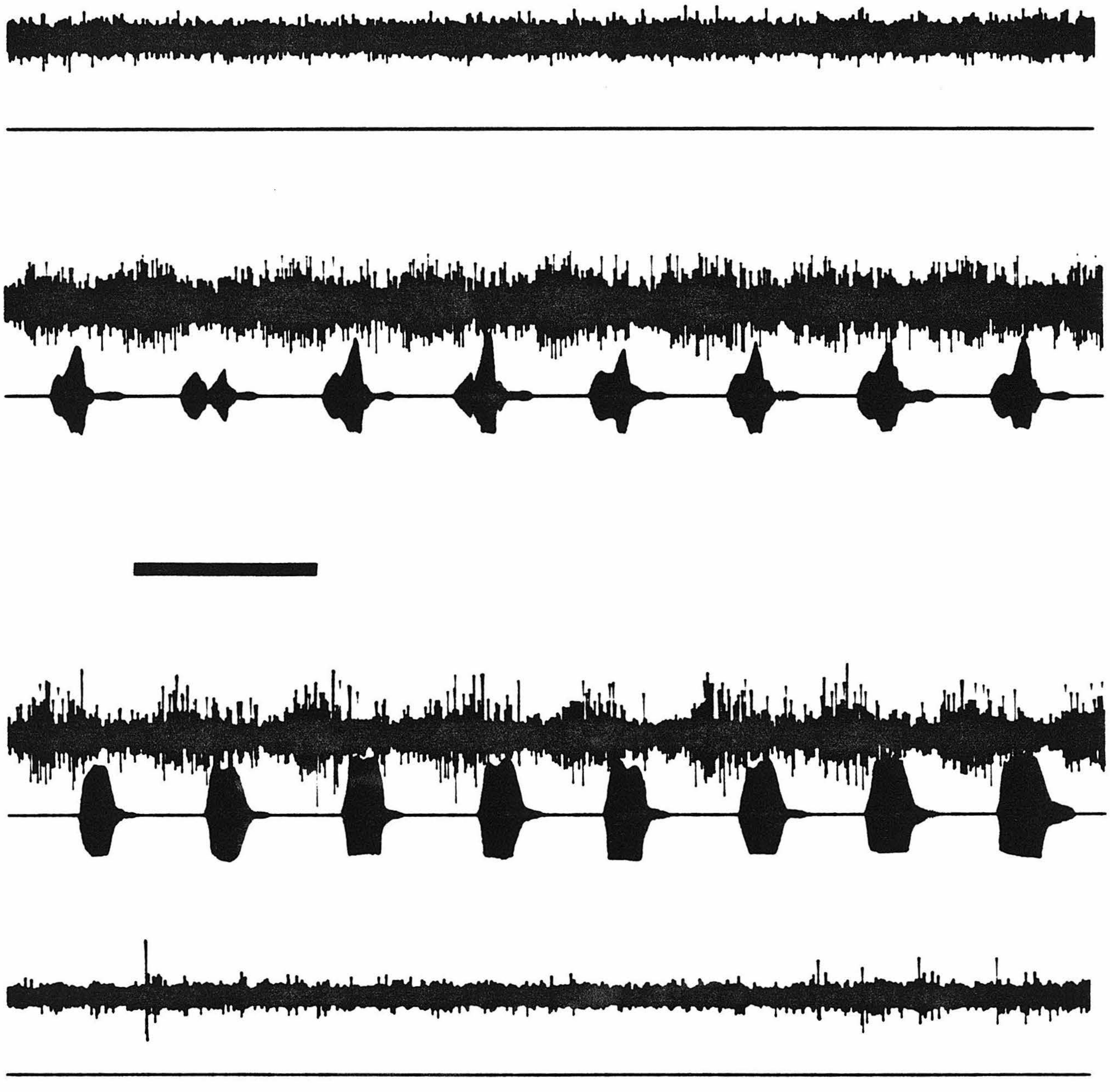
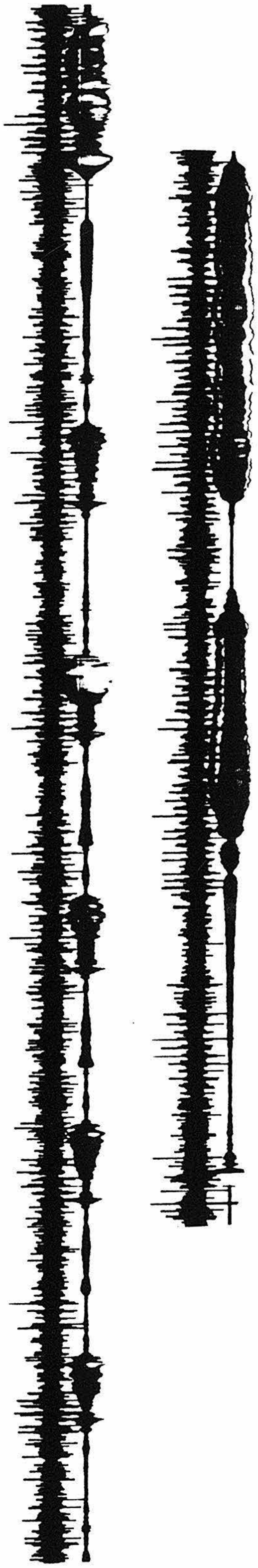
Figure 3. Temporal relationship between neural activity in right and left HVc of the same bird. Each of the three photographs represents a different subject. Upper records: song produced by a normal Wasserschläger canary, together with right HVc activity. Lower: same song elements produced by the same canary, together with left HVc activity. For each song syllable, there was a recognizable pattern of activity which appeared to be very similar whether recorded from left or right HVc. Bar = 100 msec.







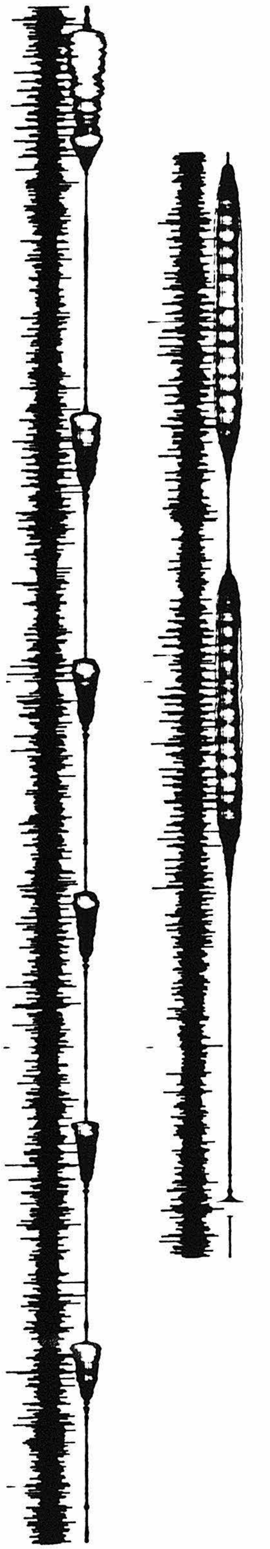
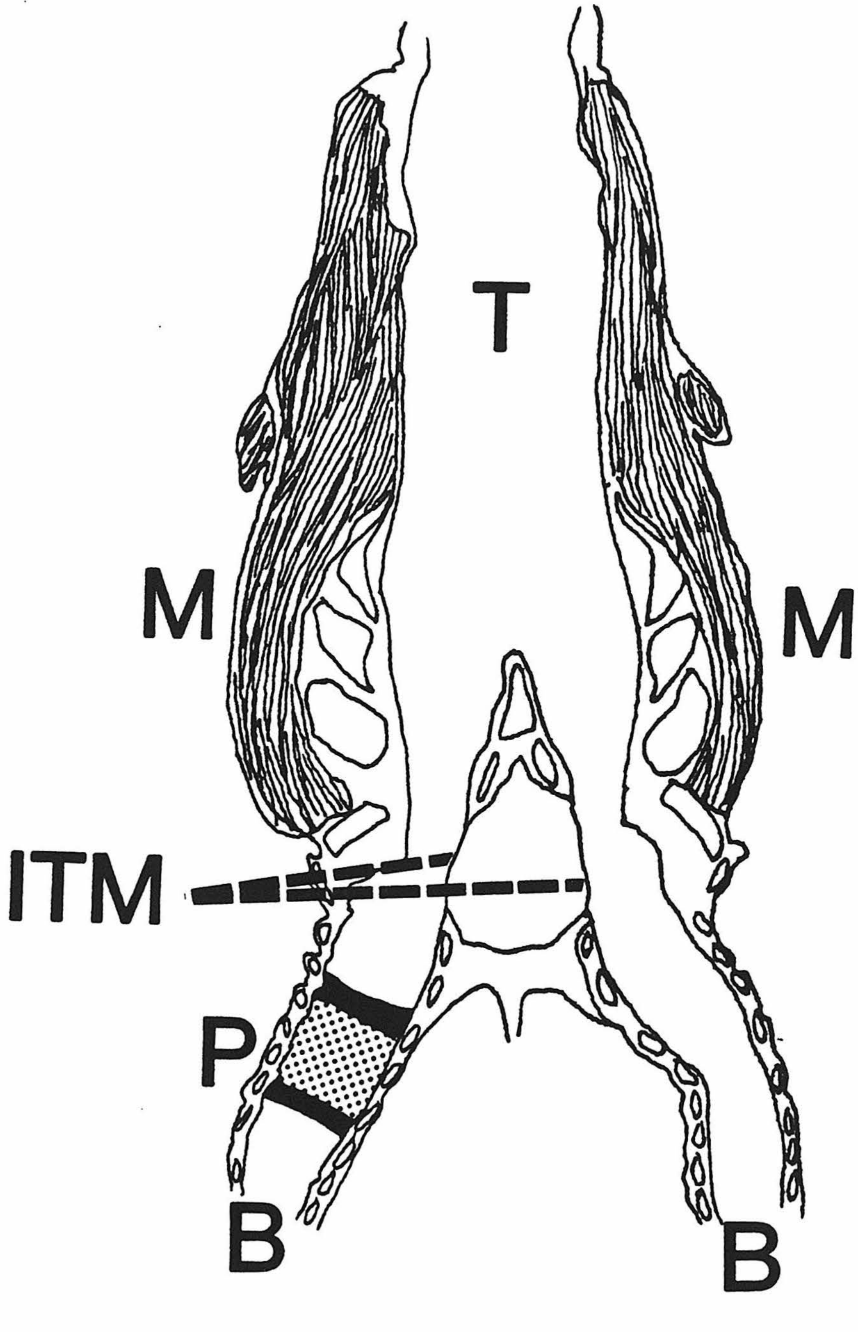


