Behavioral State Modulation of Auditory Activity in a Vocal Motor System

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Neurons of the song motor control nucleus robustus archistriatalis (RA) exhibited far weaker auditory responses in awake than in anesthetized zebra finches. Remarkably, sleep induced complex patterns of bursts in ongoing activity and uncovered vigorous auditory responses of RA neurons. Local injections of norepinephrine suggested that the changes in response strength occur through neuromodulatory control of the sensorimotor nucleus HVC, which projects to RA. Thus, motor access to auditory feedback, which zebra finches require for song learning and maintenance, may be regulated through neuromodulation. During sleep, the descending motor system may gain access to sensorimotor song memories represented as bursting patterns of activity.

Changes in behavioral state are accompanied by changes in the functional properties of forebrain neurons. As animals transition to sleep, neurons may exhibit reduced responsiveness to external stimuli, reduced ongoing (“spontaneous”) firing rates, and increased bursting and synchronization. The cellular mechanisms for such changes are mediated by the actions of neuromodulators, including norepinephrine (NE). Behavioral and comparative studies have suggested that sleep may play a role in the stabilization of certain types of memory, including the learning of fine motor tasks, but, in general, the behavioral implications of sensory gating are not as well established (2). Here we report that neuromodulatory regulation within the bird vocal motor (“song”) system controls the expression of activity patterns associated with learned auditory information. In contrast to the pattern typical for other systems, sensory responsiveness increases during sleep.

Song learning requires auditory feedback, and the role of auditory feedback is modulated during development (3). In the forebrain, the nucleus HVC and its afferents are the principal targets of auditory input to the song system (4). Neurons in HVC project to one of two pathways, either the descending motor pathway through a projection to the forebrain nucleus robustus archistriatalis (RA) or the anterior forebrain pathway (AFP) that eventually projects back to RA (Fig. 1A). Whereas HVC and RA are necessary for singing, the AFP is necessary for the development of normal song, but lesions of AFP nuclei in the adult have little effect on singing in zebra finches (5).

We recorded single neurons in the HVC and RA of awake, freely moving animals (6). Numerous previous studies, mostly conducted in urethane-anesthetized animals, have shown that HVC (7,8) and RA and AFP (9) neurons have auditory responses that are specific for acoustic features of the individual bird’s own song (BOS) and are selective for BOS relative to conspecific songs. We also observed such selectivity in the auditory responses of single HVC neurons in awake birds but, surprisingly, failed to observe any auditory response whatsoever in RA neurons recorded under the same conditions (9). The RA neurons exhibited fast regular oscillatory spiking patterns that lacked the occasional bursts observed in recordings from anesthetized birds. The complete absence of an auditory response in RA may have been the result of a neuromodulatory response related to stress induced during a brief period when the animals were manually restrained to achieve single-unit isolation (6) (see below).

Exploring under what behaviorally relevant conditions RA neurons exhibited the auditory responsiveness observed in anesthetized animals, we discovered that when birds fell asleep, RA neurons acquired complex bursting in their ongoing activity and, remarkably, gained auditory responsiveness to BOS. At night, birds prepared for chronic recordings of RA neurons were presented with continuous playback of BOS (6). When the cage lights were turned off, motion in the cage (as judged by lack of audible movements) eventually ceased and the birds fell asleep. Without fail, sleep was accompanied by slower, less regular firing (14 single units, five birds; Fig. 1B) and a dramatic increase in the auditory response to BOS (10 of 14 single units and six multiple units in four birds were tested); the effect was sufficiently reliable to be easily seen in multiple unit traces (Fig. 1D). The RA “sleep” state of ongoing activity and BOS responsiveness was observed whenever we sampled RA during the night. The only

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exceptions were during brief episodes of audible movements, implying periods of wakefulness, or brief intervals (typically <30 s) during which ongoing activity transiently increased. The latter may reflect changes in stages of sleep, which in birds occur frequently and for brief intervals (10). The transition between sleeping and waking states could be rapid. For example, when a bird was awakened by a loud sound, RA neurons rapidly returned to the awake pattern of regular ongoing activity (Fig. 1C) and loss of auditory responsiveness (Fig. 1E).

