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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 (*1*). 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 (*8*) 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

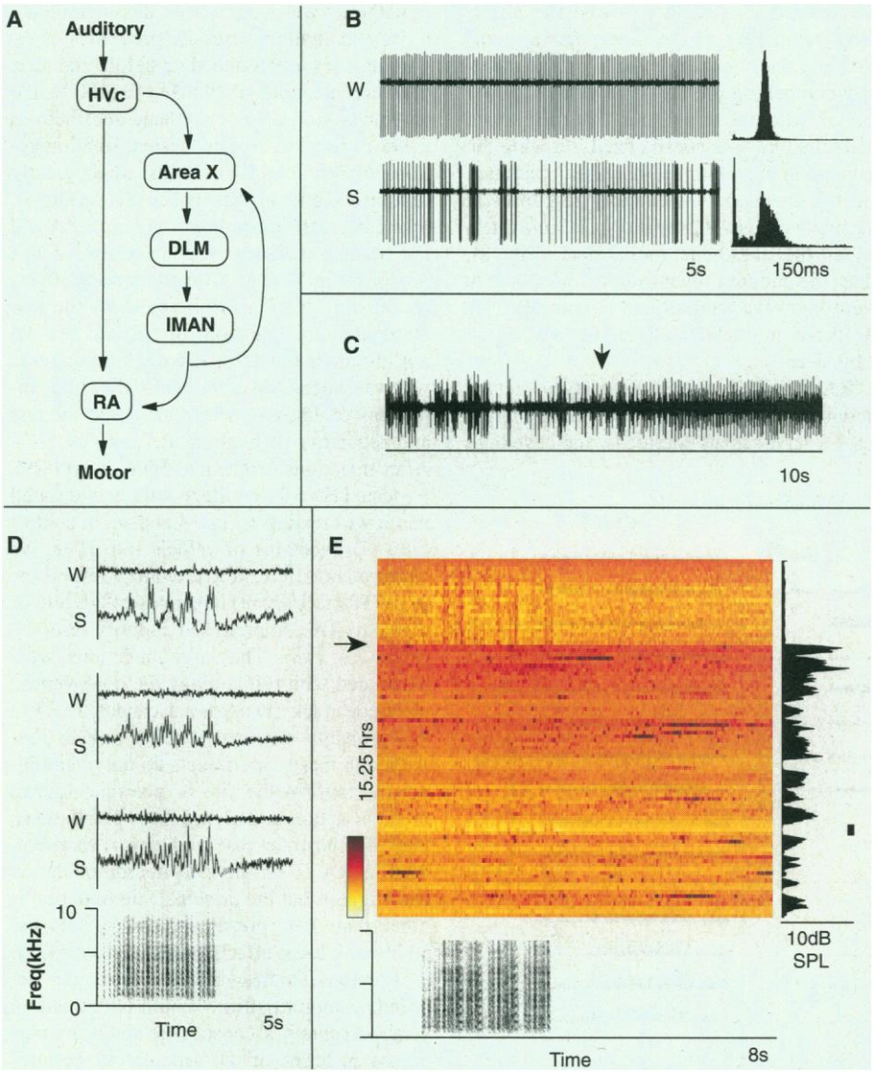

Fig. 1. (**A**) Schematic of the song system. (**B**) RA ongoing activity of the same neuron during wakefulness (W) and sleep (S) in an adult bird. (left) Traces of 5 s of single-unit activity; (right) ISI histograms derived from the two states. The fast, regular activity while awake resulted in a single-mode 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 multiunit 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 multiunit 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.