Figure 4. Diagrammatic view of the canary syrinx in frontal section, with plug (P) inserted in left bronchus (B). Note the positioning of the plug below the internal tympaniform membrane (ITM). The left-right asymmetry in the intrinsic musculature (M) is exaggerated for emphasis. T, lumen of trachea. (After Nottebohm and Nottebohm, 1976).


T
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ITM
P
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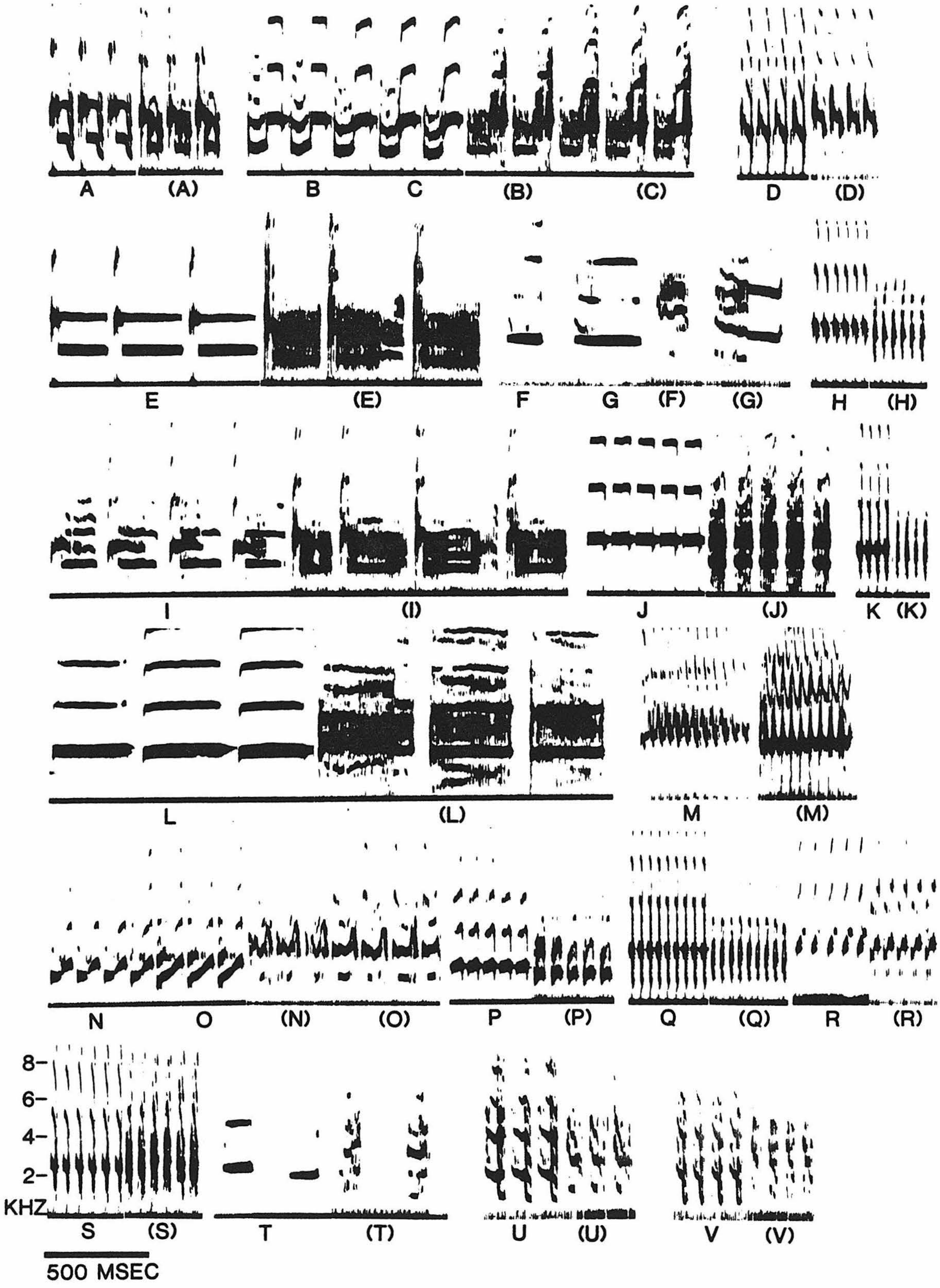
B

