

Auditory Responses in Multiple Sensorimotor Song System Nuclei Are Co-Modulated by Behavioral State

Jessica A. Cardin and Marc F. Schmidt

Department of Biology and Neuroscience Graduate Group, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Submitted 22 September 2003; accepted in final form 8 January 2004

Cardin, Jessica A. and Marc F. Schmidt. Auditory responses in multiple sensorimotor song system nuclei are co-modulated by behavioral state. *J Neurophysiol* 91: 2148–2163, 2004. First published January 14, 2004; 10.1152/jn.00918.2003. Auditory responsiveness in nucleus HVC, a high-order sensorimotor area of the avian song system, is modulated by changes in behavioral state. Modulation is not observed in the primary thalamo-recipient auditory area Field L, the indirect source of auditory input to HVC. In this study, we show that auditory responsiveness in nucleus interfacialis (Nif), the immediate auditory afferent to HVC, is modulated by behavioral state. While auditory responsiveness is generally greater in Nif during wakefulness and in HVC during sedation, simultaneous recordings reveal a co-variation of auditory response magnitude. This co-variation is observed both in awake birds, where responses are spontaneously variable, and in sedated birds during manipulations of arousal levels. Auditory responses in Nif and HVC, which are selective for the bird's own song (BOS) during sedation, become predominantly unselective during wakefulness. This loss of selectivity is accompanied by a decrease in the similarity of Nif and HVC response patterns. To explore the role of Nif in shaping HVC auditory responses, we pharmacologically manipulated Nif while recording in HVC. Injection of the GABA_A agonist muscimol into Nif eliminated most spontaneous activity and all auditory responses in the ipsilateral HVC, while injections of the GABA_A antagonist bicuculline increased HVC auditory responsiveness and selectivity. These findings indicate that HVC is not the initial site of behavioral state-dependent modulation in the song system. Together with the suppression of HVC auditory responses by muscimol in Nif, these results suggest that Nif plays an important role in the flow of auditory information to HVC.

INTRODUCTION

Sensory processing in the brain is significantly affected by changes in behavioral state. In mammals, thalamic and cortical sensory processing is different during wakefulness, sleep, and anesthesia (Castro-Alamancos 2002; Coenen and Vendrik 1972; Livingstone and Hubel 1981; Morrow and Casey 1992; Poggio and Mountcastle 1963; Steriade et al. 1969; Swadlow and Weyand 1987). Sensory processing in the avian song system is also profoundly affected by changes in behavioral state (Cardin and Schmidt 2003b; Dave et al. 1998; Nick and Konishi 2001; Rauske et al. 2003; Schmidt and Konishi 1998). Behavioral state dynamically modulates both the strength and tuning of auditory responses in nucleus HVC, a high-order sensorimotor integration area of the song system (Cardin and Schmidt 2003b). HVC population auditory responses are variable and unselective during wakefulness but stable and highly selective for the bird's own song (BOS) over all other auditory

stimuli during sedation. In addition, HVC auditory responsiveness is completely suppressed by arousal.

HVC is part of both the motor and anterior forebrain pathways of the song system and receives projections from the ascending auditory pathway (see Fig. 1A). Field L, the primary avian forebrain auditory area, is directly innervated by the auditory thalamic nucleus Ovoidalis (Ov) and is tonotopically organized (Zaretsky and Konishi 1976). Field L generally responds to many different complex stimuli (Grace et al. 2003; Janata and Margoliash 1999; Lewicki and Arthur 1996; Lim and Kim 1997) and is reciprocally connected with the caudal lateral mesopallium (CLM), which sends projections to the song system nucleus interfacialis (Nif) (Vates et al. 1996). Nif, which projects directly to HVC, has been proposed as a major source of auditory input to HVC (Janata and Margoliash 1999).

Unlike HVC, Field L does not demonstrate behavioral state-dependent auditory responses (Cardin and Schmidt 2003b; Schmidt and Konishi 1998). This suggests that the observed changes in HVC activity may result from direct modulation within HVC, modulation of auditory activity in an afferent structure such as Nif, or simultaneous modulation in HVC and Nif or other afferent structures. Previous studies have suggested that HVC may be the initial site of modulation in this pathway (Dave et al. 1998; Schmidt and Konishi 1998). However, immunohistochemical evidence suggests that Nif is a candidate site of modulatory effects. Neuromodulatory inputs to Nif from cholinergic (Ryan and Arnold 1981) and catecholaminergic (Harding et al. 1998; Mello et al. 1998; Soha et al. 1996) systems could play a role in regulating auditory input to HVC.

In this study, we investigated the relationship between Nif and HVC by recording simultaneously in both structures during wakefulness and sedation. We present evidence that Nif auditory responses are co-modulated with HVC responses by changes in behavioral state. In addition, pharmacological manipulations in Nif lead to modulation of HVC auditory responsiveness. Some of these data have been previously presented in abstract form (Cardin and Schmidt 2002).

METHODS

Animals

Adult male zebra finches (*Taeniopygia guttata*) ranging from 120 to 500 days of age were obtained from our breeding colony and from a local supplier. Birds were housed under constant 12:12 light:dark conditions and given food and water ad libitum. All procedures

Address for reprint requests and other correspondence: M. F. Schmidt, 312 Leidy Labs, 415 S. University Ave., Univ. of Pennsylvania, Philadelphia, PA 19104 (E-mail: marcschm@sas.upenn.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

described here were approved by an Institutional Animal Care and Use Committee at the University of Pennsylvania.

Auditory stimuli

The song of each bird was recorded in a sound attenuation chamber and digitized at 40 kHz with Goldwave (Goldwave, St. Johns, Canada). For BOS stimuli, two song motifs were presented in normal orientation so that the bird heard the motifs as he would when singing. For reversed-BOS stimuli (REV), a two-motif segment was played backward. Auditory stimuli were presented at 70-dB sound pressure level (SPL) peak intensity. SPL measurements were made with A weighting.

Chronic recordings

Electrodes and equipment were as described in detail by Cardin and Schmidt (2003b) and Schmidt and Konishi (1998). Briefly, the bird was anesthetized with 0.07 ml ketamine/xylazine (40 mg/kg ketamine and 8 mg/kg xylazine, Phoenix Pharmaceuticals, St. Joseph, MO), and nichrome wire electrodes (0.2–0.7 M Ω) were implanted in HVC and Nif. These low-impedance electrodes record multiunit activity from a population of neurons. HVC and Nif were located in all experiments by stereotaxic coordinates and by their distinctive, coordinated activity patterns (see Fig. 1B). Electrodes were secured in place with dental cement and attached to a connector (Omnetics Connector, Minneapolis, MN) on the skull. A silver ground wire was inserted under the skull and cemented in place.

Birds were allowed to recover for several days following surgery before being placed in a recording chamber and connected to a lightweight operational amplifier (Texas Instruments, Dallas, TX) and a mercury commutator. Large multiunit neural signals were band-passed between 500 Hz and 10 kHz and digitized at 20 kHz. The birds remained connected for 3–5 days at a time and were kept on a consistent light cycle throughout the experiments. They were handled and disturbed as little as possible to observe normal variations in behavior and arousal state.

During awake auditory trials, the birds were continuously observed on a video monitor to ensure that their eyes were open and that they were moving in the chamber. All awake auditory trials were conducted with the lights on. Any trials on which a bird did any of the following were discarded: vocalized, closed its eyes for ≥ 2 consecutive s, tucked its head under a wing, or crouched on the floor of the cage without moving. Auditory experiments during wakefulness consisted of 30–60 identical auditory stimulus trials. Each auditory stimulus trial comprised 2 s of baseline recording followed by ~ 2 s of auditory stimulus and 2 additional s of baseline recording. The time interval between the end of one trial and the beginning of the next was varied during each experiment and ranged from 5 s to 3 min. An auditory response was defined as the summed neural response to a set of auditory trials.

For arousal experiments, the implanted birds were given an intramuscular injection of diazepam (Abbott Laboratories, North Chicago, IL) and allowed to rest for 15–20 min. Under diazepam sedation, HVC and Nif demonstrate robust, song-selective auditory responses. As described in previous work (Cardin and Schmidt 2003b), this dose of diazepam resulted in a state of mild sedation from which birds were easily aroused. Arousal experiments consisted of a block of 30–60 identical BOS stimuli. The time interval between trials ranged from 5 s to 3 min. At the beginning of randomly selected trials, the bird was aroused by a light touch to the chest feathers with a padded tool.

Acute recordings

Surgery procedures were as described in detail by Cardin and Schmidt (2003b). A head post was cemented to the skull of each bird during a short initial surgery under ketamine-xylazine anesthesia. On

the day of an auditory experiment, the bird was given an intramuscular injection of 7.5 mg/kg diazepam and 0.1 ml 5% dextrose (Abbott Laboratories). The bird was secured in a stereotaxic apparatus by the head post and received a small initial injection of 2% lidocaine hydrochloride (Phoenix Pharmaceuticals) subcutaneously to the scalp. To ensure normal audition and decrease discomfort, neither ear bars nor a beak bar were used.

Windows were opened in the skull over HVC and Nif and small incisions made in the dura in each location, through which glass (5–20 M Ω) electrodes were lowered. Sharp glass (World Precision Instruments, New Haven, CT) electrodes were pulled on a Micropipette Puller P-97 (Sutter Instrument, Novato, CA). Glass electrodes were filled with isotonic saline. Once the electrodes were in place, a series of auditory trials using the BOS and REV stimuli was run to confirm song-selective HVC auditory responses.

Prior to the beginning of each acute recording experiment, we assessed the effectiveness of an air puff to the chest in arousing the bird as described in Cardin and Schmidt (2003b). A bird was considered aroused by the air puff if we observed behavioral indicators such as the eyes opening for several seconds, feathers ruffling, and tail movements. Behavioral indicators of arousal lasted 3–10 s. When the air puff was confirmed to consistently arouse the bird, the stereotaxic apparatus was moved into the sound-attenuating chamber, and the door was closed.

Arousal experiments were composed of 30–60 identical auditory stimulus trials. All auditory stimuli were examples of the bird's song recorded in a sound attenuation chamber and digitized at 40 kHz. The stimuli were played at 70 dB (SPL) through an HLS410 speaker (JBL, Northridge, CA) in the sound chamber via a Crown D-45 Amplifier (Crown International, Elkhart, IN). The time interval between trials was varied during each experiment and ranged from 5 s to 3 min. A set of 30 auditory trials usually spanned 10–18 min. On randomly interleaved trials, the bird was aroused by a puff of air to the chest 1 s before the onset of the auditory stimulus. Neural data from the acute experiments was amplified and bandpassed between 500 Hz and 10 kHz and digitized at 20 kHz. Acquisition software was written in Labview (National Instruments) by A. Leonardo.

Pharmacology

For pharmacology experiments, birds were lightly sedated with diazepam and secured in the stereotaxic apparatus with a head post as described above. Electrodes were placed in HVC and Nif, and a series of auditory stimulus trials was run to confirm song-selective auditory responses in both areas. The Nif electrode was replaced by a glass pipette connected to a Hamilton syringe containing the drug solution. A small amount of drug (0.2 μ l) was injected into Nif over the course of 2 min. We used either muscimol (8 mM in 0.9% saline, Sigma-Aldrich, St. Louis, MO) or bicuculline methiodide (10 mM in 0.9% saline, Sigma-Aldrich). After waiting for an additional 2 min, the pipette was slowly removed. Several additional sets of auditory trials were run to assess the HVC auditory response.

During all pharmacology experiments, the drug solution contained 10% biotinylated dextran amine (BDA; Molecular Probes, Eugene, OR). The bird was killed immediately following the experiment, and the tissue was fixed and cryoprotected. Staining for BDA allowed confirmation of the location and approximate spread of the microinjection (see Fig. 12A). Data from experiments in which the staining spread beyond the borders of Nif were discarded.

Histology

After chronically implanted birds had been recorded for 2–10 days, they were deeply anesthetized with 0.1 ml 50 mg/ml Nembutal (Abbott Laboratories) and transcardially perfused with 0.9% saline and 4% paraformaldehyde. Brains were cryoprotected in 30% sucrose and sectioned at 50 μ m on a freezing microtome. Electrode placement was

confirmed using cresyl violet staining. For identification of microinjection sites, sectioned tissue was processed with an avidin-biotin-horseradish peroxidase complex kit followed by a reaction with a peroxidase substrate kit (Vector Laboratories, Burlingame, CA).

Data analysis

Data were analyzed as described in detail by Cardin and Schmidt (2003b) using Matlab (The Mathworks, Natick, MA) routines written by J. A. Cardin and M. F. Schmidt. Spike events in the multiunit data from acute and chronic experiments were measured by using a peak-detection algorithm. For each data set, the threshold was visually positioned at a point clearly above background noise but low enough to detect all observed spike events. Peristimulus time histograms (PSTHs) were calculated by binning spike events (bin size = 10 ms) during each trial and summing the resulting raster plots over 15–30 auditory trials. Bin size did not affect the results of the data analysis described below.