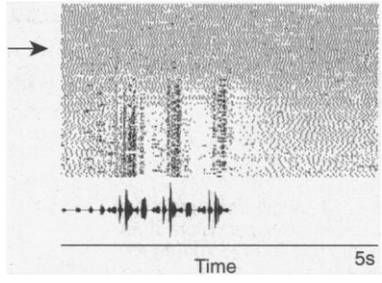

Fig. 2. A raster plot of activity of a single RA unit. Each tick mark represents the time of occurrence of a spike, relative to the multiple presentations of the BOS stimulus. BOS is shown as a spectrograph in the bottom panel. The bird was initially awake and restrained and then was administered a single 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 anesthetization, all other recordings from RA sites (N = 4) also failed to show any auditory activity; most (N = 5/6) RA sites recorded after the illustrated site showed auditory responses to BOS (*13, 28*).

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-

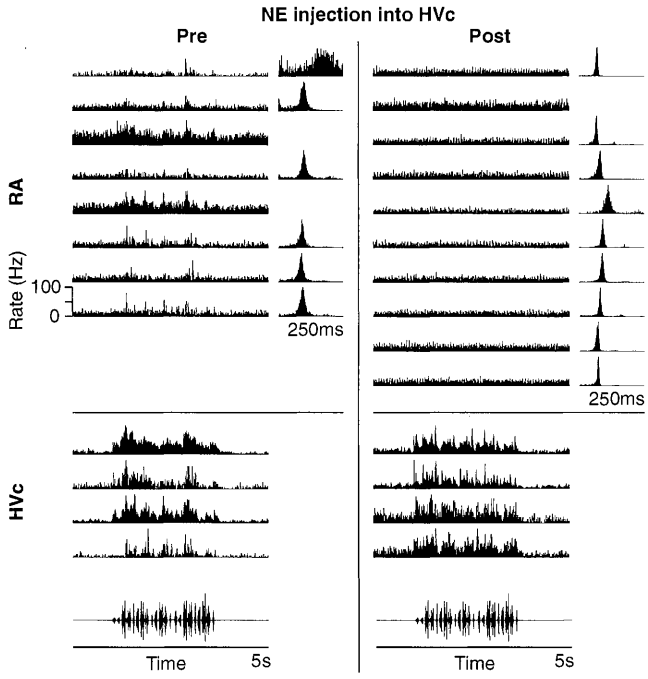

Fig. 3. PSTH representations of RA single units (with corresponding ISI distributions of ongoing activity to the right of the PSTH) and multiunit RA activity (lacking ISI distributions) from a single bird before (left panels) and after (right panels) perfusion of HVc with 200 to 250 nl of 20 mM NE. Each bin in the PSTH represents the firing rate averaged over all presentations of BOS over a 10-ms interval. The middle panels show HVc multiunit responses, and the bottom panels show the BOS stimulus. Note the absence of auditory responses in RA but not in HVc and the changes in ISI distributions, subsequent to administration of NE into HVc.

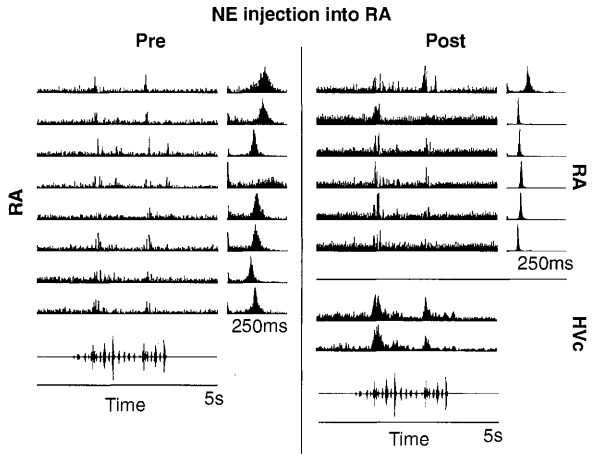

Fig. 4. RA single-unit responses before and after perfusion of RA with 200 to 250 nl of 20 mM NE. Similar layout as for Fig. 3. RA auditory responses are present after administration of NE, but there is an effect on the ISI distributions (see text).