Figure 5A. Comparision of song syllables produced by Wasserschlager canaries before and after unilateral bronchus plug. Song syllables produced by a canary whose left bronchus was plugged, leaving only the right syringeal half in operation. Preoperative syllables are indicated by capital letters; postoperative counterparts are shown by the same letters enclosed in parentheses. In the majority of cases there was an obvious correspondence between preoperative and postoperative syllables in both temporal and spectral features; in many cases these matches could be confirmed by the frequency of occurrence in a syllable in song and by the relative position within the song. In at least two cases (G and I), the simultaneous production of two fundamentals or voices in postoperative syllables was reminiscent of two-voice production in the preoperative syllables; this phenomenon was clearly incompatible with the original two-voice theory of Greenewalt.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8-
6-
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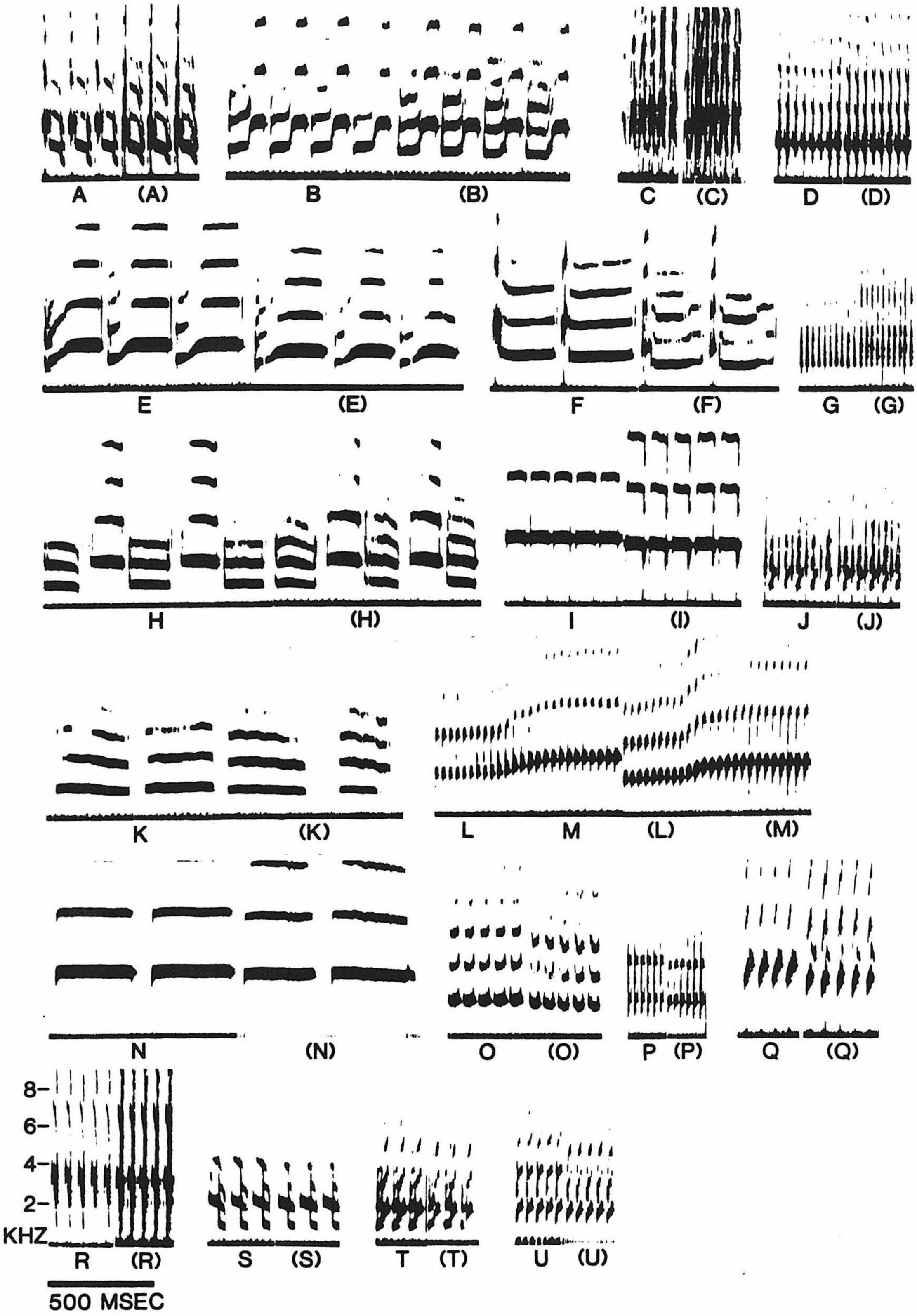
2-
KHZ
S
(S)
T
(T)
U
(U)
V
(V)
500 MSEC

Figure 5B. Song syllables produced by a canary whose right bronchus was plugged, leaving the left syringeal half in operation. Nomenclature is the same as in (A). Again, there was an obvious correspondence between most preoperative and postoperative syllables, and in this case the distortions produced by the plugging operation were noticeably less severe than those associated with the left bronchus plug.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500 MSEC

Chapter 4

INTERACTION BETWEEN MOTOR AND AUDITORY ACTIVITIES IN AN AVIAN SONG CONTROL NUCLEUS

Introduction

The discovery of a set of discrete brain nuclei involved in the control of vocalization in songbirds (Nottebohm et al., 1976) 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 following damage to either of the forebrain nuclei HVc (Hyperstriatum ventrale, pars caudale) or RA (Nucleus robustus archistriatialis). Behavioral studies (Thorpe, 1958; Marler, 1970; Marler and Tamura, 1964) 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 which 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 (Konishi, 1965), suggesting that there should be a regulatory connection between the auditory and the vocal motor control systems.

Katz and Gurney (1981) 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 (Kelley and Nottebohm, 1979). While the significance of auditory responses in HVc is 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. The development of single-unit and multiple-unit techniques for recording neuronal activity in freely moving, vocalizing birds enabled me to directly examine the roles of auditory and motor activity in song control nuclei. Results presented here show that the song-correlated pattern of neural activity persists even in the deaf bird. Furthermore, they demonstrate two types of auditory-motor interactions in the normal singing bird: motor inhibition of excitatory auditory responses, and auditory inhibition of spontaneous activity in some cells which show specific motor or premotor activity.

Results

Representative song samples and correlated neuronal activity from HVc of the white-crowned sparrow are shown in Fig. 1. As discussed in Chapters 2 and 3, all recordings obtained from within the HVc boundary, in all 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 correlated with song.

To show that the activity patterns observed during singing were not simply due to auditory reponses to song elements, I studied neuronal activity in HVc of two zebra finches before and after deafening by bilateral removal of the cochlea (Konishi, 1963) (Fig. 2). I found that deafening the adult zebra finch did not obviously 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, my 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 (see Chapter 2), then we may infer that a learned central motor program for song production is manifested in HVc.

In an effort to assess the functional significance of auditory inputs to HVc, I 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. While a detailed investigation of the selectivity of auditory responses in HVc is beyond the scope of this report, I was able to draw several conclusions from my 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, I showed that responses were not limited to the sound patterns of the bird's own song. In the case of the Wasserschläger canary, there were clear responses to some elements of the song of another non-sibling Wasserschläger individual. These responses were much more pronounced than those to song elements of a canary of a non-Wasserschläger 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, my 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 non-singing periods. Both sensory and motor activity patterns are "segregated" in time, in that a period of brisk activity is followed by a period of relative inactivity (see Chapters 2, 3, Figs. 4 and 5). Careful comparisons of the time courses of these two patterns of activity, in both the white-crowned sparrow and canary, showed clearly that many playback-correlated spikes occurred in time segments during which no comparable spikes were seen in the motor records (Figs. 4A, 5A). 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 (Figs. 4B, 5B). Thus there is manifested in HVc of the singing bird an interaction between motor and auditory activity, in that the expected auditory response is absent when the bird sings.

In order to investigate the nature of this motor-auditory interaction more fully, I performed an interference experiment consisting of presentation of various sound stimuli while the bird is singing, and during the period after song termination. Interference may be defined as a change in song-correlated activity which is attributable to the auditory stimulus. I 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; that is, the pattern of activity should be the same as that seen in the normal case, without playback.

The results of this experiment, performed in a canary, revealed an interesting phenomenon. So far as I 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, following a normal song of approximately 10-20 sec, no recognizable auditory resposnes could be elicited to any sound tested, even to song elements which produced very strong responses at other times. This general inhibition of responses decayed slowly, over a period of seconds (Fig. 6). 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 following short songs of 2-6 sec.

In an effort to determine the site of auditory inhibition, I made multi-unit recordings from Field L, the avian homologue of auditory cortex, during song playback and song production by a canary. Playback of this bird's own song elicited auditory responses which were similar to those seen in HVc in their segregated patterning (unpublished observations). These responses could also be seen, with similar time course, when the bird sang.

I also observed a tendency, on many occasions, for the canary to terminate his song abruptly as the playback trial was initiated. In twenty 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 twenty normal songs (17.0 sec) uninterrupted by playback. This observation strongly suggests that a singing bird is able to respond behaviorally to sounds, even though auditory activity in HVc is inhibited.

Motor-auditory interactions at the single-cell level. The introduction of a movable-electrode technique for recording neural activity from the singing bird (Chapter 2) has made possible the examination of sensorimotor interactions at the cellular level. One such interaction, in mockingbird HVc, was exhibited by the unit shown in Fig. 7. In the singing bird, this cell demonstrated premotor specificity (see Chapter 2) by producing premotor activity (of variable latency) for some song syllables and remaining silent for others. In the quiescent bird, the spontaneous activity of this unit was markedly inhibited by playback of all song syllables. This inhibition was statistically highly significant (two-tailed *t* test, *P* <0.001). The same type of inhibition was observed in the "trill-specific" neuron described in Chapter 2, Fig. 10. Because these inhibitory responses to song playback are confined to *non*singing periods, they represent exactly the opposite of the effect described earlier.