Auditory responses in awake birds were frequently observed to involve changes in mean firing rate, changes in the temporal distribution of spike events, or both (Cardin and Schmidt 2003b). We therefore evaluated auditory responses based on both response strength and variance measurements taken from PSTHs. The number of spikes in each PSTH bin was defined as the bin total. Thus we could compare the set of bin totals from the stimulus period to the set of bin totals from the baseline period. For each PSTH, we performed two calculations: 1) an unpaired *t*-test comparing the set of bin totals representing the BOS stimulus period to the set of bin totals representing a baseline period of equivalent duration and 2) an *F* test to compare the variance of the sets of PSTH bin totals representing the summed neural activity during the same stimulus and baseline periods. The recorded response was defined as a significant auditory response if $P < 0.01$ for either of the above tests.

Index values

To quantitatively represent changes in both firing rate and temporal distribution of spike events during auditory responses in awake and sedated birds, we used a combination of response strength and variance measurements (see Cardin and Schmidt 2003b). Variance (V) was measured as the variance of the set of PSTH bin totals resulting from a set of auditory trials. The response strength index (RS_{INDEX}) for each auditory trial was calculated by dividing the difference between mean BOS and baseline firing rates by their sum

$$RS_{INDEX} = (\overline{FR}_{BOS} - \overline{FR}_{BASE}) / (\overline{FR}_{BOS} + \overline{FR}_{BASE})$$

$\overline{FR}_{BOS}$ is the mean firing rate during BOS stimulus presentation, and $\overline{FR}_{BASE}$ is the mean firing rate during the preceding baseline period. RS_{INDEX} for each set of auditory trials is shown as mean $\pm$ SE. While the RS_{INDEX} measure does not include an assessment of variance, conclusions reached using a *z*-score method did not differ from those presented here. The variance index (V_{INDEX}) for each set of auditory trials was calculated as follows

$$V_{INDEX} = (V_{BOS} - V_{BASE}) / (V_{BOS} + V_{BASE})$$

V_{BOS} is the variance of the set of BOS bin totals, and V_{BASE} is the variance of the set of baseline bin totals.

Index values range from -1 to 1 . An RS_{INDEX} or V_{INDEX} value of 0 indicates no difference between the BOS response and baseline activity. A positive RS_{INDEX} value indicates a greater response during the BOS presentations than during the baseline periods, while a negative RS_{INDEX} value indicates a smaller response during the BOS stimulus presentations than during the baseline periods. Similarly, a positive V_{INDEX} value indicates a greater variance of bin totals during the stimulus period than during the baseline period, while a negative

V_{INDEX} value indicates lower variance of bin totals during the stimulus period than during the baseline period.

Response pattern

To compare HVC and Nif auditory responses to successive sets of BOS stimuli at different times, the patterns of successive auditory responses at a given recording site were compared in two ways. Each auditory response was the summed neural activity during a set of 30–60 auditory trials. Each PSTH resulting from a set of auditory trials was normalized by dividing all bin totals by the mean of the baseline bin totals. To investigate the prevalence of variability of awake auditory responses, we used the set of normalized bin totals corresponding to the BOS stimulus period of the PSTH. The sets of PSTH bin totals were compared with the corresponding bin totals from the next response by a paired *t*-test.

To evaluate the similarity of successive auditory response patterns, we performed linear correlation analyses of pairs of PSTHs normalized as described above. Several pairs of responses were analyzed for many birds. To avoid biasing the analysis toward birds whose auditory responses were sampled most often, we computed mean *r* values for each recording site in each bird before comparing data sets from different behavioral states. All linear correlations were Pearson correlations.

We evaluated the relationship between Nif and HVC auditory responsiveness on two time scales. For the longer time scale of variation from set to set of auditory trials over a day, we used index values to represent the HVC and Nif auditory responses to each set of auditory trials. Linear correlations were performed on the paired HVC and Nif RS_{INDEX} and V_{INDEX} measurements taken over the course of the day for each bird.

For the short time scale of stimulus-evoked changes in HVC and Nif firing rates during individual trials within a set, we assessed RS at each recording site for all the auditory trials in a set. RS is a simple measure of the change in mean firing rate during the auditory stimulus on each trial

$$RS_{BOS} = \overline{FR}_{BOS} - \overline{FR}_{BASE}$$

The relationship between HVC and Nif RS measurements was tested by linear correlation. All linear correlations were Pearson correlations.

Spontaneous activity

To assess spontaneous firing rates in Nif and HVC, we used samples of neural activity recorded in the absence of auditory stimuli. Thirty 2-s samples of spontaneous multiunit population activity were taken from recordings of Nif and HVC activity from each bird during each behavioral state. A mean firing rate was calculated for HVC and Nif for each bird in units of spike counts/s. Nif and HVC firing rates were compared across the population of birds by paired *t*-test. Mean firing rates for the population of birds were calculated by finding the mean firing rate for all Nif and all HVC samples.

Selectivity

The selectivity of awake auditory responses was measured only if the auditory response to the BOS stimulus was significantly different from baseline activity. We did not observe any instances of a significant response to the REV stimulus in the absence of a response to the BOS stimulus. The selectivity of a recording site for BOS versus REV stimuli was measured using *d'* values (Green and Swets 1966; Janata and Margoliash 1999; Mooney 2000; Solis and Doupe 1997; Theunissen and Doupe 1998). The *d'* value comparing the response to BOS stimuli relative to REV stimuli was calculated as follows

$$d' = \frac{2(\overline{RS}_{BOS} - \overline{RS}_{REV})}{\sqrt{(\sigma_{BOS}^2 + \sigma_{REV}^2)}}$$

$\overline{RS}_{BOS}$ is the mean response strength to a set of BOS auditory stimuli and was calculated as described above. $\overline{RS}_{REV}$ is the mean response strength to the corresponding set of REV stimuli. σ_{BOS}^2 and σ_{REV}^2 represent the variance of $\overline{RS}_{BOS}$ and $\overline{RS}_{REV}$, respectively. A d' value of 0.5 or greater was used as the criterion for a response selective for BOS over REV stimuli (Solis and Doupe 1997). Population d' values are shown as mean $\pm$ SE.

RESULTS

We assessed the relationship between the auditory response properties of the song system nuclei HVC and Nif during

wakefulness and sedation. Using carefully controlled acute and chronic recording paradigms, we tested the hypothesis that auditory responses in Nif and HVC are co-modulated by arousal. Finally, we used *in vivo* pharmacological manipulations to determine whether changes in Nif neural activity lead to modulation of HVC auditory responsiveness.

Characterization of Nif auditory responses in awake birds

To investigate the relationship between Nif and HVC during wakefulness, we recorded simultaneous multiunit activity from ipsilateral Nif-HVC pairs in 23 chronically implanted birds. Electrode locations were verified in three ways, as shown in the examples from one chronically implanted bird in Fig. 1, B–D. First, Nif and HVC were identified by their coordinated bursts

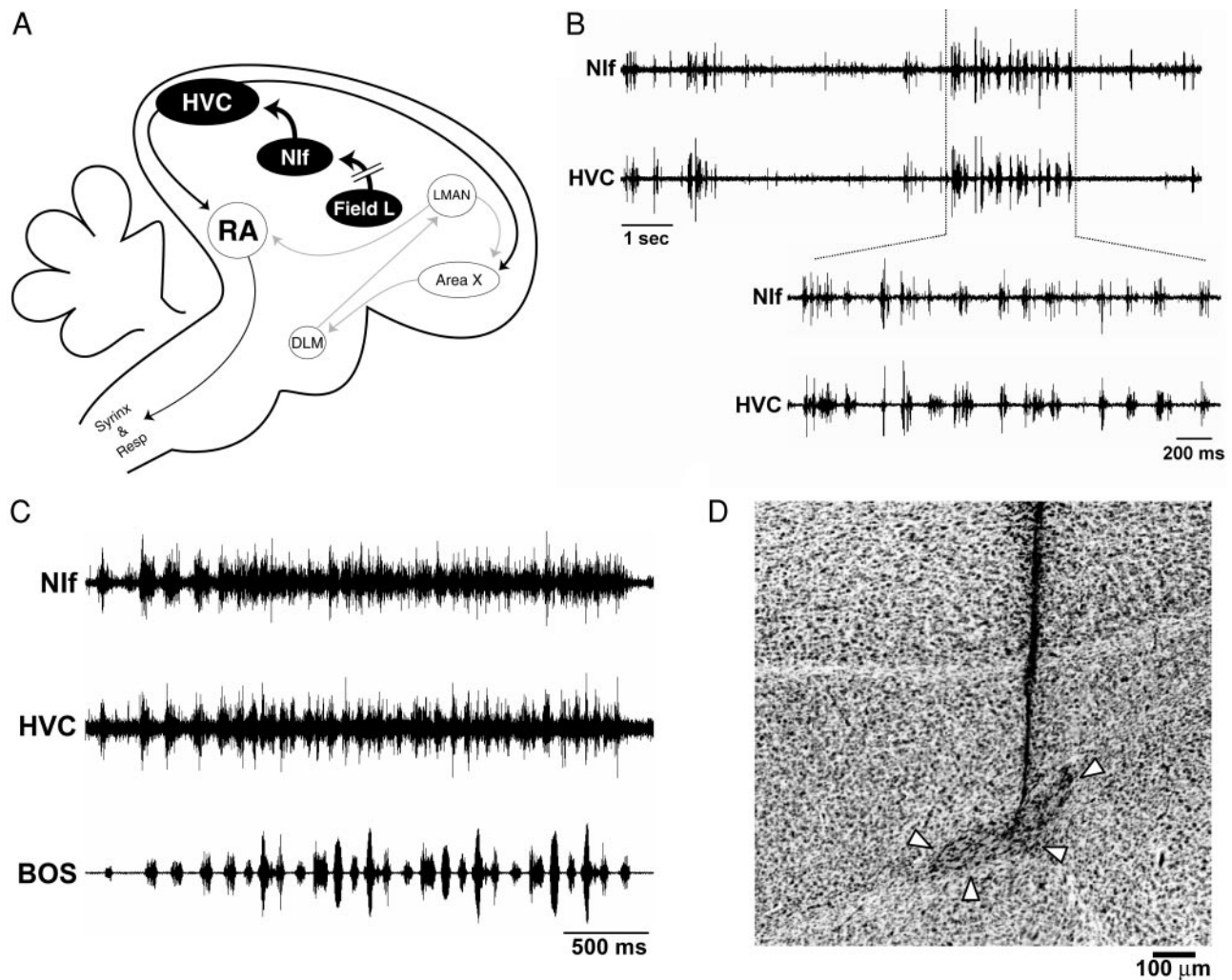


FIG. 1. Song system schematic and criteria for confirming Nif location. A: schematic diagram of the 3 major song system pathways: motor, anterior forebrain, and ascending auditory. HVC (used as proper name), which is part of both the anterior forebrain pathway and the vocal motor pathway, receives auditory input indirectly from Field L via Nif. The anterior forebrain pathway includes HVC, Area X, DLM, LMAN, and RA. The motor pathway includes Nif, HVC, and RA. Area X, song-related region of the basal ganglia; DLM, medial portion of the dorsal lateral nucleus of the anterior thalamus; Nif, nucleus interfascialis of the nidopallium; LMAN, lateral portion of the magnocellular nucleus of the anterior nidopallium; RA, robust nucleus of the arcopallium; Resp, respiratory areas of the brain stem; Syrinx, avian vocal organ. B: typical coordination of bursting activity at chronically implanted Nif and HVC electrodes in a lightly sedated bird (ZF160). Traces show simultaneously recorded spontaneous multiunit activity in both structures. The expanded section shows a portion of the *top trace* in more detail. C: premotor Nif and HVC activity in the same bird while singing several introductory notes and 4 song motifs. D: histology from the same bird showing the chronic electrode track to Nif. Arrowheads indicate borders of Nif. Dorsal surface is at the *top* and posterior is to the *right*.

of activity during sedation, as observed by Janata and Margo-liash (1999). Figure 1B shows an example of spontaneous multiunit activity recorded simultaneously from chronically implanted electrodes in an ipsilateral Nif-HVC pair during light sedation. Second, as observed previously (McCasland 1987), both Nif and HVC demonstrated robust premotor activity during song. Figure 1C shows premotor activity in Nif and HVC during vocal production of several introductory notes and four complete song motifs. Finally, electrode location was confirmed by cresyl violet histology. An example of a chronic electrode track to Nif is shown in Fig. 1D. This study only includes data from recording sites confirmed by all three criteria.

We observed that neurons in both Nif and HVC can demonstrate auditory responses to the BOS stimulus during wakefulness. Each bird was presented with 1–12 sets of BOS stimuli over the course of 1–4 days. In total, 85 sets of auditory stimuli were presented to 23 chronically implanted birds during wakefulness. We measured multiunit neural responses to each set of BOS stimulus trials to assess whether they were significantly different from baseline activity on the basis of response strength, variance, or both (see METHODS). Using this method, 96.5% (82/85) of the sets of auditory trials generated significant auditory responses in HVC, and 98.8% (84/85) of the sets generated significant auditory responses in the ipsilateral Nif. All birds (23/23) demonstrated at least one significant awake auditory response in both HVC and Nif.