To investigate the robustness of the day-night dichotomy and the role of behavioral state in modulating RA auditory responses, we tested RA multineuronal activity in three birds for responses to BOS beginning at night and continuing into the following day (6). In birds varying from posthatch day (PHD) 58, when juveniles are undergoing the process of learning their song, to adulthood, the results consistently demonstrated that during sleep, RA neurons responded strongly to BOS, whereas when animals were awake, responses were much weaker. Over all recording sites, daytime responses averaged 6 ± 8% (SD) of the responses at night or 9.3 ± 10.1% for the eight sites with statistically significant daytime auditory responses (11). In both adults and juveniles, the strongest auditory responses during the day were observed when birds were relatively silent and inactive (Fig. 1E). Restricting the analysis to the single period of behavioral quiescence with the strongest neuronal response during the day, one per recording site, the response was still just 17.1 ± 13.15% of the response at night. In two cases with birds that were accustomed to the chronic recording situation, we housed a female in an adjacent half-cage beginning in the middle of the day. The birds engaged in countercalling, the male’s singing and activity levels increased and the male sang “directed” songs toward the female, but there were no changes in the very weak auditory responses to BOS.

**Fig. 1.** (A) Schematic of the song system. (B) RA ongoing activity of the same neuron during wakefulness (W) and sleep (S) in an adult bird. (left) Traces of 5 s of single-unit activity; (right) ISI histograms derived from the two states. The fast, regular activity while awake resulted in a single-mode ISI. During sleep, the neuron was slower, less regular, and bursting, resulting in a rightward shift, broadening, and anisotropy of the major ISI mode and emergence of a second mode near zero. (C) The bird was asleep and then suddenly awoke when a loud sound was presented (at arrow). Note rapid change in RA ongoing activity. (D) Three sites from one adult bird recorded on different nights. Each pair of traces represents the rectified averaged multunit response to multiple presentations of BOS during wakefulness or sleep (top to bottom, n = 20, 50, or 60 repetitions, respectively). The bottom panel is a spectrograph, frequency versus time representation of the BOS stimulus. (E) RA multunit activity in response to BOS presented throughout the night and continuing past when cage lights were turned on (at arrow). Each row represents 10 min of the experiment (20 presentations of BOS, once per 30 s). For each row, neuronal activity for 8 s starting 1 s before BOS was averaged over the 20 presentations. Response strength is represented by the color scale from white (weakest) to black (strongest). BOS is shown as a spectrograph (frequency versus time) in bottom panel. The graph to the right shows the average RMS power of sounds recorded in the cage over the same intervals during which the neuronal signals were recorded. The constant RMS contribution from the stimulus presentation was removed. The remaining sounds arise from the bird’s vocalizations and movement-generated cage noises and are a reflection of general activity levels. Note the strong stimulus-aligned neuronal responses at night, the sudden transition to unresponsive state at the start of day, and the occasional daytime responses that are observed during times of reduced activity levels. During these periods, the bird appeared to be resting. The period of behavioral quiescence used to estimate the strongest daytime responses is shown by the vertical bar to the right. The response over that interval was 49.6% of the response at night, the strongest daytime response observed at any of the 14 sites analyzed.

**Fig. 2.** A raster plot of activity of a single RA unit. Each tick mark represents the time of occurrence of a spike, relative to the multiple presentations of the BOS stimulus. BOS is shown as a spectrograph in the bottom panel. The bird was initially awake and restrained and then was administered a single injection of anesthetic (50 μl, 20% urethane intramuscular) at the arrow. The response to BOS developed as the anesthetic took effect. In this bird, before the anesthetic took effect. In this bird, before
responses. Thus, RA neurons are considerably less sensitive to auditory stimulation during wakefulness than during sleep; this sensitivity may vary with the level of stress or alertness but is not apparently engaged by social interactions.