Discussion

In Chapter 2, I demonstrated that HVc, as well as other song control nuclei, 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 may conclude that HVc generates or relays learned cues for song.

The present results 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 interference 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 non-spiking interactions between cells. Thus it is tempting to speculate that the motor-auditory interaction I 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 inputs following 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, since 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 (Judge et al., 1980) and vocalization-related suppression of auditory inputs in the bat (Suga and Schlegel, 1973). 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 (Muller-Preuss and Ploog, 1981), is also confined to roughly the period of stimulated vocalization. I know of no previously documented case in which prolonged inhibition of central sensory activity can be attributed to normal performance of a motor task.

The alternation of motor and auditory activity in the white-crowned sparrow and canary song segments illustrated in Figs. 4 and 5 suggests a mechanism by which auditory feedback may be used in song development and/or song maintenance. If we assume that the segregation of motor activity into active and silent intervals is a necessary feature of the adult pattern, then we may also assume that this segregation develops as the song develops. Since normal song development requires access to auditory feedback, it is quite likely that auditory responses in song system nuclei play a role in development. The timing of the multi-unit responses described here suggests that their role may be relatively straightforward: to inhibit concurrent motor activity so as to achieve segregation in the motor output from HVc. This hypothesis implies mutual inhibition between auditory and motor activities in HVc, and in its simplest form is dependent upon a mechanism by which auditory inputs can produce inhibition without producing spike activity within HVc. Because recordings from Field L neurons indicate auditory responses which persist during singing, it is likely that all sensorimotor interactions occur within song system nuclei. The postulated auditory inhibition of motor activity could be mediated by collaterals from auditory input fibers which are not themselves inhibited and whose activity does not appear in our recordings. Alternatively, it could be mediated by inhibitory interneurons whose activity has not appeared in my electrode placements. The concept of mutual inhibition is supported by the demonstration in single-unit recordings of playback-mediated inhibition of spontaneous activity in some motor-specific or premotor-specific neurons. Qualitatively, this interaction is exactly the opposite of the motor inhibition of auditory responses discussed above, thus illustrating that either category of activity can, under appropriate conditions, inhibit the other.

Canary males tend to sing during intervals when other males are silent. This tendency probably accounts for the 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 (1981), HVc neurons which respond to auditory stimuli project to a nucleus (Area X) of unknown function, whereas those which 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 was therefore 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 (Leppelsack and Vogt, 1976; Scheich et al., 1979); this approach should make it possible to localize the source of inhibition. My results indicate that at least some neurons in Field L respond while the bird is singing.

Auditory responses in HVc very probably play a role in development and/or maintenance of the crystallized song pattern. Nothing is known as yet about the nature of these responses in a young bird which 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.

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Figure 1. Relationship between neural activity in HVc and whistle song elements produced by a white-crowned sparrow. In each record, the upper trace represents multi-unit activity recorded from left HVc; the lower trace is the amplitude envelope of the sound produced by the bird. Note the time-locked correspondence of HVc activity and song elements, and the absence of increased activity in cases where the third whistle is not produced. Time calibration = 100 msec.

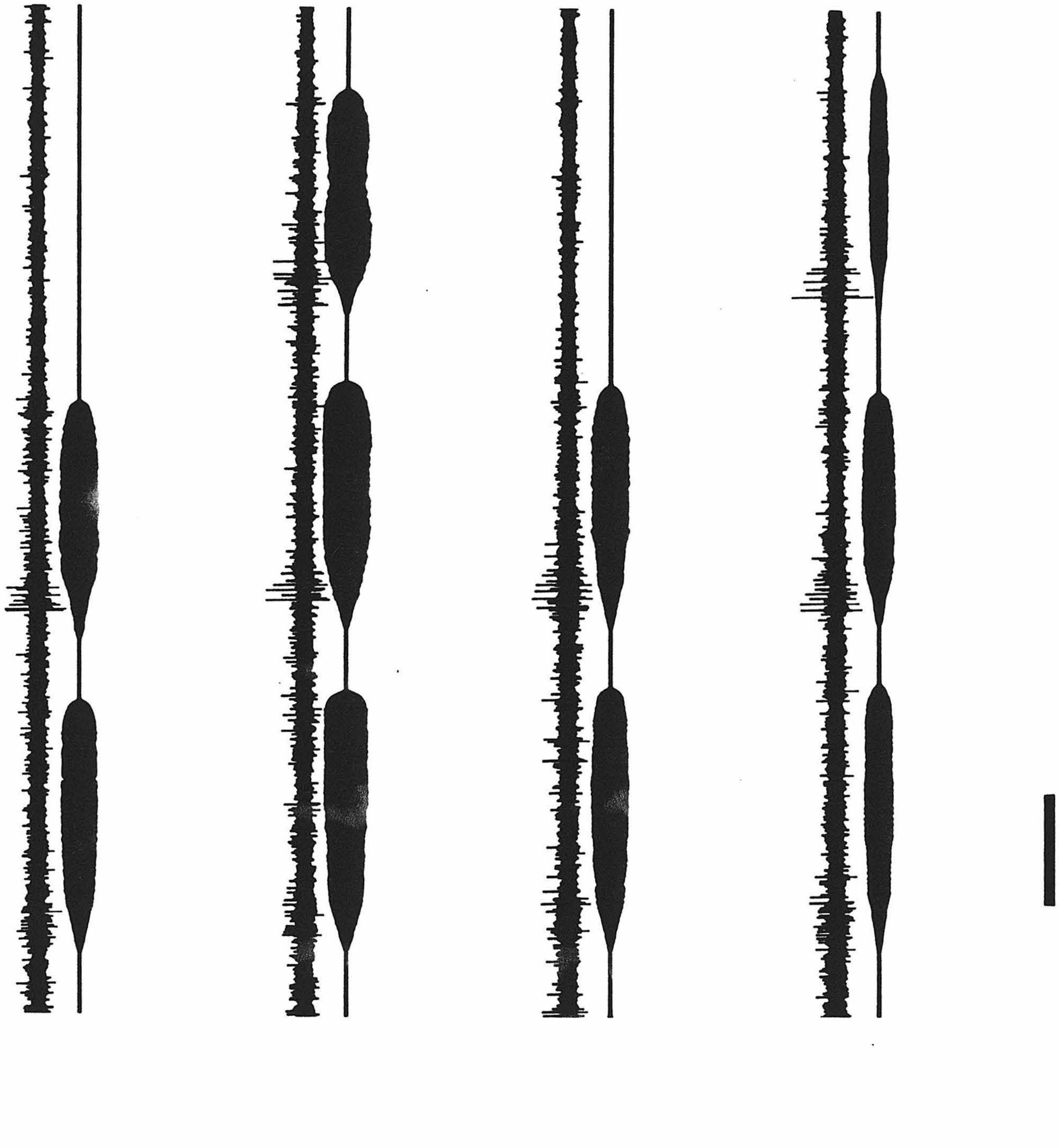


Figure 2. Persistence of song-related discharge in HVc of the deafened bird. Upper record: baseline activity in left HVc of a nonvocalizing, deafened zebra finch. Lower: song produced by a deafened zebra finch, together with left HVc activity. The complex sound patterns of song in this species were reflected in the relatively complex spike discharge patterns during song. Nevertheless, our data showed patterns of activity which were clearly correlated with production of song elements. Time calibration = 100 msec.