While the prevalence of awake auditory responses in HVC and Nif was similar, the distribution of response characteristics was quite different. In both our previous (Cardin and Schmidt 2003b) and current experiments, many HVC auditory responses involved not an increase in mean firing rate but a change in the temporal distribution of spike events. However, auditory responses recorded simultaneously in Nif predominantly involved a change in mean firing rate (Fig. 2). To quantify the distribution of these response characteristics, we used only the auditory responses previously determined to be significantly different from baseline activity, as described above. We found that 17.1% (14/82) of the HVC auditory responses were significant for response strength alone (RS only), 57.3% (47/82) were significant for response strength and

variance (RS and V), and 25.6% (21/82) were significant for variance alone (V only; Fig. 2, left). In contrast, most Nif auditory responses fell into the RS and V category: 90.6% (76/84) were significant for both response strength and variance, while 7.1% (6/84) were significant for response strength alone, and only 2.3% (2/84) were significant for variance alone (Fig. 2, right). Thus Nif auditory responses almost always involved a change in both mean firing rate and temporal distribution of spike events, while one-fourth of the HVC responses involved only a change in the temporal distribution of spike events.

Nif auditory responses are variable during wakefulness

Our previous work has indicated that HVC auditory responses vary during wakefulness. Because HVC receives a strong input from Nif, this modulation of HVC activity may reflect varying auditory responsiveness in Nif. To compare the stability of Nif and HVC auditory responses in awake birds, we presented 20 chronically implanted birds with 2–12 sets of 30 identical BOS stimuli at different times on a single day. The mean interval between the end of one set of auditory stimuli and the beginning of the next set was 73.6 ± 31.2 min. While we observed auditory responses in both HVC and Nif in awake birds, auditory responses in both nuclei varied greatly over time. Figure 3A shows an example of simultaneous recordings in an ipsilateral HVC and Nif pair during two awake auditory trial sets separated by 10 min. At the first time point (*left panels*), both Nif (*top*) and HVC (*bottom*) demonstrated robust, if different, auditory responses to 30 presentations of the BOS stimulus. However, at the second time point (*right panels*), the auditory responses were greatly diminished in both nuclei.

We assessed the prevalence of this modulation by comparing the response patterns of pairs of successive auditory responses recorded at each multiunit electrode. An auditory response was defined as the summed neural activity during a set of auditory stimulus presentations. The PSTH of each auditory response was normalized to the mean baseline activity, and successive PSTHs were compared by paired *t*-test (see METHODS). We found that 18/20 birds demonstrated significant changes ($P < 0.01$; paired *t*-test) in Nif auditory response characteristics within a relatively short period of time (5–180 min). Similarly, 17/20 birds demonstrated significant changes ($P < 0.01$; paired *t*-test) in HVC auditory response characteristics. Thus both Nif and HVC demonstrate modulation of auditory responsiveness during wakefulness.

Next, we used linear correlation analyses to test the hypothesis that the observed modulation in auditory responsiveness is specific to wakefulness. Figure 3B shows mean correlation coefficients for pairs of Nif and HVC auditory responses during wakefulness and sedation. Only immediately successive pairs of auditory responses recorded within a short interval (5–180 min) were compared. In both Nif and HVC, pairs of auditory responses were much less correlated in awake than in sedated birds. The mean *r* value for pairs of Nif auditory responses was 0.39 ± 0.03 during wakefulness ($n = 49$ comparisons in 20 birds) and 0.71 ± 0.04 during sedation ($n = 7$ comparisons in 6 birds; $P < 0.01$; Mann-Whitney *U* test; Fig. 3B, left). Similarly, the mean *r* value for pairs of successive awake HVC auditory responses (0.33 ± 0.03 ; $n = 49$ compar-

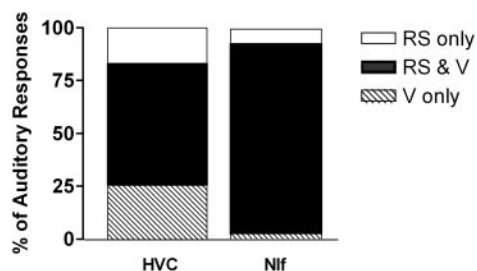


FIG. 2. Nif and HVC awake auditory responses demonstrate different characteristics. Awake auditory responses could be identified as significant on the basis of response strength alone (RS only), response strength and variance (RS and V), or variance alone (V only). Eighty-five sets of stimuli were presented to 23 birds in total and resulted in 84 significant auditory responses in Nif and 82 significant responses in HVC. In HVC, 17% (14/82) of the auditory responses fell into the RS alone category, 57.3% (47/82) into the RS and V category, and 25.6% (21/82) into the V alone category. In contrast, 90% (76/84) of the Nif responses fell into the RS and V category, 7.1% (6/84) into the RS alone category, and only 2.3% (2/84) into the V alone category.

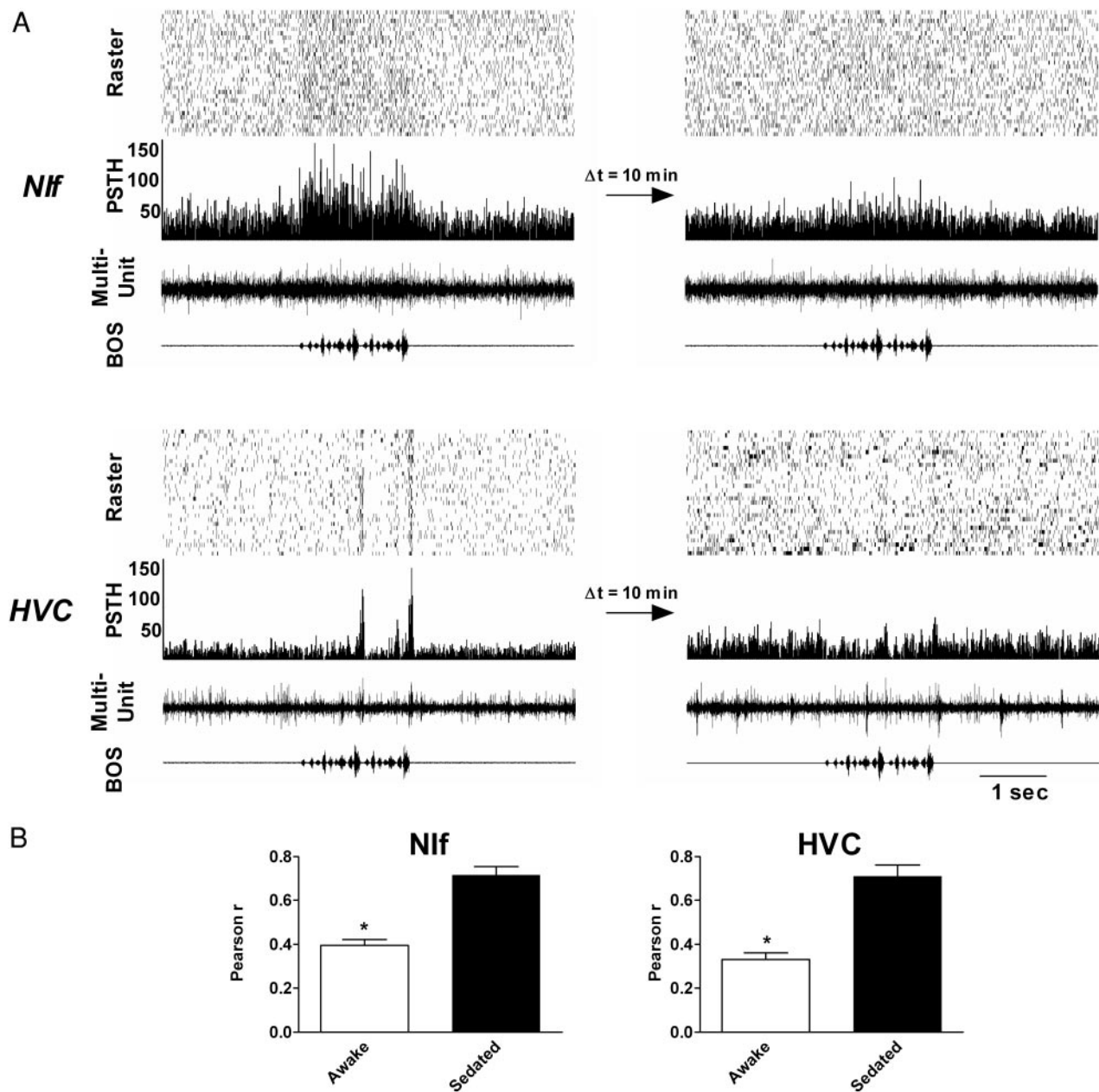


FIG. 3. Variable awake auditory responses in Nif and HVC. A: 2 sets of simultaneously recorded multiunit population auditory responses were recorded in an ipsilateral Nif-HVC pair in an awake bird (ZF169). Nif activity is shown in *top panels* and corresponding HVC activity is shown in *bottom panels*. Time interval between the 2 sets of auditory trials was 10 min. While both recording sites demonstrated robust auditory responses to the 1st set of bird's own song (BOS) stimuli (*left panels*), responses to the 2nd set of BOS stimuli (*right panels*) were greatly diminished at both recording sites. Note also that the Nif response in the *top left panel* demonstrates a greater degree of auditory responsiveness and a different response pattern than the simultaneously recorded HVC response. *Top section*: raster plot of auditory trials. *Middle section*: peristimulus time histograms (PSTHs) resulting from the sum of raster plots in units of spike events per bin (bin size = 10 ms). Trace is an example of multiunit activity during 1 auditory trial. *Bottom section*: amplitude waveform of auditory stimulus. B: Nif and HVC auditory response patterns are variable during wakefulness and stable during sedation. Linear correlation analyses were used to compare pairs of immediately successive multiunit auditory responses recorded at each electrode site. Pairs of successive Nif auditory responses (*left*) were less similar during wakefulness (open bars) than during sedation (black bars). Mean Nif correlation coefficient during wakefulness ($n = 49$ comparisons in 20 birds) was significantly smaller than during sedation ($n = 7$ comparisons in 6 birds; $P < 0.01$; Mann-Whitney U test). Likewise, the simultaneously recorded pairs of successive awake HVC responses (*right*) were less similar during wakefulness ($n = 49$ comparisons in 20 birds) than during sedation ($n = 7$ comparisons in 6 birds; $P < 0.01$; Mann-Whitney U test).

isons in 20 birds) was significantly smaller than that for sedated HVC responses (0.71 ± 0.05 ; $n = 7$ comparisons in 6 birds; $P < 0.01$; Mann-Whitney U test; Fig. 3B, *right*). The mean interval between awake responses was 48.2 ± 16.3 min, while

the mean interval between sedated responses was 41.6 ± 4.9 min. These results indicate that the observed modulation of auditory responses in both Nif and HVC is predominantly restricted to the state of wakefulness.

Co-variation of auditory responses in Nif and HVC during wakefulness

The above data indicate that both Nif and HVC responses vary in awake birds. To evaluate the relationship between awake auditory responses in Nif and HVC, we compared Nif and HVC auditory responses from individual birds over two ranges of time intervals: 1) long intervals, from set to set of auditory trials during the day, and 2) short intervals, over the course of the 30 auditory trials within a set. We calculated index values to quantitatively represent the response strength (RS_{INDEX}) and variance (V_{INDEX}) properties of neural responses to each set of auditory trials. Index values represent all possible auditory responsiveness measurements within a range of -1 to 1 (see METHODS). Only data from birds with six or more awake auditory trial sets on the same day ($n = 4$ birds) were used. The mean interval between sets of awake auditory trials was 31.5 ± 10.4 min.

The example in Fig. 4 illustrates the observed co-variation of Nif and HVC auditory responsiveness over 1 day. Figure 4 shows Nif and HVC RS_{INDEX} and V_{INDEX} data from one bird during 11 sets of awake auditory trials in 1 day. Auditory responsiveness values in Nif and HVC were highly correlated ($P < 0.01$) over the day. The correlation coefficients for Nif and HVC RS_{INDEX} (Fig. 4A) and V_{INDEX} (Fig. 4B) values were 0.84 and 0.90 , respectively (Pearson linear correlation). Analysis of auditory responses from all four birds showed that, in each bird, both Nif and HVC RS_{INDEX} values ($r = 0.74 \pm 0.07$) and V_{INDEX} values ($r = 0.78 \pm 0.07$) were significantly correlated over the course of 1 day ($P < 0.01$). Taken together,

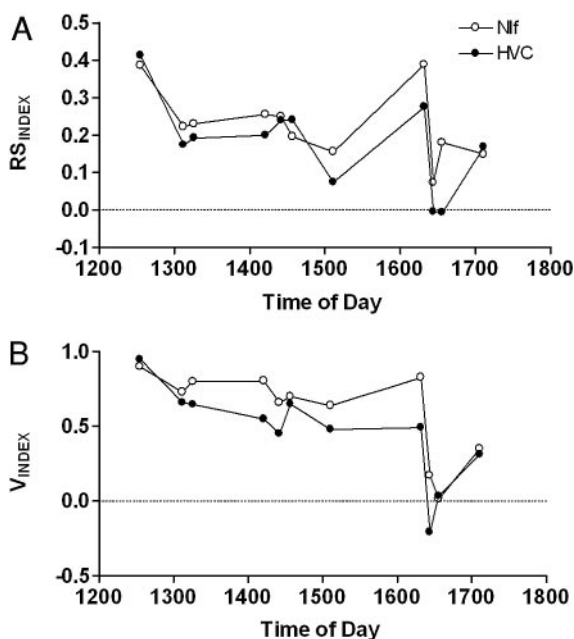


FIG. 4. Nif and HVC auditory responses co-vary over time. Simultaneous multiunit auditory responses in an ipsilateral Nif-HVC pair in 1 chronically implanted bird (ZF170) during wakefulness on a single day. The bird was presented with 30 identical BOS stimulus trials at 11 times during the day. $\circ$, Nif auditory response measurements; $\bullet$, HVC auditory response measurements. A: Nif and HVC RS_{INDEX} values co-varied over the day ($r = 0.84$; $P < 0.01$; linear correlation). B: similarly, Nif and HVC V_{INDEX} values co-varied over the day ($r = 0.90$, $P < 0.01$; linear correlation). Dotted lines, baseline activity levels.

these results indicate that awake auditory responsiveness in Nif and HVC co-varies over the relatively long time scale of 1 day.