The properties of RA neurons during sleep are similar to those observed in anesthetized animals, which could provide a useful experimental paradigm. To quantify the differences between awake and anesthetized states, we recorded a sample of RA and HVc neurons in urethane-anesthetized birds. Simultaneous recordings showed that bursting activity in RA and HVc was correlated (12). We also recorded from the RA of two awake-restrained animals and a third animal that had participated in the awake-chronic RA recordings, before and after the animals were anesthetized. In all three birds, RA neurons failed to show auditory responses while the animals were awake but exhibited strong auditory responses after the animals were anesthetized (13). Rates of ongoing activity declined and neurons commenced to burst as animals were anesthetized; this trend followed the differences comparing awake, freely moving animals and anesthetized animals (14). Particularly compelling are the three cases, one per animal, where a single unit was maintained while the bird was anesthetized, showing the decrease in ongoing rate, increase in bursting, and the emergence of auditory responses at the single cell level over the short time interval as the anesthetic took effect (Fig. 2). Thus, the auditory input to RA is presumably latent in awake animals and is unmasked by anesthesia in qualitatively similar ways as it is by sleep.

RA and HVc receive a variety of neuromodulatory inputs, including noradrenergic input from the locus coeruleus, and have high concentrations of adrenergic receptors (15). We postulated that changes in concentrations of norepinephrine (NE) may affect expression of RA auditory responses. To test this hypothesis, we characterized the ongoing activity and auditory response properties of recording sites in RA and HVc before and after pressure injections of 20 mM NE (200 to 250 nl) into RA or HVc of urethane-anesthetized zebra finches. In all birds tested, small injections of NE into HVc abolished or greatly diminished auditory responses in RA (Fig. 3), whereas small injections of NE into RA did not abolish auditory responsiveness of RA neurons (Fig. 4) (16). Complementary effects on bursting were also observed for the two manipulations. Injections of NE into RA did not eliminate bursts in RA ongoing activity, whereas injections of NE into HVc did; injections of either structure increased the rate and regularity of ongoing RA activity (17). After injections of NE into HVc or into RA, we tested HVc for auditory activity and found that it was retained (Figs. 3 and 4). In control (200 nl) injections of vehicle into HVc, we observed no effect on RA auditory responses; large (500 to 1000 nl) injections of NE into or dorsal to RA compromised auditory responses in RA (18). The large injections were associated with reflux along the dorsoventral electrode track (that passed caudal to HVc) and probably involved HVc as well as RA. Although these experiments do not unambiguously confirm the site or pharmacological mode of action underlying these phenomena, they demonstrate that auditory responsiveness in RA is sensitive to neuromodulation and suggest that the principal site of action is other than RA, presumably HVc. NE has additional, local effects on RA activity (19).

Previous studies suggested that the descending motor pathway could participate in song perception if conspecific songs are perceived in terms of the articulatory gestures necessary to produce those songs. This parallels the “motor” theory of speech perception by reference to production (20). The theory for birds, however, was based on recordings in anesthetized animals of auditory responses in the brainstem hypoglossal nucleus that arise from HVc input (21). The present data suggest that the hypoglossal responses are likely to be very weak or nonexistent in awake animals, making this pathway an unlikely candidate to contribute to conspecific song perception.

Adult zebra finches require auditory feedback to maintain their songs (22). During sleep, RA neurons exhibited bursting activity correlated with complex bursts typical of HVc neurons. An intriguing possibility is that auditory feedback processed during the day modifies the bursting behavior of HVc, which would then be communicated to RA during the night. HVc activity patterns, in-
including bursting, are synchronized across a large spatial extent of the nucleus (23). Information encoded in these bursts may stabilize aspects of the vocal motor program encoded in the neuronal population activity patterns. A similar scheme has been suggested for stabilizing patterns of hippocampal neurons recruited during exploration of novel extra-personal space (24).