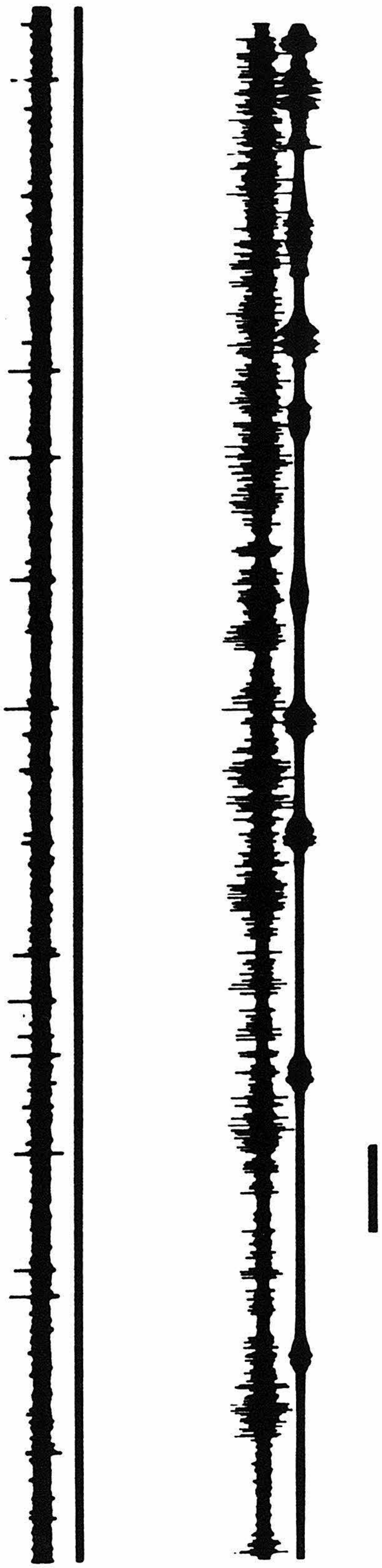


Figure 3A. Auditory responses to normal and reverse playback of a bird's own song. Upper: a portion of song played back to a quiescent, nonvocalizing canary, and the neural responses of left HVc neurons to those sounds. Lower: the same song elements played in reverse, during the same recording session, 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. Time calibration = 100 msec.

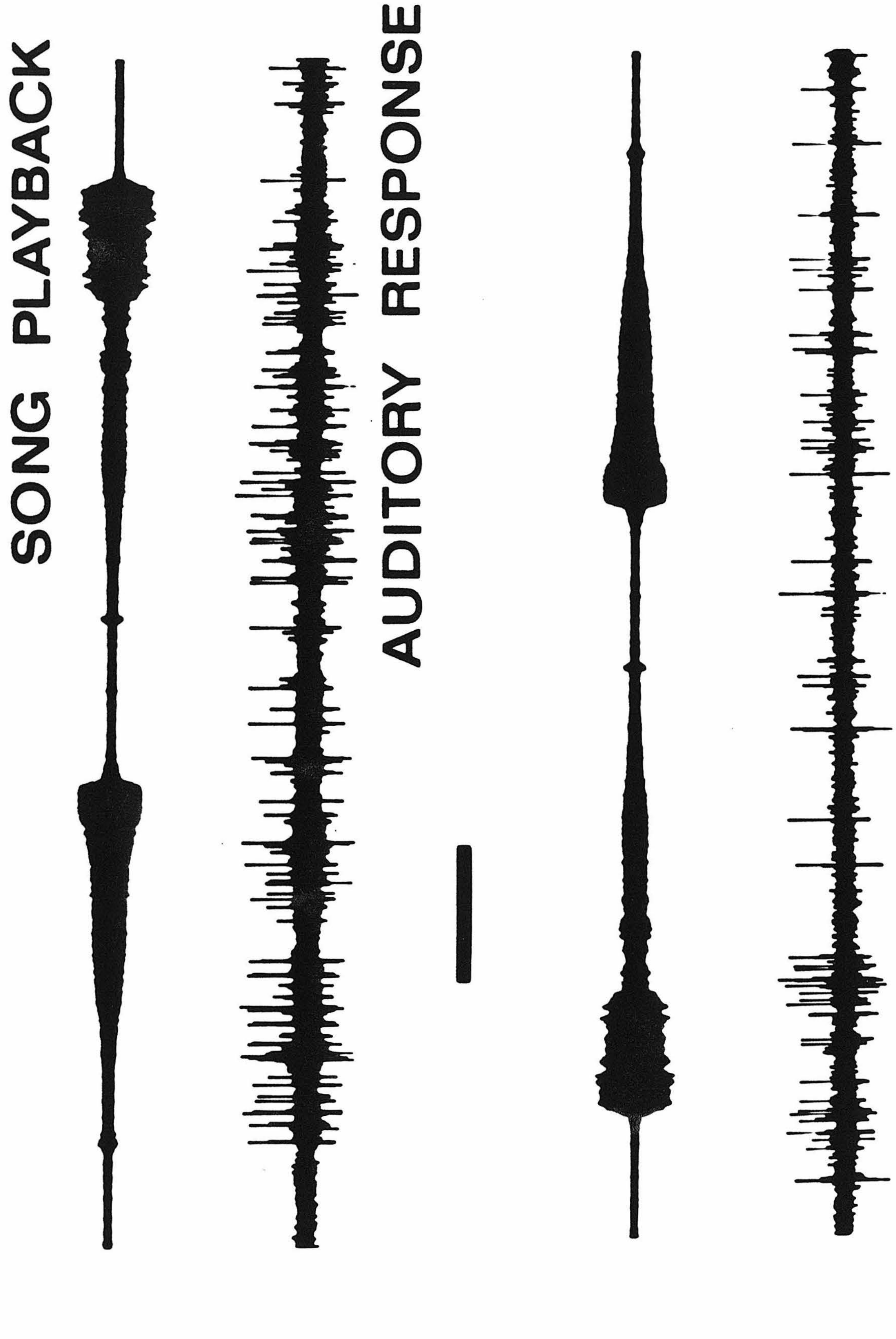

SONG PLAYBACK
AUDITORY RESPONSE

Figure 3B. 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 Figure 3A. Center: 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 above. 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.