cluding 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 extrapersonal 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 concentrations of neuromodulators. Neuromodulators 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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9. 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) records. Of 29 HVc single units (19 sites, 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 = 19 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, four birds).
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11. RA activity was recorded from one adult (> PHD 150) (three sites, 3 days), from a PHD 59 to 69 juvenile (four sites, 4 days), and from a PHD 58 to 81 juvenile (eight sites, 7 days). BOS was presented at 30-s intervals starting 3.8 to 8.0 hours before light onset and thereafter throughout the day (*6*). Multiunit responses were averaged for each 20 repetitions (1-ms bins), and then the mean of the ongoing activity (the average over 2 to 5 s starting 1 s after stimulus offset) was subtracted. At each site, the presence of an auditory response during sleep or wakefulness was determined by comparing distributions (t tests) of the RMS values calculated over the duration of BOS and during ongoing activity. By this measure, 14/14 sites showed auditory activity during sleep ($P \leq 0.0054$), and 8/14 sites (3/3 birds) showed auditory activity during wakefulness ($P < 0.05$) (data from one site were lost).
12. Ongoing unit activity from HVc and RA was binned (1-ms resolution) and cross correlated (16 pairwise comparisons, 11 sites, three birds). A bootstrap procedure showed that the peak correlation was significant ($P < 0.02$) in 15/16 comparisons, with HVc leading RA by 2 to 14 ms in 12/15 cases (two birds). A third bird contributed three cases; for all three, RA led HVc (by 1, 28, and 44 ms). Correllelograms were much broader for these three paired recordings than observed for any of the recordings in which HVc led RA.
13. Firing rates were estimated from peristimulus time histograms (PSTHs) with 50-ms bins. The strength of response was quantified from the PSTH as the ratio of the variance in firing rate during stimulus presentation over the variance in ongoing activity beginning 1 s after the stimulus offset. This measure is sensitive to slight stimulus-driven changes in temporal patterns of spike trains. Nevertheless, 33 recordings sites (28 single units, 15 multiunits) from four adult awake, freely moving birds gave a response strength for BOS of 1.00 ± 0.40 (1 = no response). In contrast, 72 recording sites (71 single units, 33 multiunits) from 12 urethane-anesthetized birds gave a response strength for BOS of 18.71 ± 33.81. This difference was significant (Mann-Whitney U, Z = −9.004, $P < 0.0001$). Twenty-two recordings sites (14 single units, 15 multiunits) from five awake but restrained birds gave a response strength for BOS of 2.77 ± 2.62. In only one awake-restrained bird were most sites auditory (six single units, six multiunits, seven sites). One of the animals with chronic headgear and two of the awake-restrained animals were subsequently anesthetized, resulting in average increases in response strength of 111, 1860, and 634%.
14. In awake animals, the distribution of interspike intervals (ISIs) of ongoing activity of RA single units had a single mode corresponding to the mean of the regular pattern of firing. In anesthetized animals, ISI distributions typically had two modes, a long-interval (major) mode reflecting the regular firing pattern and a short-interval (minor) mode reflecting the complex bursts. The major mode was fit with a Gaussian, and then the Gaussian was subtracted from the data. The number of residual intervals from 0 to the Gaussian mean −2 SD, as a percentage of the total number of intervals, was taken as the index of bursting. With these procedures, 29 recording sites (33 single units) from five awake, freely moving animals had average ISIs 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 = −3.724, $P < 0.0003$).
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16. Two birds received two left and one right hemisphere injections of NE into RA, with 27 single and multiple units 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 23/27 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 = −0.06, P = 0.9519). In contrast, in three birds injected with NE into HVc, we recorded 27 units in RA before and 30 units after injection. There were significant changes in the number of responsive units and the magnitude of the responses. Before and after injections of NE into HVc, there were 21/27 and 5/30 BOS responsive units, respectively, recorded in RA. The average response strength was 8.5 ± 6.6 before and 2.0 ± 1.6 after injections; the difference was significant (Z = −5.090, $P <$ 0.001).
17. For NE injections into RA, the mean of the major mode of the ISI distributions for RA neurons decreased significantly from 98.7 ± 34.7 ms before injections to 63.1 ± 22.0 ms after injections (Z = −3.842, $P < 0.0002$). The SD of the major mode also decreased significantly from 16.2 ± 16.8 to 3.6 ± 2.3 ms (Z = −6.308, $P < .0001$), indicating an increase in the regularity of firing. The burstiness changed from 17.1 ± 8.9 to 14.0 ± 5.2%; this change was not significant (Z = −1.146, P = 0.2516). For NE injections into HVc, all three measures of RA ongoing activity changed significantly ($P < 0.05$) (mean ISI: 123.0 ± 26.9 to 102.4 ± 24.0 ms; irregularity: 29.0 ± 20.9 to 16.3 ± 15.6 ms; bursting: 14.3 ± 7.5 to 9.5 ± 5.7%).
18. In one bird, vehicle (artificial cerebral spinal fluid stained with dextran-rhodamine) was injected into HVc. All RA sites before (N = 7) and after (N = 8) the injection were auditory. Postmortem histology indicated that the injection filled but was restricted to HVc. Three birds received large injections of NE targeted toward RA but that probably involved large parts of the caudal forebrain (see text). On the basis of the criterion of response strength > 3 (*16*), in the three birds, 28/35 and 8/26 RA units were auditory before and after injections, respectively.
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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 an inhomogeneous 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