Accounts such as the template theory of birdsong learning posit sensory and sensorimotor phases of development but do not address the role of behavioral state. Our data indicate that sensory properties of neurons in the motor pathway for song are sensitive to changes in behavioral state throughout the day as well as during sleep. Indeed, in adults, the auditory response properties of HVc neurons are apparently suppressed during singing (7). Singing recruits most if not all HVc neurons (25) and can involve a preparatory phase characterized by increasing ongoing activity starting up to 5 s before the onset of singing (26). Such dynamic reconfiguration of the network may involve local changes in conjunctions of neurotransmitters. Neuronal modulators exhibit a complex developmental regulation in the song system (27), which could contribute to a possible modulation of the effect of auditory feedback on HVc neurons during the sensorimotor phase of song learning.

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4. Responses were computed as the percentage of change in the average spiking rate during the stimuli (typically 30 repetitions) relative to the ongoing rate. Ongoing rates were computed from long (>300 s) recordings of 20-26 neurons (seven birds) that exhibited premotor activity in relation to singing, 19 also responded to broadcast sounds. When those neurons were presented with BOS and reversed BOS (REV) (N = 17 units, 13 recording sites, six birds) or BOS and conspecific songs (CON) (N = 13 units, six recording sites, two birds), they exhibited much stronger responses to BOS (BOS > REV: paired t = 3.42, df = 18, P < 0.004; BOS > CON: paired t = 4.14, df = 12, P < 0.002). In contrast, no RA neurons recorded under the same conditions exhibited any statistically significant response to BOS (N = 28 SU, 33 recording sites, 17 birds). On the basis of these procedures, 25 sites from five awake, freely moving animals had average SI5 of 30.7 ± 7.4 ms (32.6 Hz) for the major mode. The irregularity of firing for the major mode was 5.6 ± 4.9 ms (determined from the distribution of SD from the Gaussians). In contrast, data from 60 recording sites (68 single units) from 12 urethane- anesthetized birds resulted in an average ISI of 98.9 ± 32.6 ms (10.1 Hz) for the major mode. The irregularity of firing for the major mode was 20.9 ± 17.2 ms. These differences are significant (average ISI: z = 8.095, P < 0.0001; irregularity: Z = 6.774, P < 0.0001). The index of bursting was 7.5 ± 8.3% for data from awake animals and significantly higher at 12.8 ± 7.4% from urethane-anesthetized birds (z = 3.742, P < 0.001).


6. Two birds received two left and one right hemisphere injections of NE into RA with 27 and single multiple sites recorded in RA before injection and 17 after injection. Using the measure of response strength for single units (13) and a criterion of response strength > 3, we found that before and after injections of NE into RA, there were 23/26 and 16/17 BOS responsive units, respectively, recorded in RA. The average response strength was 19.7 ± 34.8 before and 16.4 ± 16.0 after injections; the difference was not significant (Z = 0.064, P = 0.95), indicating that the increased NE into IT might affect, but three in RA. Neurons increased in response strength of 111, 1860, and 634%. In one bird, vehicle (artificial cerebral spinal fluid) was presented every 30 s throughout the night that was preceded or followed within 30 s by singing. After obtaining a stable multi-unit activity, the bird was restrained during recording. In both restrained birds gave a response strength for BOS of 1.00 ± 0.000 (28 single units, 15 multiunits) from four adult birds, three neurons had similar changes in response strength of 111, 1860, and 634%. In anesthetized animals, auditory stimuli elicited immediate vocalizations. Daytime videoaudiotape recordings facilitated visual assessment of the bird’s activity.