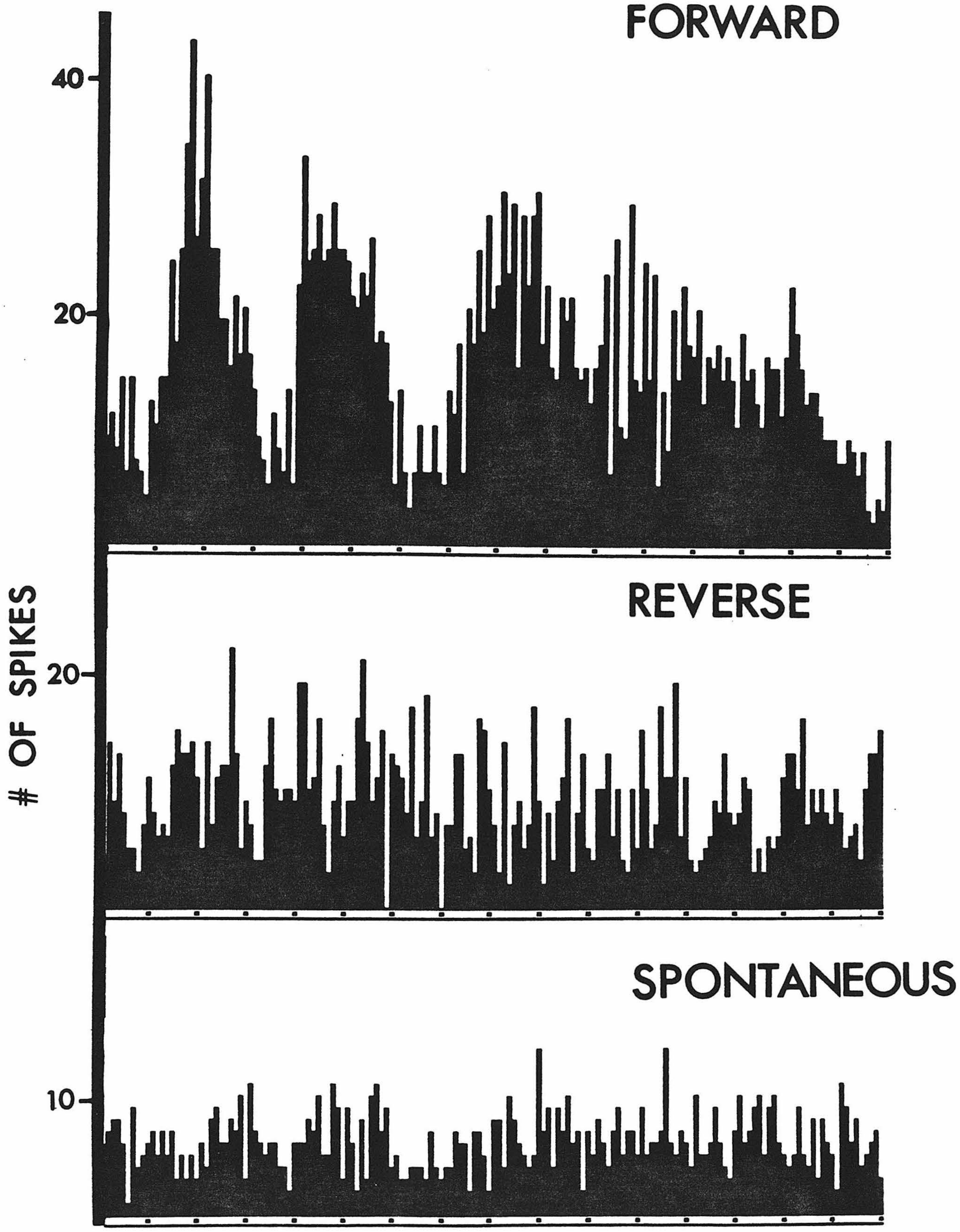

FORWARD
40
20
REVERSE
20
OF SPIKES
SPONTANEOUS
10

Figure 4A. 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. Center 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 following the song element. No spikes comparable in amplitude to the auditory response spikes could be seen at the same latencies in the motor records. Time calibration = 100 msec.

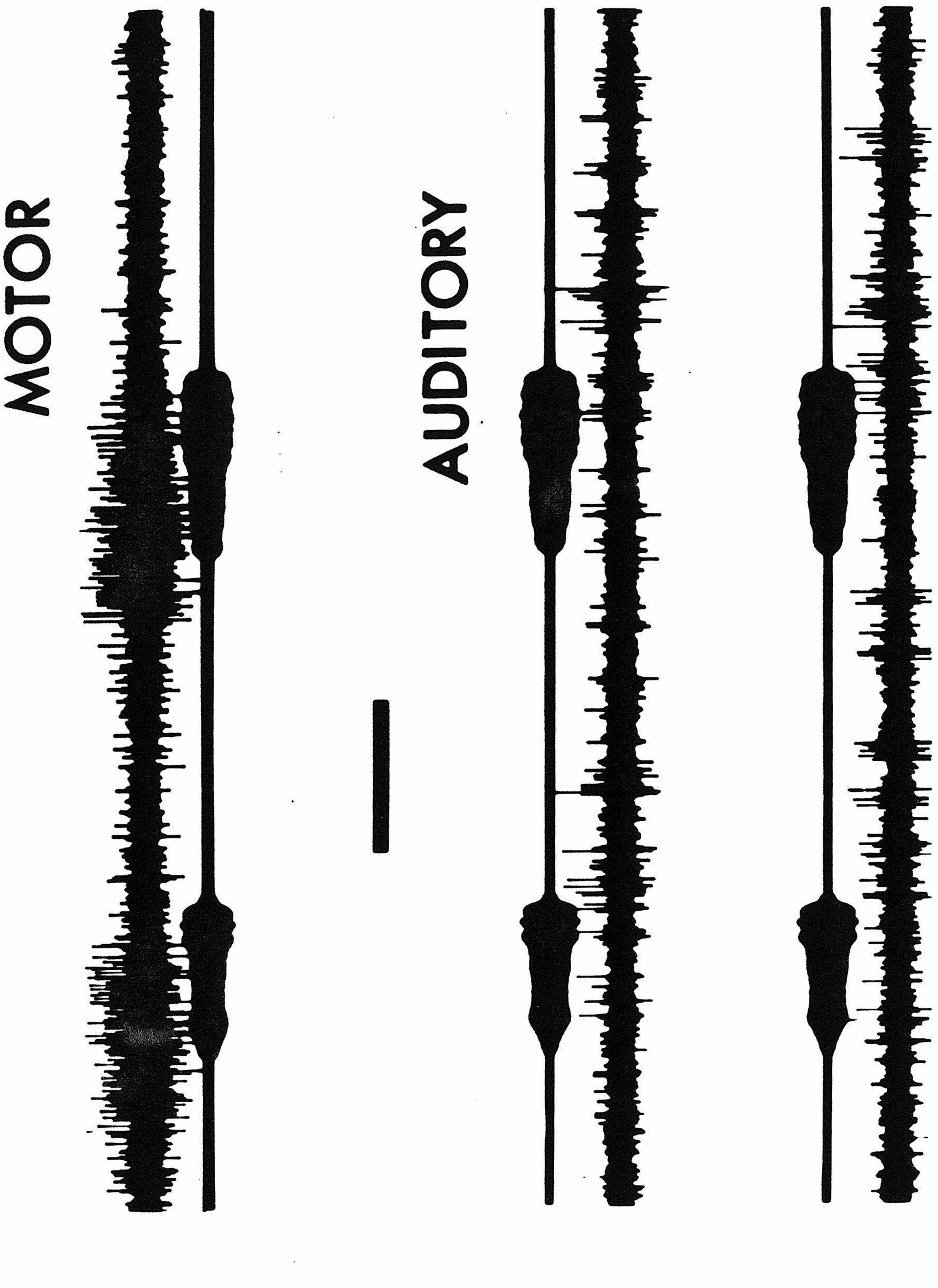

MOTOR
AUDITORY

Figure 4B. 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 elements shown in Fig. 4A (second and third peaks in the histogram). Lower: summed responses to 30 repetitions of the same 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 approximately 1.7 sec.

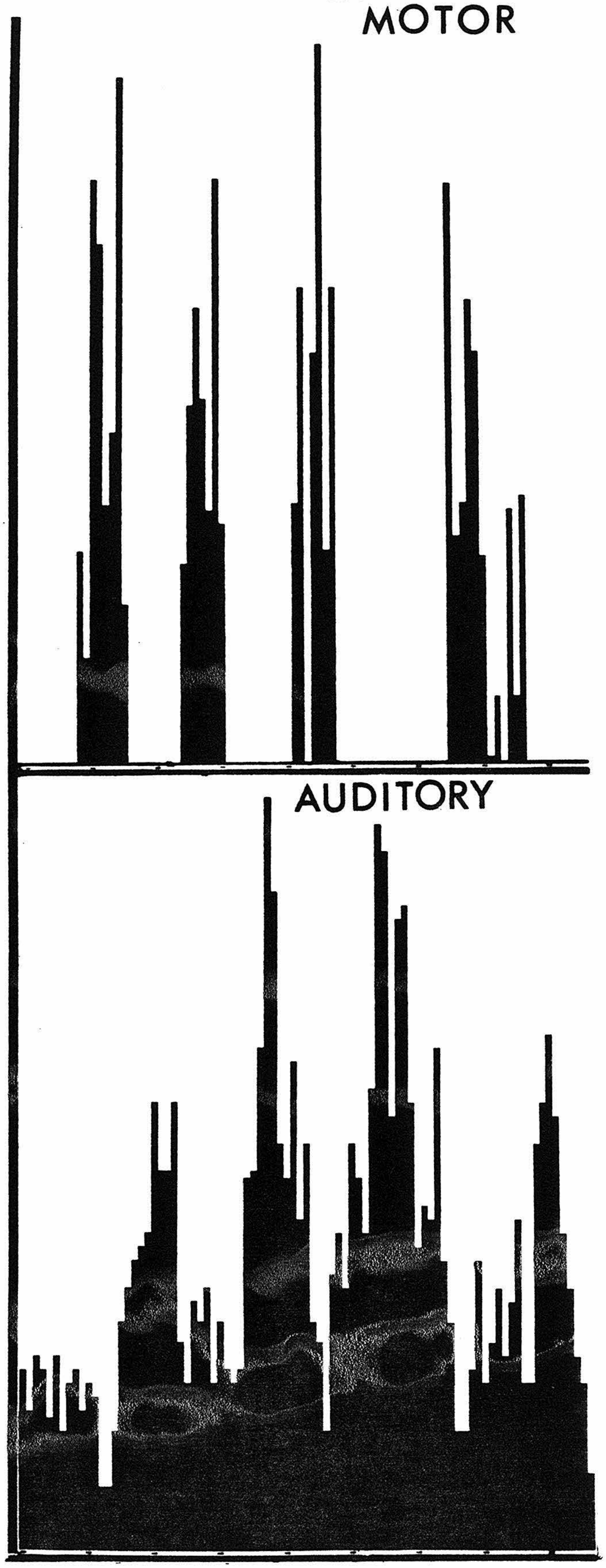

MOTOR
AUDITORY

Figure 5A. Comparison of motor activity and auditory responses to the repeated trill element in the song of a white-crowned sparrow. Upper three records: motor activity for the trill segment of song (as in Fig. 4A). Lower three records: same-day auditory responses to the same trill elements (as in Fig. 4B). The relationship between motor and auditory activity was qualitatively similar to that in the canary (Fig. 4): auditory response spikes occurred at times when motor activity was generally absent. Time calibration = 500 msec.