To explore the trial-to-trial relationship between neural activity in Nif and HVC, we calculated RS, a simple measure of the stimulus-evoked change in mean firing rate during each auditory trial in a set (see METHODS). We performed linear correlation analyses of Nif and HVC RS values for each set of awake auditory trials ($n = 85$ sets in 23 birds; data not shown). In 72.9% (62/85) of the sets of awake auditory trials, Nif and HVC RS values co-varied significantly ($r = 0.62 \pm 0.18$; $P < 0.01$; Pearson linear correlation). In all 23 birds, there was at least one auditory trial set in which Nif and HVC response strength values were significantly correlated on this shorter time scale.

Behavioral state alters the relationship between neural activity in Nif and HVC

HVC auditory responses are modulated by changes in behavioral state (Cardin and Schmidt 2003b; Nick and Konishi 2001; Rauske et al. 2003; Schmidt and Konishi 1998). We therefore explored the impact of behavioral state on the relationship between spontaneous and evoked neural activity in Nif and HVC. To quantify spontaneous firing rates in Nif and HVC during wakefulness, we measured mean multiunit firing rate in the absence of auditory stimuli in 23 awake chronically implanted birds (see METHODS). A subset of 14 birds was then sedated, and multiunit spontaneous activity was again measured. Figure 5A shows examples of spontaneous firing rates in Nif and HVC during wakefulness (top) and light sedation in one bird (bottom). During wakefulness, Nif demonstrated a higher level of population activity than HVC. During sedation in the same bird, spontaneous activity in Nif and HVC exhibited coordinated bursting activity, as shown previously by Janata and Margoliash (1999). The graphs to the right show mean multiunit Nif and HVC activity levels for the entire group of birds during wakefulness and sedation. Mean spontaneous multiunit Nif firing rates in awake birds (142.5 ± 26.8 spike events/s) were significantly higher than during sedation (27.5 ± 6.8 spike events/s; $P < 0.01$; $n = 14$ birds; paired t -test). In contrast, there was no significant difference between mean multiunit HVC firing rates in awake (18.2 ± 4.0 spike events/s) and sedated (16.9 ± 4.2 spike events/s) birds, despite the change from bursting to tonic activity (NS; $n = 14$ birds; paired t -test). Thus while the overall level of activity in HVC did not change with behavioral state, multiunit firing rates in Nif were significantly greater during wakefulness than during sedation.

To assess whether the relationship between Nif and HVC auditory responsiveness also changes with behavioral state, awake and sedated auditory responses recorded from chronically implanted birds were compared. Figure 5B shows Nif and HVC auditory responsiveness during wakefulness and sedation for 85 sets of auditory trials in 23 birds. In awake birds, both Nif RS_{INDEX} (left) and V_{INDEX} (right) values were usually greater than the simultaneously recorded HVC values, as shown by the large number of points below the solid unity line in each graph ($P < 0.01$; 2-tailed Wilcoxon signed-rank test). These results are well illustrated by the example shown in Fig. 3A (left panels), where both Nif and HVC demonstrate audi-

Spontaneous Activity

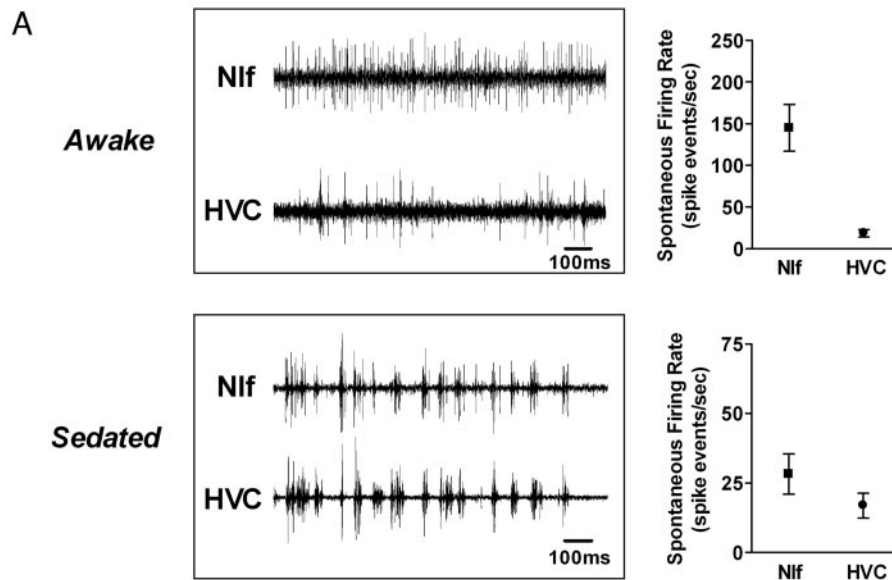
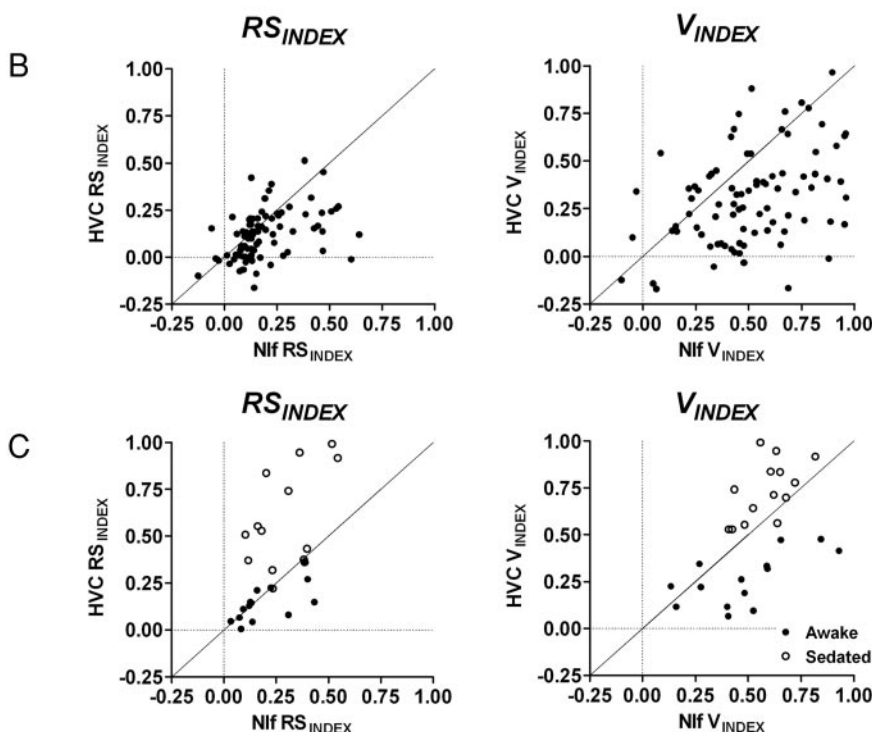


FIG. 5. Relationship between activity in Nif and HVC changes with behavioral state. **A:** spontaneous multiunit activity in Nif and HVC was quite different during wakefulness and sedation. Boxed examples show 1-s traces of simultaneously recorded spontaneous multiunit activity in Nif and HVC in 1 chronically implanted bird (ZF88) during wakefulness and sedation. During wakefulness, Nif showed a high rate of spontaneous activity (80.9 ± 14.9 spike events/s), while HVC showed a significantly ($P < 0.01$) lower mean firing rate (21.8 ± 5.9 spike events/s). Graph on right shows mean spontaneous firing rates across the population of 23 birds. During sedation, Nif (26.1 ± 6.8 spike events/s) and HVC (25.9 ± 9.5 spike events/s) showed coordinated bursting activity, illustrated by the boxed example from the same chronically implanted bird (ZF88). Graph on right shows mean firing rate for all sedated birds ($n = 14$). Mean Nif spontaneous multiunit firing rates were significantly higher during wakefulness than during sedation ($P < 0.01$; paired t -test). Mean spontaneous multiunit firing rates in HVC were not affected by behavioral state (NS; paired t -test). **B:** response strength (RS_{INDEX}) and variance measurements (V_{INDEX}) for simultaneously recorded Nif and HVC auditory responses to sets of BOS stimuli during wakefulness ($n = 85$ sets of BOS stimuli in 23 birds). During wakefulness, Nif RS_{INDEX} was larger than the corresponding HVC RS_{INDEX} (left), indicated by the proportion of points below the unity line ($P < 0.01$; 2-tailed Wilcoxon signed-rank test). Similarly, Nif V_{INDEX} during wakefulness was also larger than the corresponding HVC V_{INDEX} (right; $P < 0.01$; 2-tailed Wilcoxon signed-rank test). These results indicate greater auditory responsiveness in Nif than in HVC during wakefulness. **C:** in a subset of the birds ($n = 14$), auditory responsiveness was assessed during both wakefulness and sedation. While Nif auditory responsiveness, as measured by RS_{INDEX} (left) and V_{INDEX} (right) values, was greater than that in HVC during wakefulness (RS_{INDEX} , $P < 0.01$; V_{INDEX} , $P < 0.01$; paired t -test), HVC auditory responsiveness was greater during sedation (RS_{INDEX} , $P < 0.01$; V_{INDEX} , $P < 0.01$; paired t -test). $\circ$, sedation; $\bullet$, wakefulness. Dotted lines, no difference from baseline activity; solid line, unity.

Evoked Activity



tory responses but the Nif response is greater than the simultaneously recorded HVC response.

A subset ($n = 14$) of the chronically implanted birds was lightly sedated after completion of the awake auditory trials. During sedation, the relationship between Nif and HVC responses was altered. Figure 5C shows mean Nif and HVC RS_{INDEX} and V_{INDEX} values for awake and sedated auditory responses. Nif RS_{INDEX} and V_{INDEX} values were significantly greater than HVC values during wakefulness ($P < 0.01$; paired t -test). However, during sedation, this relationship was re-

versed, and HVC RS_{INDEX} and V_{INDEX} values were greater than Nif values ($P < 0.01$; paired t -test). These data suggest that the relationship between auditory responsiveness in Nif and HVC is greatly altered by changes in behavioral state.

Auditory response patterns in Nif and HVC are more similar during sedation than during wakefulness

While the degree of auditory responsiveness in Nif and HVC co-varied during wakefulness, the specific patterns of simulta-

neously recorded Nif and HVC auditory responses could still be quite different, as shown by the example in Fig. 3A (*left*). To investigate the effect of behavioral state on the relationship between Nif and HVC response patterns, we compared simultaneously recorded auditory response PSTHs from Nif and HVC by linear correlation (see METHODS). Figure 6A shows examples of simultaneous Nif and HVC auditory response PSTHs in one bird during wakefulness and sedation. While the awake response patterns are quite different ($r = 0.21$), with peaks at different time points, the sedated response patterns are more similar ($r = 0.63$; Pearson linear correlation). Analysis of data from all birds demonstrated that the mean correlation coefficient for pairs of simultaneous Nif and HVC responses was significantly smaller during wakefulness ($r = 0.31 \pm 0.03$; $n = 85$ comparisons in 23 birds) than during sedation ($r = 0.62 \pm 0.05$; $n = 22$ comparisons in 14 birds; $P < 0.01$; Mann-Whitney U test; Fig. 6B). These results suggest that during sedation, both auditory responsiveness, as measured by index values, and response patterns are similar in Nif and HVC. In contrast, while overall Nif and HVC auditory respon-

siveness values co-vary during wakefulness, the patterns of simultaneous awake auditory responses in Nif and HVC differ significantly.

Auditory tuning in Nif is altered by behavioral state

We have observed that multiunit activity in HVC is highly selective for the BOS stimulus during sedation but unselective during wakefulness (Cardin and Schmidt 2003b). Because Nif has been proposed as a main auditory input to HVC, we predicted that Nif would not demonstrate song selectivity during wakefulness. To test this hypothesis, we presented 51 sets of randomly intermixed BOS and REV stimuli to 23 chronically implanted awake birds. Song selectivity was assessed by calculating d' values; a d' of >0.5 was considered selective for BOS over REV, while a value of less than -0.5 was considered selective for REV over BOS (see METHODS). Figure 7A shows an example of awake auditory responses in Nif to interleaved presentations of BOS and REV stimuli. In this bird, the Nif responses to the BOS (*left*) and REV (*right*) stimuli were quite similar ($d' = 0.09$). Figure 7B (*left*) shows Nif d' values plotted against HVC d' values for each of the 51 BOS-REV comparisons. While we observed some ($n = 14/51$) auditory responses with Nif or HVC $d' > 0.5$ in awake birds, mean d' measurements for Nif (0.11 ± 0.06) and HVC (0.22 ± 0.05) in awake birds revealed that both structures were predominantly unselective. Nif d' values during wakefulness were significantly smaller than those from HVC ($P < 0.01$; paired t -test). During sedation, both Nif and HVC demonstrated significant song selectivity for the BOS stimulus. Mean sedated Nif d' was 0.87 ± 0.16 , while mean sedated HVC d' was 2.89 ± 0.45 ($n = 12$ comparisons in 12 birds; $P < 0.01$; paired t -test; Fig. 7B, *right*). Thus both Nif and HVC demonstrate song selectivity during sedation but not during wakefulness. These results indicate that behavioral state profoundly affects the fundamental characteristics of auditory processing throughout this ascending auditory pathway.