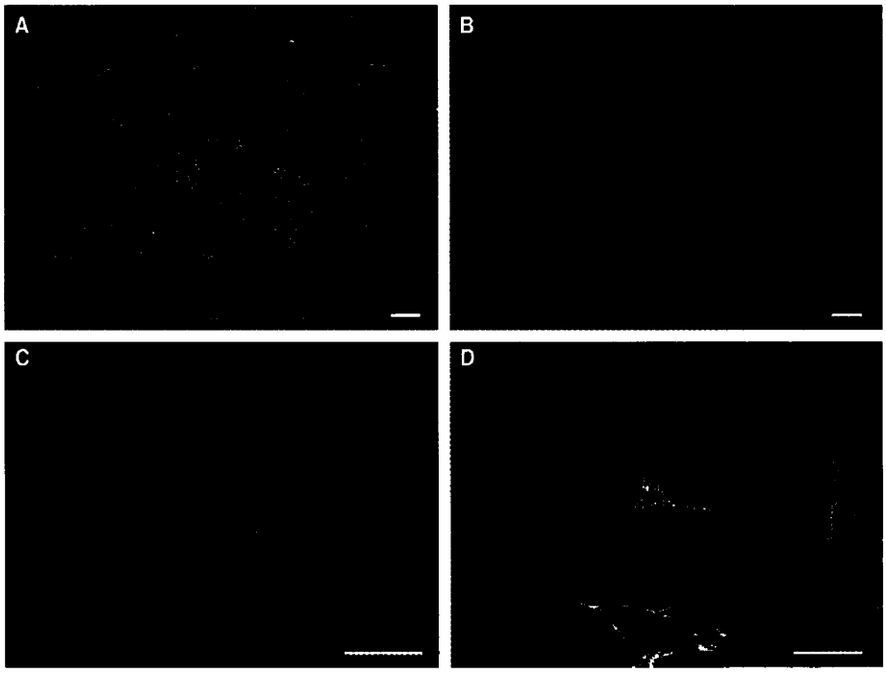

Fig. 1. (**A**) Cluster of bacteria around a lysed ciliate in a seawater sample tracked (*21*) over 2 s (velocity v = 25 μm s^{-1}, run duration τ = 0.3 s). (**B**) Cluster of large bacteria in a cloud of attractant in a seawater sample tracked over 2 s (v = 50 μm s^{-1}, τ = 0.5 s). (**C**) Bacteria 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 μm s^{-1}, τ = 0.3 s). (**D**) Simulation as described in Fig. 2 of the scenario shown in (C). Bars, 50 μm.

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