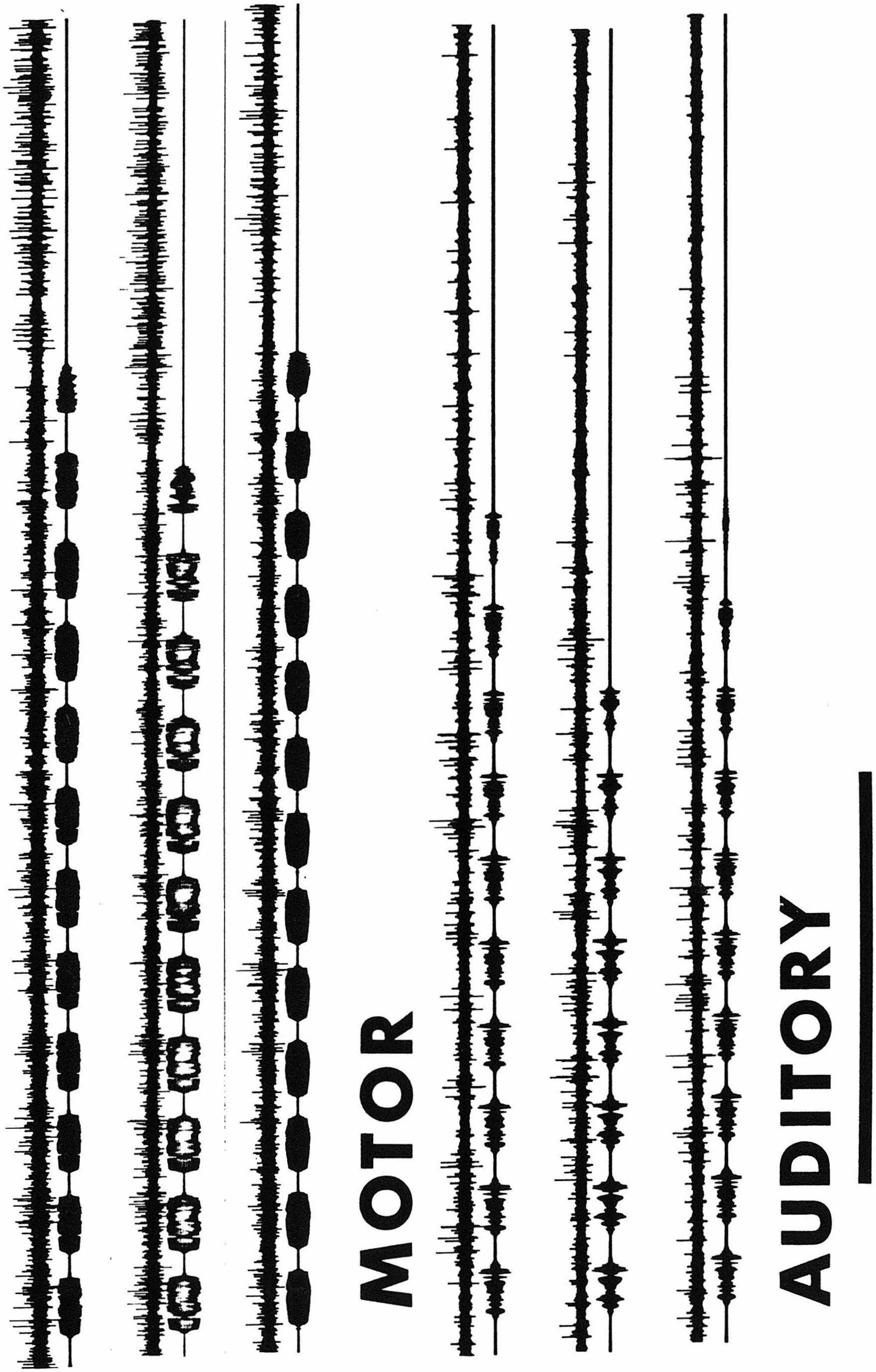
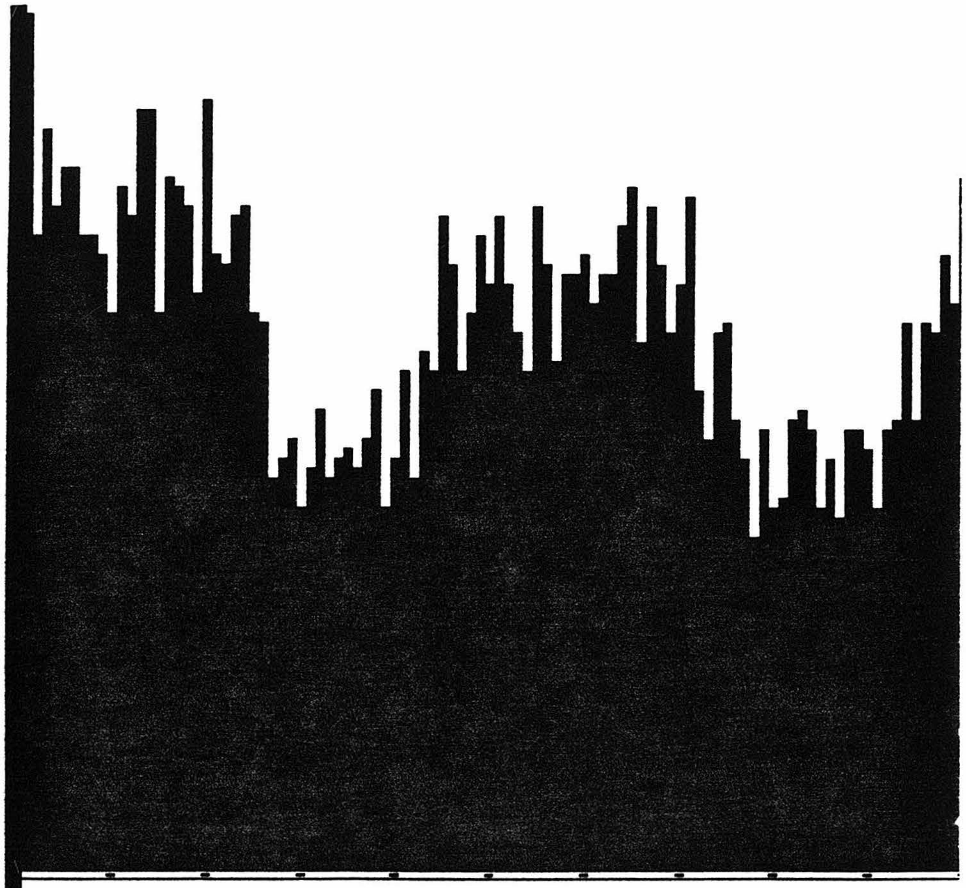
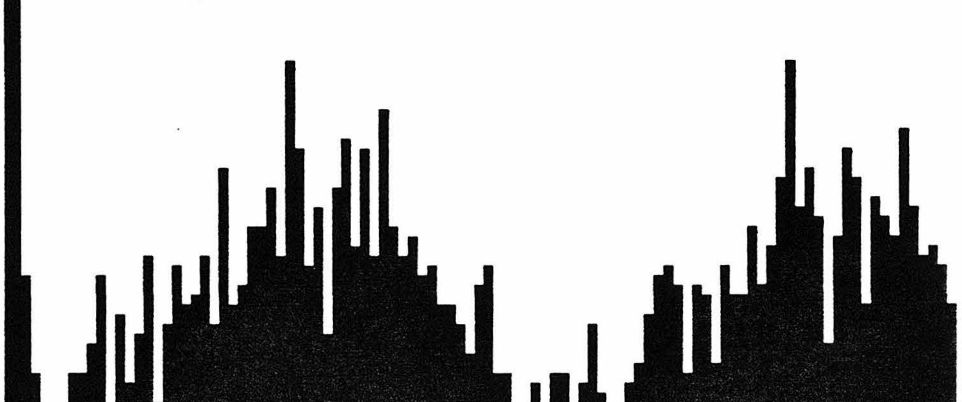

MOTOR
AUDITORY

Figure 5B. Histograms of motor and auditory activity in the white-crowned sparrow. Upper: Summed motor activity from 30 repetitions of trill segments as shown in Fig. 5A. Duration of each histogram sweep was 200 msec, or approximately two full periods of trill element production. Lower: Summed auditory responses to 30 repetitions of the same trill segments presented as auditory stimuli. Both motor and auditory patterns showed a clear oscillation between active and silent periods. The peaks in auditory responses corresponded to the gaps in motor activity.