Nif auditory responses are suppressed by arousal

Our observations of co-variation of Nif and HVC auditory activity in awake birds suggest that auditory activity in Nif, like that in HVC, may be modulated by behavioral state. To explore this hypothesis, we used an acute recording paradigm in which we recorded simultaneously from ipsilateral Nif and HVC pairs while repeatedly changing the bird's behavioral state. All recording sites in both Nif and HVC demonstrated a song-selective response to the BOS stimulus prior to the beginning of the arousal experiments (data not shown). In these experiments, lightly sedated birds were intermittently aroused by an air puff on randomly selected auditory trials (see METHODS). Using this arousal paradigm, we recorded from 13 pairs of Nif and HVC multiunit recording sites in nine birds. All of the Nif and HVC recording sites (13/13) showed a significant suppression of auditory responsiveness following arousal. Figure 8 shows data from a typical Nif-HVC arousal experiment. During resting trials (*left panels*), both Nif (*top*) and HVC (*bottom*) demonstrated robust responses to the BOS stimulus. In contrast, auditory responses in both Nif and HVC were completely suppressed by arousal on randomly intermixed trials (*right panels*).

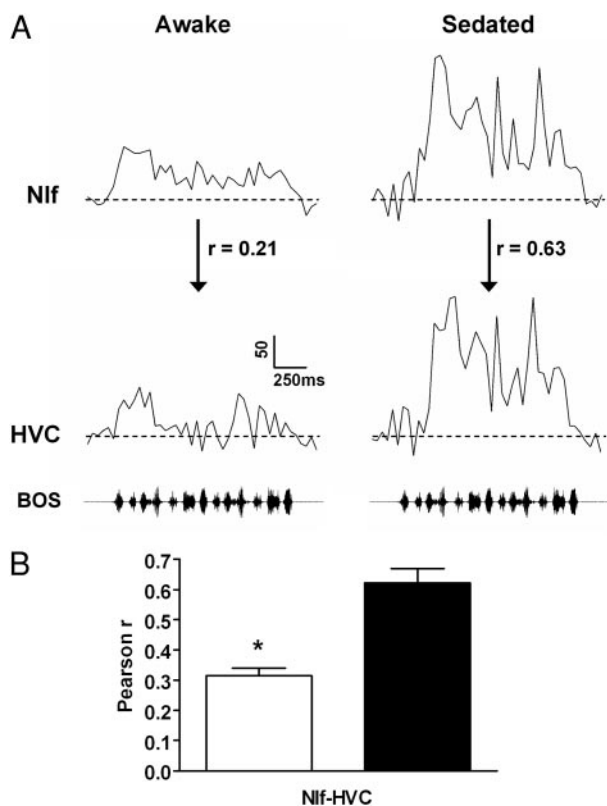


FIG. 6. Auditory response patterns in Nif and HVC are less similar during wakefulness than during sedation. A: examples of PSTHs recorded from an ipsilateral Nif and HVC pair in 1 bird (ZF160) during wakefulness (*left*) and sedation (*right*). For purposes of illustration, these PSTHs are shown with a bin size of 50 ms. During wakefulness, the Nif and HVC auditory responses were quite different ($r = 0.21$; NS; Pearson linear correlation). During light sedation immediately thereafter, auditory responses at the same recording sites became more similar ($r = 0.63$; $P < 0.01$; linear correlation). Dashed lines represent mean baseline activity. Bottom trace: amplitude waveform of the BOS stimulus. B: cumulative data for simultaneous Nif and HVC auditory responses during wakefulness (open bar) and sedation (black bar). Mean correlation coefficient for simultaneous Nif and HVC auditory responses during wakefulness ($n = 85$ comparisons in 23 birds; $r = 0.31 \pm 0.03$) was significantly smaller than during sedation ($n = 22$ comparisons in 14 birds; $r = 0.62 \pm 0.05$; $P < 0.01$; Mann-Whitney U test).

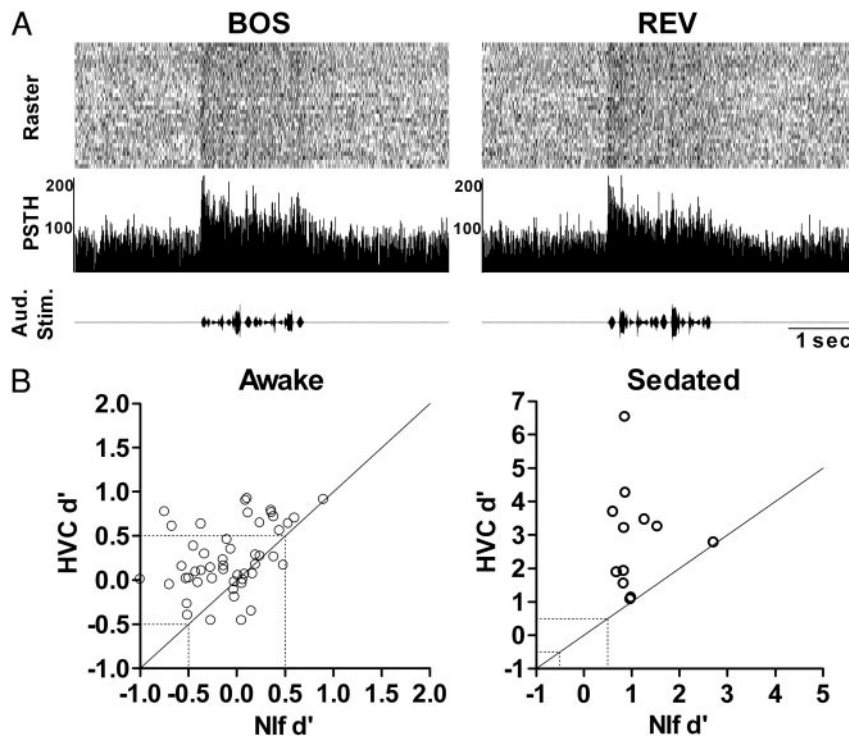


FIG. 7. Nif auditory responses during wakefulness are not song selective. Interleaved BOS and reversed-BOS (REV) stimuli were presented to awake birds. Selectivity was measured by calculating d' values; a d' of -0.5 or less was defined as selective for REV over BOS and a d' of 0.5 or more was defined as selective for BOS over REV. A: typical Nif responses to interleaved BOS and REV stimuli in 1 bird (ZF80), showing the lack of selectivity for either stimulus. The d' value for this pair of responses was 0.09 . Each PSTH shows the summed neural activity in units of spike counts per bin (bin size = 10 ms). B: cumulative selectivity data for awake and lightly sedated chronically implanted birds. In each panel, Nif d' values are plotted against the simultaneously recorded HVC d' values. While we observed a few responses with $d' > 0.5$ during wakefulness (left), both Nif ($d' = 0.11 \pm 0.06$) and HVC ($d' = 0.22 \pm 0.05$) were generally unselective. In contrast, both Nif and HVC showed significant BOS selectivity during sedation (right), although Nif ($d' = 0.87 \pm 0.16$) was significantly less selective than HVC ($d' = 2.89 \pm 0.45$; $n = 12$ sets of stimuli in 12 birds; $P < 0.01$; paired t -test).

Figure 9 shows cumulative data from all 13 paired Nif and HVC recording sites. RS_{INDEX} and V_{INDEX} values were calculated for the resting and aroused trials at each recording site. Figure 9A shows the index values from Nif and HVC for the experiment shown in the previous figure (Fig. 8). RS_{INDEX} and V_{INDEX} values in both Nif and HVC tended to be large and positive during resting trials and small during arousal trials (Fig. 9B). Mean Nif RS_{INDEX} was 0.44 ± 0.03 during resting trials and -0.24 ± 0.05 during arousal trials ($P < 0.01$; paired t -test; Fig.

9C, top). Similarly, mean HVC RS_{INDEX} during resting trials was 0.39 ± 0.03 and during arousal trials was -0.20 ± 0.05 ($P < 0.01$; paired t -test; Fig. 9D, top). Mean Nif V_{INDEX} was 0.62 ± 0.04 during resting trials and -0.33 ± 0.08 during arousal trials ($P < 0.01$; paired t -test; Fig. 9C, bottom). Likewise, mean HVC V_{INDEX} during resting trials was 0.67 ± 0.04 and during arousal trials was -0.26 ± 0.06 ($P < 0.01$; paired t -test; Fig. 9D, bottom). These results indicate a rapid and consistent suppression of both Nif and HVC auditory responsiveness by arousal.

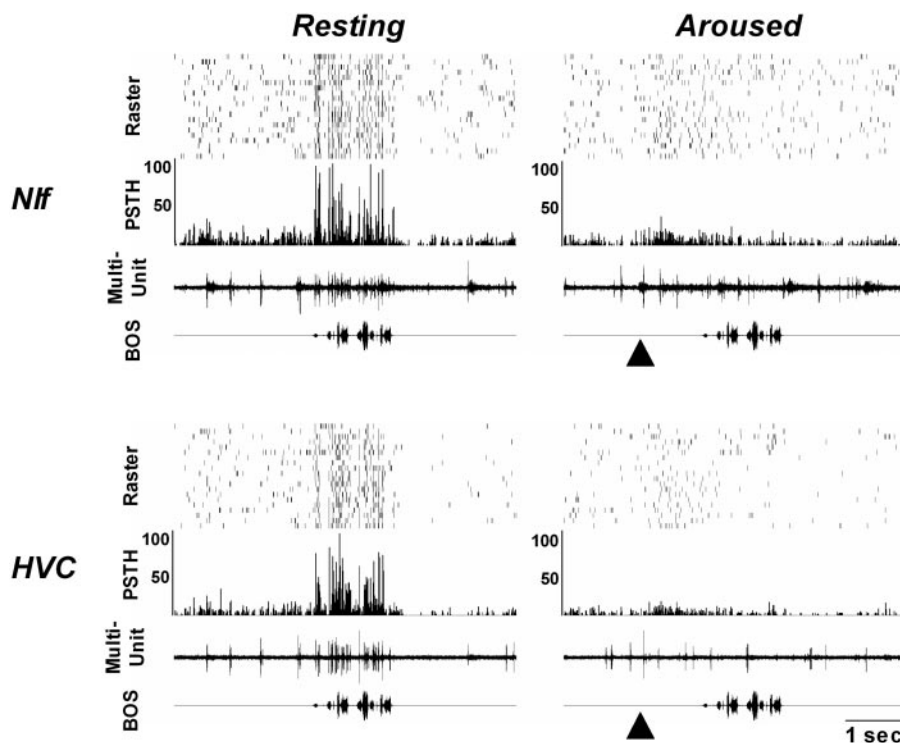


FIG. 8. Suppression of Nif auditory responsiveness by arousal. Representative data from 1 acute recording experiment showing arousal-mediated suppression of Nif and HVC auditory responses. BOS stimulus trials were presented to a lightly sedated bird while recording multiunit activity from an ipsilateral Nif-HVC pair. On randomly interleaved trials, the bird was aroused by a puff of air to the chest 1 s before the onset of the auditory stimulus. The 2 types of trials were separated for analysis. During resting trials (left), there were robust auditory responses to the BOS stimulus in both Nif (top) and HVC (bottom). However, arousal (right) completely eliminated the auditory responses in both nuclei. Each PSTH shows the summed neural activity in units of spike counts per bin. Timing of the air puff is indicated by the arrowheads.

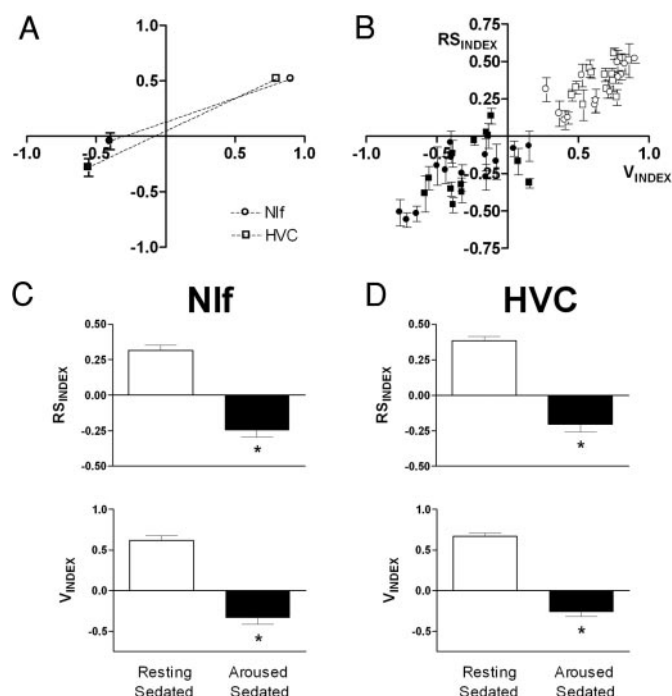


FIG. 9. Quantification of arousal effects on Nif and HVC auditory responsiveness. Data from all acute recording arousal experiments. A: plot of RS_{INDEX} and V_{INDEX} measurements from Nif (circles) and HVC (squares) during the experiment shown in Fig. 8. $\circ$, data from resting trials; $\bullet$, data from arousal trials. B: data from all 13 acute recording arousal experiments. Again, data from Nif recordings are represented by circles and simultaneously recorded data from the ipsilateral HVC are represented by squares. C: both mean Nif RS_{INDEX} and mean Nif V_{INDEX} were significantly smaller in the aroused state than in the resting state ($P < 0.01$; paired t -test). Open bars, data from resting trials; black bars, data from arousal trials. D: similarly, both mean HVC RS_{INDEX} and mean HVC V_{INDEX} were significantly smaller during arousal trials than during resting trials ($P < 0.01$; paired t -test).