9. BOS was presented every 30 s throughout the night that was preceded or followed within 30 s by singing. After obtaining a stable multi-unit activity, the bird was restrained during recording. In both restrained birds gave a response strength for BOS of 1.00 ± 0.000 (28 single units, 15 multiunits) from four adult birds, three neurons had similar changes in response strength of 111, 1860, and 634%. In anesthetized animals, auditory stimuli elicited immediate vocalizations. Daytime videoaudiotape recordings facilitated visual assessment of the bird’s activity.
Microscale Nutrient Patches in Planktonic Habitats Shown by Chemotactic Bacteria

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Are nutrients available to microbial communities in micropatches long enough to influence growth and competition? And what are the sources of such patches? To answer these questions, the swimming behavior of chemotactic bacteria in seawater samples was examined. Clusters of bacteria formed in conjunction with cell lysis and excretion by protozoa. These point sources of nutrients spread into spherical patches a few millimeters in diameter and sustained swarms of bacteria for about 10 minutes. Within that time, a large proportion of the nutrients was encountered by bacteria, chemotactic and nonchemotactic alike. Chemotaxis is advantageous for bacteria using patches over a certain size.

The existence of microscale nutrient patches in pelagic habitats has important implications for microbial ecology (1). Patches represent resources that are available within limited time and space. This creates situations that encourage competitive foraging, and elevated concentrations within patches increase transfer rates of nutrients into the food web. One line of evidence that patches exist is based on observations that a proportion of aquatic bacteria swim, an effort that is beneficial only in a heterogeneous nutrient environment (2). The nature of targets for chemotactic bacteria has remained largely a matter of speculation, although bacterial chemotaxis is stimulated by organic and inorganic compounds. Interest has focused on algal exudation since the discovery of symbiosis between bacteria and species of terrestrial plants (3–5), but experimental evidence has been contradictory (4, 6). Point-source releases of nutrients have been suggested to result in patches that are consumed before dispersing to background levels (7). We attempted to resolve the question by direct observation of microbial communities.

Observations of seawater samples (8) revealed that clusters of bacteria continuously formed and dispersed. Some sources of attractants were identified as the autolysis of a large microbe, such as an algal cell or a protozoan (Fig. 1A). Other clusters formed without any visually distinct source (Fig. 1B). Various species of ciliates were often seen at the center of these clusters, which we assumed were related to the discharge of undigested organic matter and inorganic nutrients from food vacuoles (9). Other zooplankton excrete plumes of nutrients (10). Studies have focused mainly on the importance of these nutrient plumes for phytoplankton growth (10, 11), and conclusions have been largely negative. However, bacteria have 100 times the uptake potential of phytoplankton and are therefore potential key players in rapidly consuming dissolved nutrients from patches and transferring them into the food web.

The current model of bacterial chemotaxis is based on swimming behavior of the enteric bacterium Escherichia coli (12). Reports of different swimming behavior displayed by strains of marine bacteria (13) pose the question of whether the model is widely applicable. To test this, we studied swimming behavior of bacteria from seawater enrichments under conditions of low oxygen saturation (14), where they were observed to form clusters around algae producing oxygen (Fig. 1C). Motility patterns could be reproduced by simulations (Figs. 1D and 2). At an intermediate distance, the mean radial component of runs toward the source increased by a factor of 2.5. This local maximum was a result of an alignment phenomenon, where runs heading...

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Fig. 1. (A) Cluster of bacteria around a lysed ciliate in a seawater sample tracked (21) over 2 s (velocity $v = 25 \mu m \cdot s^{-1}$, run duration $\tau = 0.3 s$). (B) Cluster of large bacteria in a cloud of attractant in a seawater sample tracked over 2 s ($v = 50 \mu m \cdot s^{-1}$, $\tau = 0.5 s$). (C) Bacteria cultured on 0.02% tryptic soy broth swarming around an individual Pavlova lutheri cell as a response to the oxygen gradient tracked over 16 s ($v = 25 \mu m \cdot s^{-1}$, $\tau = 0.3 s$). (D) Simulation as described in Fig. 2 of the scenario shown in (C). Bars, 50 $\mu m$.

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