MOTOR



AUDITORY

Figure 6. Inhibition of auditory responses following song production. Upper: responses in left HVc of a canary to two conspecific song elements played back in a non-singing period. Center: 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 a partial release from inhibition. Time calibration = 100 msec.

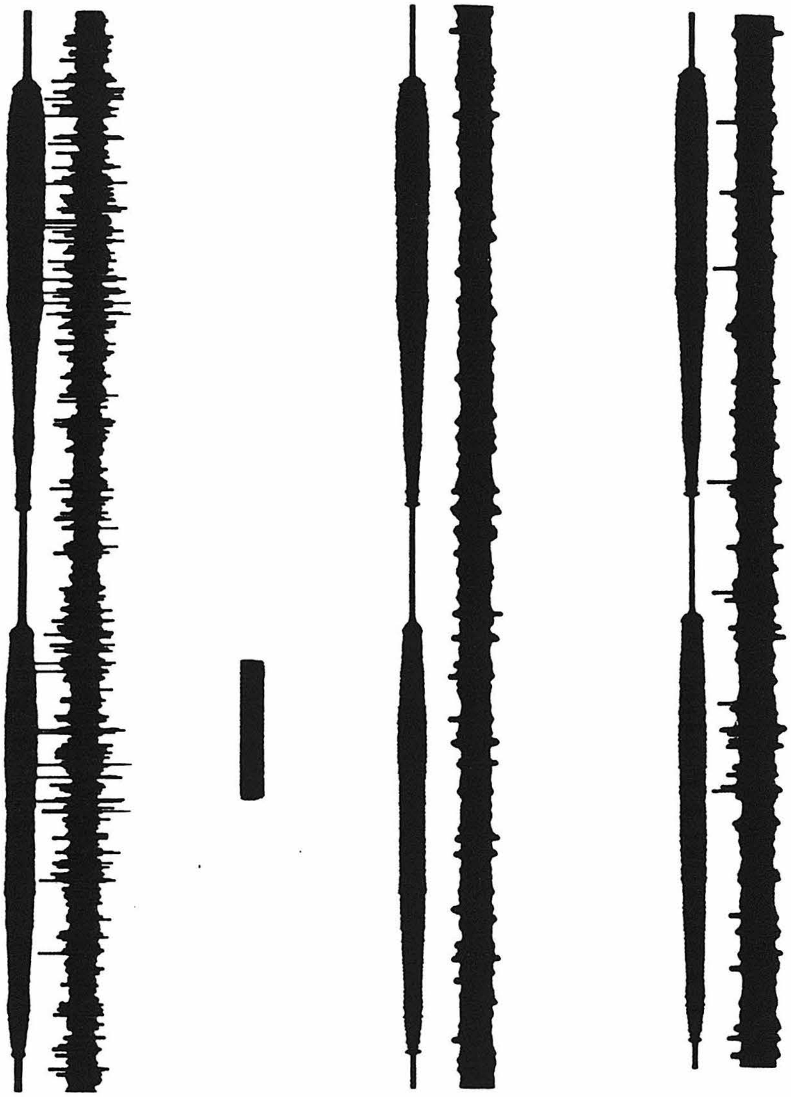
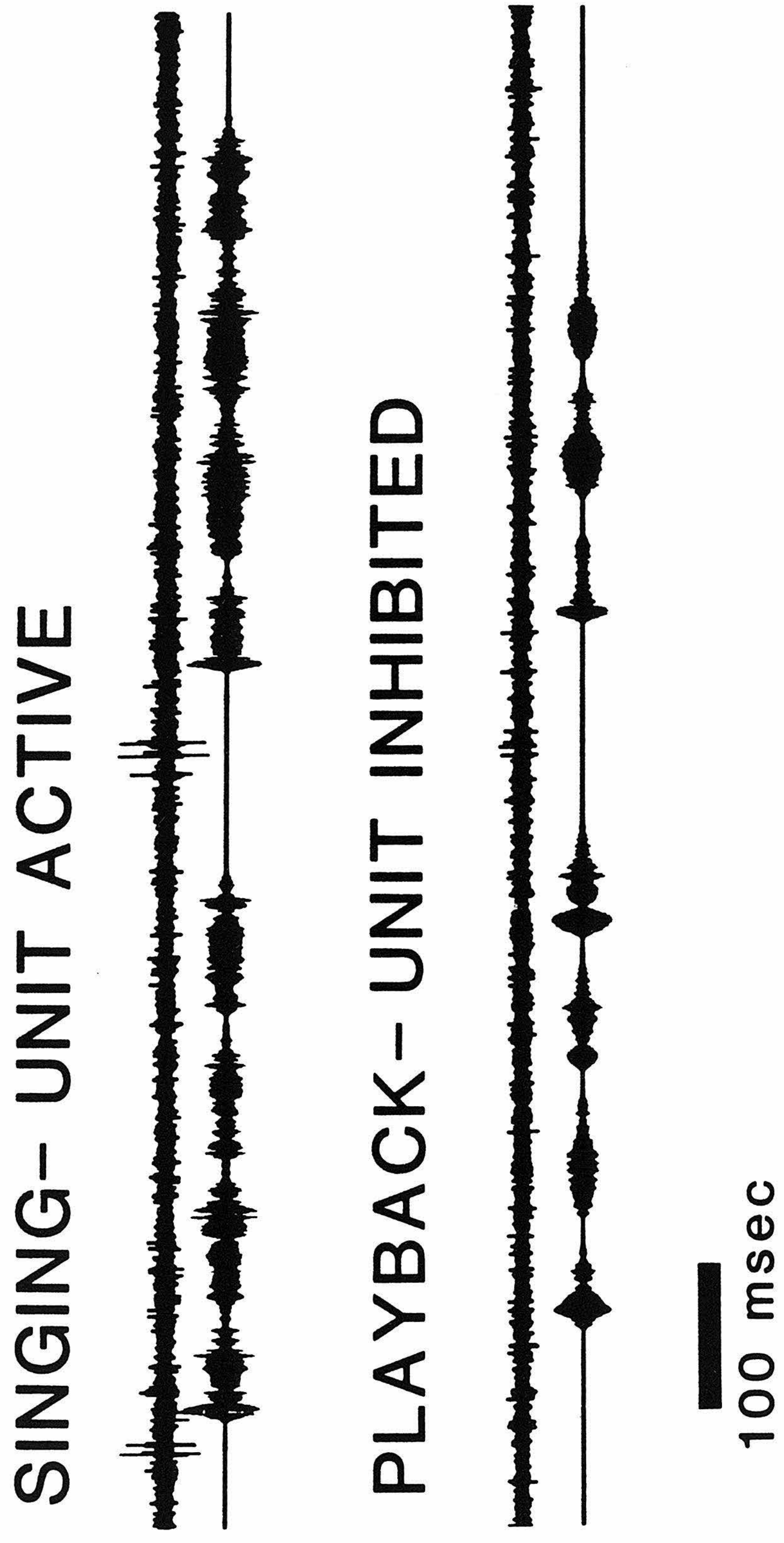


Figure 7. Example of a single-unit motor-auditory interaction. This call, recorded from mockingbird HVc, exhibited premotor specificity in the singing bird by firing in advance of only a few syllables, including that shown in the upper record. During nonsinging periods, the unit was inhibited by playback of all song syllables, as illustrated in the lower record. This inhibition was statistically highly significant.


SINGING- UNIT ACTIVE
PLAYBACK- UNIT INHIBITED
100 msec