In addition to evaluating the effects of behavioral state on overall Nif and HVC auditory responsiveness, we also assessed the co-modulation of Nif and HVC auditory activity on a trial-by-trial basis throughout the arousal experiments. RS, a simple measure of the stimulus-evoked change in mean firing rate, was calculated for Nif and HVC during each trial of an arousal experiment. Linear correlation analysis was used to measure the degree of co-variation between Nif and HVC RS values. Figure 10 shows RS data from Nif and HVC during one arousal experiment, which was comprised of a randomly intermixed set of 30 resting and 30 arousal BOS stimulus trials (data from this experiment are also shown in Figs. 8 and 9A). Nif and HVC RS values are shown for each auditory trial in the set. The dotted line represents baseline activity levels. Nif and HVC RS values were highly correlated throughout the experiment ($r = 0.97$; $P < 0.01$). Analysis of all 13 Nif-HVC arousal experiments demonstrated a consistently high degree of correlation between Nif and HVC RS values from trial to trial (mean $r = 0.83 \pm 0.10$; $P < 0.01$).

To compare further the effects of behavioral state manipulations on Nif and HVC auditory responses, we recorded from chronically implanted birds using an arousal paradigm similar to that described above (see METHODS). In all experiments (8/8 birds), the auditory responses recorded in both Nif and HVC during resting trials were completely suppressed by arousal. Figure 11A shows resting and arousal data from all birds. Mean

Nif RS_{INDEX} was 0.34 ± 0.05 during resting trials and 0.05 ± 0.01 during arousal trials ($P < 0.01$; paired t -test; Fig. 11B, top). Similarly, mean HVC RS_{INDEX} was 0.42 ± 0.08 during resting trials and -0.01 ± 0.02 during arousal trials ($P < 0.01$; paired t -test; Fig. 11C, top). Mean Nif V_{INDEX} was 0.58 ± 0.05 during resting trials and 0.16 ± 0.04 during arousal trials ($P < 0.01$; paired t -test) (Fig. 11C, bottom). Likewise, mean HVC V_{INDEX} was 0.73 ± 0.08 during resting trials and -0.04 ± 0.09 during arousal trials ($P < 0.01$; paired t -test; Fig. 11C, bottom). These results, like those from the acute recordings described above, demonstrate a significant co-modulation of Nif and HVC auditory responses by arousal.

In addition to effects on auditory processing, arousal also altered spontaneous activity in both Nif and HVC ($n = 8$ birds). Mean spontaneous activity levels in Nif were significantly higher during periods of arousal (60.1 ± 14.4 spike events/s) than during periods of rest (27.9 ± 6.8 spike events/s; $P < 0.01$; paired t -test). In contrast, mean spontaneous activity levels in HVC were significantly lower during arousal (12.9 ± 3.1 spike events/s) than during resting (20.2 ± 5.4 spike events/s) periods ($P < 0.01$; paired t -test). As the results from awake and sedated birds, these data indicate that spontaneous activity in Nif and HVC is modulated by behavioral state.

Neural activity in Nif affects HVC auditory responsiveness

Taken together, the preceding results from both awake and sedated experiments indicate that the observed modulation of HVC auditory responses is correlated with changes in Nif auditory responsiveness. However, little is known about the relationship between neural activity in Nif and HVC. We recorded HVC auditory responses in lightly sedated birds while pharmacologically manipulating neural activity in the ipsilateral Nif. In all pharmacology experiments, the injected solution included BDA. Experiments in which BDA staining extended beyond Nif were excluded. An example of a BDA-labeled injection site is depicted in Fig. 12A, left. As shown by comparison to the cresyl violet staining of the same section of tissue (right), the injection site was restricted within the borders of Nif.

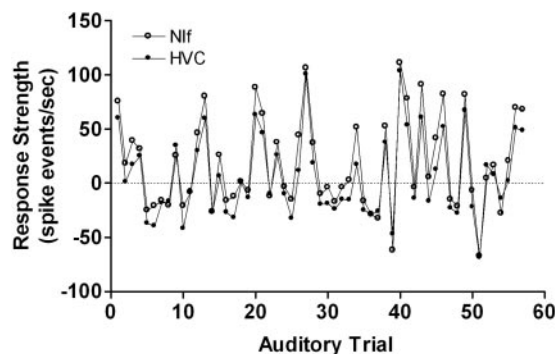


FIG. 10. Stimulus-evoked neural activity in Nif and HVC co-varies during modulation of behavioral state. RS measurements were used to quantify the change in mean firing rate in Nif and HVC evoked by the BOS stimulus. Nif and HVC RS values were measured for every trial during an acute recording experiment. The bird was aroused by an air puff on randomly interleaved trials. Shown here are the RS values for Nif and HVC auditory responses during the experiment shown in Figs. 8 and 9A. $\circ$, Nif data; $\bullet$, simultaneously recorded data from the ipsilateral HVC. Nif and HVC RS values co-varied significantly throughout the experiment ($r = 0.97$; $P < 0.01$; Pearson linear correlation). Dashed line, baseline activity levels.

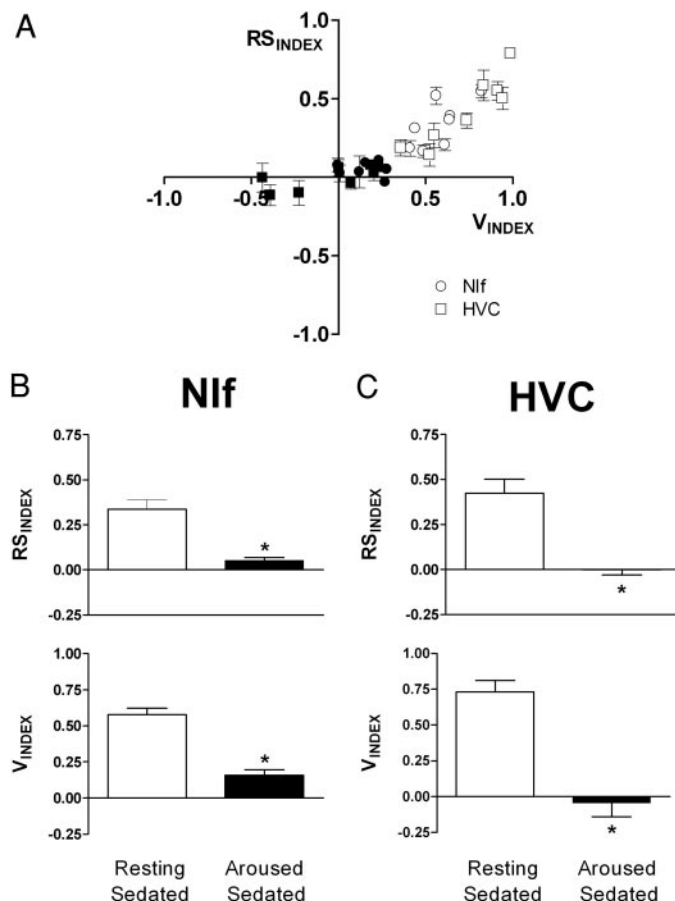


FIG. 11. Arousal modulates Nif auditory responsiveness in chronically implanted birds. For these experiments, an arousal paradigm similar to that described above was used to modulate the behavioral state of chronically implanted birds. Birds were aroused on randomly selected BOS stimulus trials. A: data from all Nif-HVC arousal experiments on chronically implanted birds ($n = 8$ birds); data from Nif are shown as circles while simultaneously recorded data from the ipsilateral HVC are shown as squares. Resting trials are shown as open symbols and arousal trials as filled symbols. B: during arousal experiments, both mean Nif RS_{INDEX} and mean Nif V_{INDEX} were significantly reduced by arousal ($P < 0.01$; paired t -test). C: both mean HVC RS_{INDEX} and mean HVC V_{INDEX} were significantly reduced by arousal ($P < 0.01$; paired t -test).

We first assessed the effects of suppressing activity in Nif on auditory responsiveness in HVC. For these experiments, we recorded multiunit activity in HVC while injecting muscimol, a GABA_A receptor agonist, to the ipsilateral Nif ($n = 5$ Nif-HVC pairs in 4 birds). Figure 12B shows data from a typical experiment. During baseline recording, there was a robust HVC auditory response to the BOS stimulus (Fig. 12B, left). Immediately following a small injection (0.2 μ l) of muscimol (8 mM in 0.9% saline) to the ipsilateral Nif, the entire HVC auditory response, and indeed most spontaneous HVC activity, was eliminated (Fig. 12B, right). In all experiments (5/5), muscimol injection to Nif completely eliminated all ipsilateral HVC auditory responses (Fig. 12C). Mean HVC RS_{INDEX} was 0.38 ± 0.04 before muscimol and 0.05 ± 0.03 after muscimol injection to Nif ($P < 0.01$; paired t -test; Fig. 12C, left). Mean HVC V_{INDEX} before muscimol was 0.64 ± 0.1 and after muscimol to Nif was -0.09 ± 0.16 ($P < 0.01$; paired t -test; Fig. 12C, right). After 15–40 min of recovery, both mean HVC RS_{INDEX} (0.34 ± 0.03) and mean HVC

V_{INDEX} (0.44 ± 0.09) returned to preinjection levels. Injection of muscimol to Nif also had a suppressive effect on spontaneous activity in HVC. While spontaneous HVC activity was robust prior to drug injection (33.7 ± 9.9 spike events/s), it was significantly lower following muscimol treatment (5.12 ± 3.9 spike events/s; $P < 0.01$; paired t -test).

The above results suggest that HVC auditory responsiveness depends on neural activity in Nif. To further test this hypothesis, we injected bicuculline, a GABA_A receptor antagonist, into Nif while recording from the ipsilateral HVC ($n = 4$ Nif-HVC pairs in 4 birds). Injection of a small (0.2 μ l) amount of bicuculline (10 mM in 0.9% saline) to Nif caused an increased auditory response to the BOS stimulus in the ipsilateral HVC. Figure 13A shows data from a typical experiment. During baseline recording, an HVC auditory response to the BOS stimulus was recorded (Fig. 13A, left). After a low dose of bicuculline was injected to the ipsilateral Nif, the HVC auditory response was increased (Fig. 13A, right), and an additional PSTH peak appeared in response to the initial portion of the auditory stimulus (arrow). In all experiments (4/4), bicuculline injection to Nif increased the ipsilateral HVC auditory response (Fig. 13B). Mean HVC RS_{INDEX} before bicuculline was 0.27 ± 0.03 and after bicuculline injection to Nif was 0.41 ± 0.01 ($P < 0.01$; paired t -test; Fig. 13B, left). Mean HVC V_{INDEX} before bicuculline was 0.50 ± 0.06 and after bicuculline to Nif was 0.75 ± 0.04 ($P < 0.01$; paired t -test; Fig. 13B, right). After 30–50 min of recovery, both mean HVC RS_{INDEX} (0.33 ± 0.02) and mean HVC V_{INDEX} (0.61 ± 0.05) returned to preinjection levels.

In contrast to muscimol, bicuculline injection did not cause a significant change in spontaneous HVC activity. Mean spontaneous firing rates prior to drug injection (27.9 ± 7.2 spike events/s) were similar to those following bicuculline (27.2 ± 6.7 spike events/s). However, bicuculline injection to Nif did significantly alter HVC song selectivity. Mean HVC d' during baseline recordings was 0.89 ± 0.10 and following bicuculline injection to Nif was 1.4 ± 0.21 ($n = 4$; $P < 0.05$; paired t -test). Linear correlation analysis revealed a change in HVC auditory response patterns after bicuculline administration to Nif ($n = 4$; $r = 0.39 \pm 0.15$).

As a control, we injected saline alone into Nif and recorded from the ipsilateral HVC. Injections of small amounts of 0.9% saline (0.2 μ l) had no effect on HVC auditory responsiveness ($n = 4$ Nif-HVC pairs in 4 birds). Mean HVC RS_{INDEX} during the baseline period was 0.56 ± 0.04 and after saline was 0.53 ± 0.06 (NS; paired t -test). After 15–30 min of recovery, mean HVC RS_{INDEX} was 0.51 ± 0.05 . Similarly, mean HVC V_{INDEX} during the baseline period was 0.82 ± 0.03 and after saline was 0.76 ± 0.06 (NS; paired t -test). After recovery, mean HVC V_{INDEX} was 0.77 ± 0.06 . Thus the injection of vehicle alone to Nif does not affect auditory responses in the ipsilateral HVC.

An increase in GABAergic activation in Nif thus leads to a profound suppression of auditory responsiveness in HVC, suggesting that Nif inputs are necessary for the expression of HVC auditory responses. The reduction of spontaneous activity in HVC after muscimol to Nif indicates that input from Nif plays an important role in determining spontaneous HVC activity under sedation. In contrast, decreasing GABAergic activity in Nif leads to an increase in HVC auditory responsiveness in the absence of any change in spontaneous neural activity. These

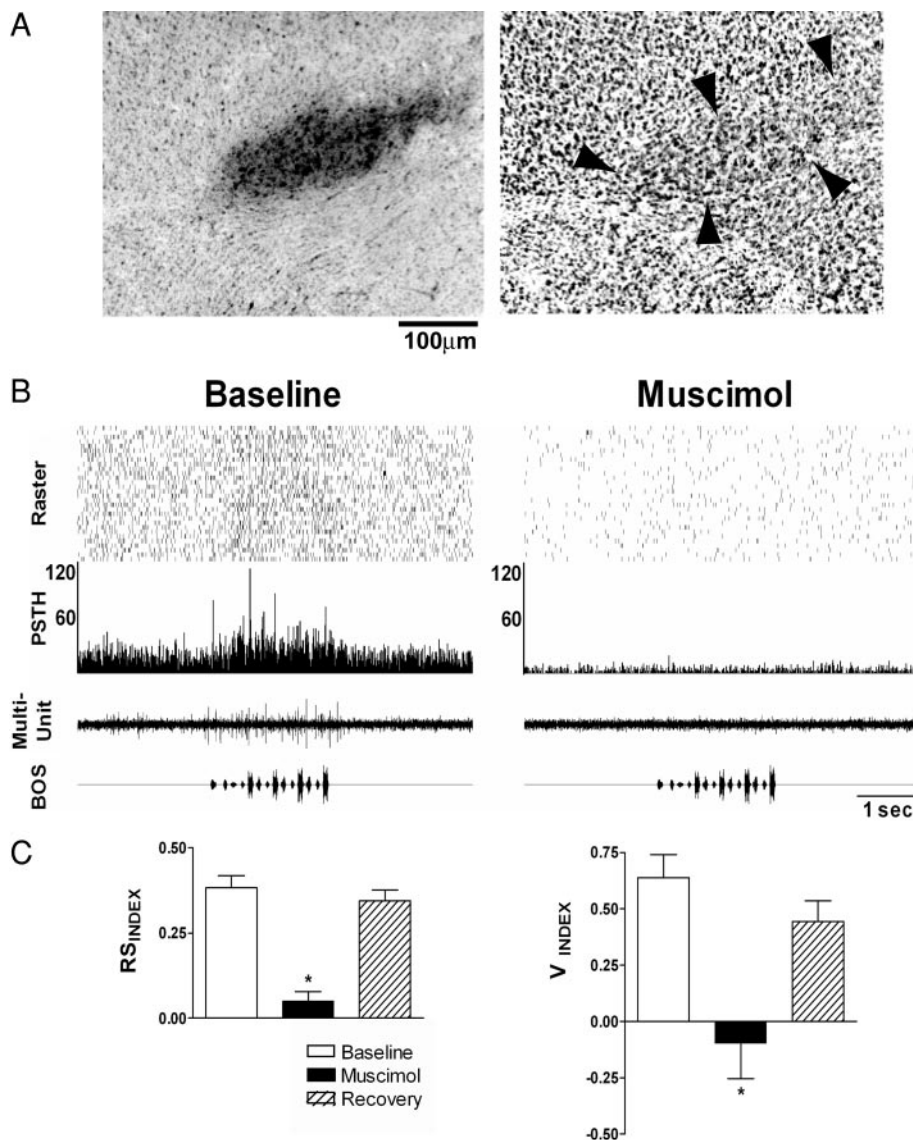


FIG. 12. Muscimol injection to Nif eliminates spontaneous activity and auditory responses in HVC. For these experiments, birds were lightly sedated and secured in the stereotaxic apparatus. Following a series of baseline auditory stimulus trials, a small dose of muscimol was injected into Nif and further auditory stimuli were presented. *A*: example of a drug injection site in Nif labeled with biotinylated dextran amine (BDA; *left*). BDA staining is restricted within the borders of Nif, as shown by comparison with the cresyl violet staining of the same section of tissue (*right*). Dorsal surface is at the *top* and posterior is to the *right*. *B*: example of acute recordings from HVC before and after injection of muscimol into Nif. During the baseline recordings (*left*), HVC showed a robust auditory response to the BOS stimulus. Injection of muscimol (0.2 μ l 8 mM muscimol in 0.9% saline) to the ipsilateral Nif caused a suppression of auditory responsiveness and most spontaneous activity in HVC (*right*). *C*: cumulative data from all muscimol experiments ($n = 5$ Nif-HVC pairs in 4 birds). Open bars, baseline recordings; black bars, recordings immediately after muscimol; hatched bars, recordings after recovery. Both mean HVC RS_{INDEX} and mean HVC V_{INDEX} were significantly reduced following muscimol injection to Nif ($P < 0.01$; paired t -test) and recovered to baseline levels after 15–40 min.

data support a role for Nif in providing auditory input to HVC and suggest that modulation of Nif activity is one possible mechanism by which HVC auditory responsiveness may be regulated.

DISCUSSION

In this study, we investigated the relationship between sensory processing in Nif and HVC during wakefulness and sedation. We recorded simultaneous multiunit Nif and HVC population auditory responses in a large number of awake birds. Like HVC, Nif demonstrated a significant degree of variation in auditory responsiveness during wakefulness. Nif and HVC auditory responsiveness co-varied over both short and long intervals in awake birds. Nif auditory responsiveness was robustly modulated by changes in the bird's behavioral state and completely suppressed by arousal. While both Nif and HVC were song-selective during sedation, both structures were predominantly unselective in awake birds. Finally, pharmacological manipulations of neural activity in Nif caused dramatic changes in HVC spontaneous and auditory activity.

Our results suggest that HVC is not the initial site of behavioral state-dependent modulation in the ascending auditory pathway. We propose that either HVC is indirectly altered by modulation arising in Nif or earlier afferent structures or Nif and HVC are co-modulated by external inputs. Alternatively, the observed changes in HVC auditory responsiveness might result from a combination of modulation in Nif feeding forward to HVC and additional modulatory effects within HVC itself.

Nif input is necessary for spontaneous and auditory activity in HVC

A previous study by Janata and Margoliash (1999) characterized the properties of Nif neurons in urethane-anesthetized birds. They found that Nif responded preferentially to the BOS stimulus but was not as song selective as HVC in the same birds. They also observed correlated firing patterns in Nif and HVC. In our experiments, we observed similar characteristics in sedated birds. Coincident bursting in Nif and HVC might be caused by Nif driving HVC activity. Alternatively, both struc-

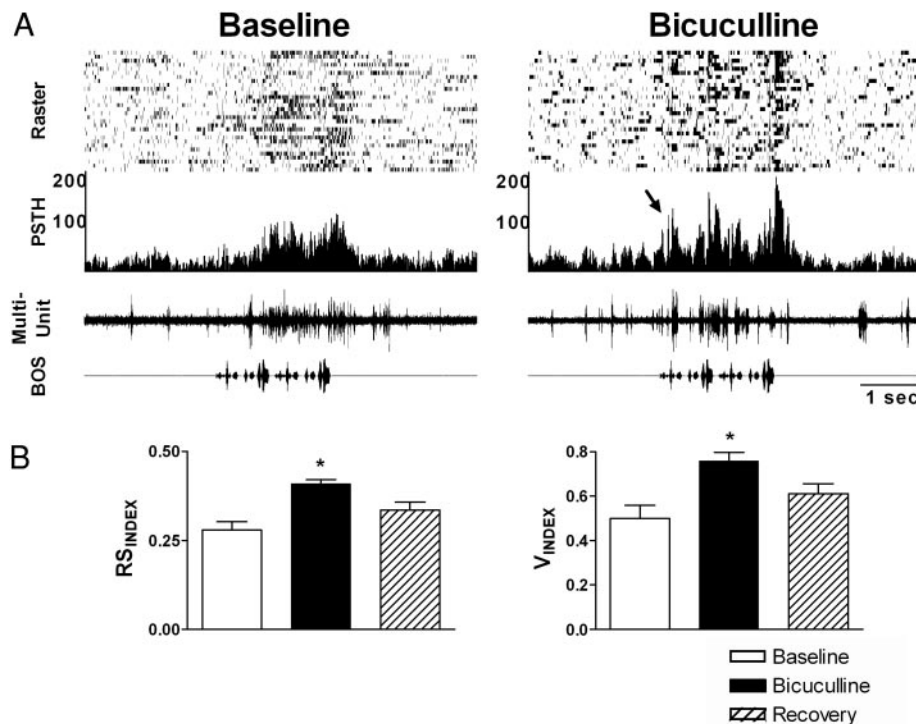


FIG. 13. Bicuculline injection to Nif increases auditory responsiveness in HVC. Neural recordings were made from HVC of lightly sedated birds before and after injection of small amounts of bicuculline to Nif. *A*: example of multiunit recordings in HVC before and after injection of a small dose of bicuculline to Nif ($0.2 \mu\text{l}$ 10 mM bicuculline in 0.9% saline). During the baseline recordings (left), there was a large HVC auditory response to the BOS stimulus. Following a small injection of bicuculline to the ipsilateral Nif, HVC auditory responsiveness was increased (right). In addition, a new peak emerged in the PSTH (arrow). *B*: cumulative data from all bicuculline experiments ($n = 4$ Nif-HVC pairs in 4 birds). Open bars, baseline recordings; black bars, recordings immediately after bicuculline; hatched bars, recordings after recovery. Both mean HVC RS_{INDEX} and mean HVC V_{INDEX} were significantly increased by bicuculline to Nif ($P < 0.01$; paired t -test) and recovered after 30–50 min.

tures might be driven by a common input such as Uva. In the latter case, Nif might provide a depolarizing offset to HVC neurons, allowing input from another source to drive HVC bursting. The almost complete loss of spontaneous HVC activity following injection of muscimol to Nif suggests that Nif inputs are necessary for spontaneous HVC activity under sedation. In contrast, levels of spontaneous activity in Nif during wakefulness are greatly increased, and Nif and HVC activity does not display any obvious degree of coordination.

Because Nif provides the strongest known anatomical input to HVC from any auditory structure (Fortune and Margoliash 1995; Nottebohm et al. 1982), it has been proposed as a major source of auditory input to HVC (Janata and Margoliash 1999). However, Field L projects to the HVC shelf region into which some HVC dendrites extend, suggesting a second possible route for auditory input to reach HVC (Fortune and Margoliash 1995; Kelley and Nottebohm 1979). Preliminary data have suggested that injections of GABA, lidocaine, or muscimol into Nif, but not Field L, affect the spontaneous and evoked activity of the ipsilateral HVC (Boco and Margoliash 2001; Fee et al. 2002). Likewise, we found in this study that injecting small doses of the GABA_A receptor agonist muscimol into Nif eliminated all auditory HVC activity. In addition, small injections of the GABA_A receptor antagonist bicuculline into Nif led to an increase in HVC auditory responsiveness. Together, these results suggest that Nif is a major source of auditory input to HVC. While it is possible that Nif inputs simply provide a depolarizing offset that allows another auditory input to drive HVC, it seems unlikely that the main auditory input to HVC comes from another source. Uva, the thalamic structure that projects to both Nif and HVC, does not demonstrate auditory responses (Wild 1994). MMAN, another forebrain structure that projects to HVC, demonstrates longer auditory response latencies than those observed in HVC (Vates et al. 1997). Finally, inputs to HVC from Field L are sparse and

primarily contact the shelf region around HVC (Fortune and Margoliash 1995; Kelley and Nottebohm 1979). If Nif is indeed a main avenue by which auditory input reaches HVC, even fine-grained changes in Nif auditory activity could have a profound impact on song system auditory processing. Such precise regulation of auditory feedback to the main song system might play a role in song learning and the maintenance of adult song.

Behavioral state dependence of the Nif-HVC relationship

We observed that both spontaneous and evoked activity in Nif and HVC was altered by changes in behavioral state. During sedation, multiunit recordings in HVC exhibited greater auditory responsiveness than those in Nif but similar response patterns to the BOS stimulus. The relationship between Nif and HVC auditory responses was greatly changed by behavioral state. While Nif and HVC auditory responses covaried in awake birds, Nif auditory responsiveness was higher than that in HVC. In addition, Nif and HVC response patterns were less similar in awake than in sedated birds. These results suggest that Nif inputs may have less impact on HVC activity during wakefulness than during sedation. Alternatively, Nif and HVC may be strongly driven by a shared input during sedation, but less so during wakefulness. Consistent with the idea of a behavioral state-dependent functional relationship between Nif and HVC, we observed consistent coordination of sedated Nif and HVC auditory activity, but found significant trial-by-trial correlation of Nif and HVC response strength in only 73% of the auditory trial sets in awake birds.

Auditory tuning in Nif was also greatly altered by changes in the bird's behavioral state. Multiunit recording sites in Nif demonstrated only a few instances of song selectivity during wakefulness but consistently strong selectivity for the BOS stimulus during sedation. Consistent with our previous exper-

iments (Cardin and Schmidt 2003b), multiunit recordings in HVC were largely unselective during wakefulness and showed robust BOS-selective responses during sedation. In a previous study, a subclass of HVC interneurons was shown to be song-selective during wakefulness (Rauske et al. 2003). Differences between those results and this study are most likely due to the fact that our recordings represent multiunit activity of a broad population of neurons, rather than single-unit recordings of a specific subpopulation. During sedation, HVC was significantly more song-selective than Nif. Interestingly, injection of bicuculline into Nif increased song selectivity in the ipsilateral HVC. Little is known about the underlying neural dynamics of Nif and the impact of Nif synapses in HVC. However, it seems likely that, as observed in HVC by Rosen and Mooney (2003), the expression of song selectivity in Nif neurons is precisely tuned by a very complex series of inhibitory and excitatory interactions. Disruption of these interactions could lead to altered song selectivity in Nif, which would then affect song selectivity in HVC.

These results support the idea of a hierarchical emergence of song selectivity along the ascending auditory pathway (Grace et al. 2003; Janata and Margoliash 1999; Lewicki and Arthur 1996), where Nif inputs provide some, but not all, of the selectivity demonstrated by HVC. The lack of song selectivity in both Nif and HVC during wakefulness suggests that these structures may be tuned to respond to a greater range of auditory stimuli during wakefulness than during sedation or sleep. This broadening of tuning may be consistent with a role for these high-order sensorimotor areas in general perception of complex auditory stimuli in awake birds.

Nif as a site of modulatory input

Several previous studies have found that auditory processing in the avian song system is modulated by the bird's behavioral state. Auditory responses in HVC are robust during sleep (Dave and Margoliash 2000; Rauske et al. 2003; Schmidt and Konishi 1998), sedation (Cardin and Schmidt 2003a,b), and anesthesia (Doupe 1997; Schmidt and Konishi 1998; Theunissen and Doupe 1998). HVC auditory responses are generally less robust in awake than in sedated birds (Cardin and Schmidt 2003b; Nick and Konishi 2001; Rauske et al. 2003; Schmidt and Konishi 1998) and are suppressed by arousal (Cardin and Schmidt 2003b). Auditory responses in Field L, which receives direct auditory thalamic input, are not affected by behavioral state (Cardin and Schmidt 2003b; Schmidt and Konishi 1998).

HVC is one potential site of direct modulatory effects. Studies in mammalian systems have indicated that the noradrenergic and cholinergic systems play roles in mediating the behavioral state-dependent modulation of sensory processing (for review, see McCormick 1992; McCormick and Bal 1997). Preliminary experiments have indicated that injection of norepinephrine to HVC disrupts auditory responses in RA (Dave et al. 1998) and that stimulation of cholinergic afferents affects the auditory responses of HVC neurons (Shea and Margoliash 2003). While HVC is thus a possible site of behavioral state-dependent modulation, the results of this study indicate that auditory responsiveness in Nif, an immediate afferent to HVC, is also robustly regulated by the bird's behavioral state. The pharmacological manipulations described here suggest that Nif does indeed provide auditory input to HVC. Together, these

results suggest that even though some modulation likely occurs within HVC, Nif is an additional target of behavioral state-dependent modulation.

The results of this study do not provide conclusive evidence that Nif, rather than another afferent structure, is the initial site of behavioral state-dependent modulation. However, immunohistochemical results indicate that Nif is a strong candidate for a site of direct modulation of song system auditory processing. Ryan and Arnold (1981) found evidence of acetylcholinesterase in Nif, suggesting a possible cholinergic innervation. In addition, fibers in Nif stain strongly for tyrosine hydroxylase in adult birds, indicating a catecholaminergic input (Soha et al. 1996). Mello et al. (1998) observed dopamine β -hydroxylase staining in Nif, suggesting that the input may be noradrenergic. Nif is thus a potential site of neuromodulatory action. While there is currently little evidence about the source of these inputs, preliminary data from our laboratory suggest that noradrenergic manipulations in Nif alter both spontaneous and stimulus-evoked activity in HVC (Cardin and Schmidt 2003a). More extensive pharmacological manipulations may help to elucidate the mechanisms of behavioral state-dependent modulation of sensory processing in this ascending sensorimotor pathway.

As a site of state-dependent modulation, Nif could provide a powerful avenue for regulation of auditory input to the rest of the song system. Auditory feedback to the song system is necessary for both song learning in juvenile birds (Konishi 1965; Price 1979) and song maintenance in adult birds (Brainard and Doupe 2001; Leonardo and Konishi 1999; Nordeen and Nordeen 1992). Nif is an auditory (Janata and Margoliash 1999), motor (McCasland 1987), and possibly, somatosensory (Wild 1994) structure and a source of auditory input to HVC. Because HVC contains neurons that project to the motor and anterior forebrain pathways, modulation of neural activity in Nif has the potential to directly affect auditory input to the rest of the song system.

ACKNOWLEDGMENTS

We thank M. J. Higley, D. J. Perkel, and the members of the Schmidt Laboratory for helpful discussions and comments during the preparation of this manuscript.

GRANTS

This research was supported by a predoctoral National Research Service Award by the National Institute of Deafness and Other Communication Disorders to J. A. Cardin and March of Dimes Basil O'Connor Starter Scholar Research Grant 5-FY00-626 and Whitehall Foundation Grant 2000-12-22-A to M. F. Schmidt.

REFERENCES

- Boco T and Margoliash D.** Nif is a major source of auditory and spontaneous drive to HVC. *Soc Neurosci Abstr* 318.2, 2001.
- Brainard MS and Doupe AJ.** Postlearning consolidation of birdsong: stabilizing effects of age and anterior forebrain lesions. *J Neurosci* 21: 2501–2517, 2001.
- Cardin JA and Schmidt MF.** Behavioral state-dependent modulation of auditory responses in HVC and Nif. *Soc Neurosci Abstr* 588.11, 2002.
- Cardin JA and Schmidt MF.** Effects of neuromodulators in Nif on auditory responses in HVC. *Soc Neurosci Abstr* 942.4, 2003a.
- Cardin JA and Schmidt MF.** Song system auditory responses are stable and highly tuned during sedation, rapidly modulated and unselective during wakefulness, and suppressed by arousal. *J Neurophysiol* 90: 2884–2899, 2003b.

- Castro-Alamancos MA.** Different temporal processing of sensory inputs in the rat thalamus during quiescent and information processing states *in vivo*. *J Physiol* 539: 567–578, 2002.
- Coenen AM and Vendrik AJ.** Determination of the transfer ratio of cat's geniculate neurons through quasi-intracellular recordings and the relation with the level of alertness. *Exp Brain Res* 14: 227–242, 1972.
- Dave AS and Margoliash D.** Song replay during sleep and computational rules for sensorimotor vocal learning. *Science* 290: 812–816, 2000.
- Dave AS, Yu AC, and Margoliash D.** Behavioral state modulation of auditory activity in a vocal motor system. *Science* 282: 2250–2254, 1998.
- Doupe AJ.** Song- and order-selective neurons in the songbird anterior forebrain and their emergence during vocal development. *J Neurosci* 17: 1147–1167, 1997.
- Fee MS, Hahnloser RHR, and Kozhevnikov AA.** The origin of a representation of time in the brain. *Soc Neurosci Abstr* 680.3, 2002.
- Fortune ES and Margoliash D.** Parallel pathways and convergence onto HVC and adjacent neostriatum of adult zebra finches (*Taeniopygia guttata*). *J Comp Neurol* 3603: 413–441, 1995.
- Grace JA, Amin N, Singh NC, and Theunissen FE.** Selectivity for conspecific song in the zebra finch auditory forebrain. *J Neurophysiol* 89: 472–487, 2003.
- Green DW and Swets JA.** *Signal Detection and Psychophysics*. New York: Wiley, 1966.
- Harding CF, Barclay SR, and Waterman SA.** Changes in catecholamine levels and turnover rates in hypothalamic, vocal control, and auditory nuclei in male zebra finches during development. *J Neurobiol* 34: 329–346, 1998.
- Janata P and Margoliash D.** Gradual emergence of song selectivity in sensorimotor structures of the male zebra finch song system. *J Neurosci* 19: 5108–5118, 1999.
- Kelley DB and Nottebohm F.** Projections of a telencephalic auditory nucleus-field L in the canary. *J Comp Neurol* 183: 455–469, 1979.
- Konishi M.** The role of auditory feedback in the control of vocalization in the white-crowned sparrow. *Z Tierpsychol* 22: 770–783, 1965.
- Leonardo A and Konishi M.** Decrystallization of adult birdsong by perturbation of auditory feedback. *Nature* 399: 466–470, 1999.
- Lewicki MS and Arthur BJ.** Hierarchical organization of auditory temporal context sensitivity. *J Neurosci* 16: 6987–6998, 1996.
- Lim D and Kim C.** Emerging auditory response interactions to harmonic complexes in field L of the zebra finch. *Auris Nasus Larynx* 24: 227–232, 1997.
- Livingstone MS and Hubel DH.** Effects of sleep and arousal on the processing of visual information in the cat. *Nature* 291: 554–561, 1981.
- McCasland JS.** Neuronal control of bird song production. *J Neurosci* 7: 23–39, 1987.
- McCormick DA.** Neurotransmitter actions in the thalamus and cerebral cortex and their role in neuromodulation of thalamocortical activity. *Prog Neurobiol* 39: 337–388, 1992.
- McCormick DA and Bal T.** Sleep and arousal: thalamocortical mechanisms. *Annu Rev Neurosci* 20: 185–215, 1997.
- Mello CV, Pinaud R, and Ribeiro S.** Noradrenergic system of the zebra finch brain: immunocytochemical study of dopamine- β -hydroxylase. *J Comp Neurol* 400: 207–228, 1998.
- Mooney R.** Different subthreshold mechanisms underlie song selectivity in identified HVC neurons of the zebra finch. *J Neurosci* 20: 5420–5436, 2000.
- Morrow TJ and Casey KL.** State-related modulation of thalamic somatosensory responses in the awake monkey. *J Neurophysiol* 67: 305–317, 1992.
- Nick TA and Konishi M.** Dynamic control of auditory activity during sleep: correlation between song response and EEG. *Proc Natl Acad Sci* 98: 14012–14016, 2001.
- Nordeen KW and Nordeen EJ.** Auditory feedback is necessary for the maintenance of stereotyped song in adult zebra finches. *Behav Neural Biol* 57: 58–66, 1992.
- Nottebohm F, Kelley DB, and Paton JA.** Connections of vocal control nuclei in the canary telencephalon. *J Comp Neurol* 207: 344–357, 1982.
- Poggio GF and Mountcastle VB.** The functional properties of ventrobasal thalamic neurons studied in unanesthetized monkeys. *J Neurophysiol* 26: 775–806, 1963.
- Price PH.** Developmental determinants of structure in the zebra finch song. *J Comp Physiol Psychol* 93: 260–277, 1979.
- Rauske PL, Shea SD, and Margoliash D.** State and neural class-dependent reconfiguration in the avian song system. *J Neurophysiol* 89: 1688–1701, 2003.
- Rosen MJ and Mooney R.** Inhibitory and excitatory mechanisms underlying auditory responses to learn vocalizations in the songbird nucleus HVC. *Neuron* 39: 177–194, 2003.
- Ryan SM and Arnold AP.** Evidence for cholinergic participation in the control of bird song: acetylcholinesterase distribution and muscarinic receptor autoradiography in the zebra finch brain. *J Comp Neurol* 202: 211–219, 1981.
- Schmidt MF and Konishi M.** Gating of auditory responses in the vocal control system of awake songbirds. *Nat Neurosci* 1: 513–518, 1998.
- Shea SD and Margoliash D.** Basal forebrain cholinergic modulation of auditory activity in the zebra finch song system. *Neuron* 40: 1213–1226, 2003.
- Soha JA, Shimizu T, and Doupe AJ.** Development of the catecholaminergic innervation of the song system of the male zebra finch. *J Neurobiol* 29: 473–489, 1996.
- Solis MM and Doupe AJ.** Anterior forebrain neurons develop selectivity by an intermediate stage of birdsong learning. *J Neurosci* 17: 6447–6462, 1997.
- Steriade M, Iosif G, and Apostol V.** Responsiveness of thalamic and cortical relays during arousal and various stages of sleep. *J Neurophysiol* 32: 251–265, 1969.
- Swadlow HA and Weyand TG.** Corticogeniculate neurons, corticotectal neurons, and suspected interneurons in visual cortex of awake rabbits: receptive-field properties, axonal properties, and effects of EEG arousal. *J Neurophysiol* 57: 977–1000, 1987.
- Theunissen FE and Doupe AJ.** Temporal and spectral sensitivity of complex auditory neurons in the nucleus HVC of male zebra finches. *J Neurosci* 18: 3786–3802, 1998.
- Vates GE, Broome BM, Mello CV, and Nottebohm F.** Auditory pathways of caudal telencephalon and their relation to the song system of adult male zebra finches. *J Comp Neurol* 366: 613–642, 1996.
- Vates GE, Vicario DS, and Nottebohm F.** Reafferent “thalamo-cortical” loops in the song system of oscine songbirds. *J Comp Neurol* 380: 275–290, 1997.
- Wild JM.** Visual and somatosensory inputs to the avian song system via nucleus uvaeformis (Uva) and a comparison with the projections of a similar thalamic nucleus in a nonsongbird, *Columba livia*. *J Comp Neurol* 349: 512–535, 1994.
- Zaretsky MD and Konishi M.** Tonotopic organization in the avian telencephalon. *Brain Res* 111: 167–171, 1976.