Dynamic control of auditory activity during sleep: Correlation between song response and EEG

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The song nucleus high vocal center (HVC) sends neural signals for song production and receives auditory input. By using electroencephalography (EEG) to objectively identify wake/sleep state, we show that HVC auditory responses change with physiological states. Comparison of EEG and HVC records revealed that HVC response to auditory stimuli is greatest during slow-wave sleep. During slow-wave sleep, HVC neurons responded preferentially to the bird's own song. Strikingly, both spontaneous and forced waking during sleep caused HVC auditory responses to cease within milliseconds of an EEG-measured state change. State-dependent phenomena in downstream nuclei, such as robustus archistriatalis, are likely to be derivatives of those in HVC.

The song system of birds contains neurons that respond to auditory stimuli, particularly the individual bird's own song (BOS) (1). Song-specific responses occur even within the pathway that conveys neural signals to the muscles of the vocal organ (2). Recent studies suggest that auditory responses within this pathway occur primarily or are greatly enhanced under anesthesia or in sleep (3–5). These artificial and natural physiological states seem to lift the gate that controls auditory input. These findings raise two important issues: one concerns the definition of relevant physiological states; the other concerns the primary site of the gate. To address the first question, we used electroencephalography (EEG) to investigate the state dependence of high vocal center (HVC) auditory responses and, further, to examine the temporal dynamics of these state changes. For the second question, we discuss the results from HVC with reference to the published data from a follower nucleus, robustus archistriatalis.

Methods

Animals and Surgery. General methods were previously described by Schmidt and Konishi (3). Eighteen adult (>120 days post-hatching) male zebra finches (*Taeneopygia guttata*) were obtained from our breeding colony or from a local vendor (Magnolia Bird Farm, Los Angeles, CA).

Birds were anesthetized with 60–90 μl intrapectoral 3 mg/ml ketamine hydrochloride (Phoenix Pharmaceuticals, St. Joseph, MO) and 1.5 mg/ml xylazine hydrochloride (Lloyd Laboratories, Shenandoah, IA) in 0.9% sodium chloride 45 min before surgery. After local injection of lidocaine hydrochloride (1% xylocaine, Astra Pharmaceutical, Worcester, MA), the skull was exposed through an incision in the scalp. Small openings were made in the skull over the right HVC, over the right hyperstriatum accessorium anterior to HVC for placement of the reference electrode, and over the right and left hyperstriatum ventrale anterior and lateral to HVC for placement of the EEG electrodes.

HVC recording electrodes were made of formvar-insulated nichrome wires (66-μm diameter; AM Systems, Everett, WA), the tips of which were coated with rhodium to lower impedance. EEG electrodes were silver wires coated with Teflon (AM Systems). Teflon was stripped from the last ≈3 mm (75-μm tip diameter). The reference electrode was also 75-μm silver wire completely stripped of Teflon. EEG and reference wires were placed between the dura mater and the brain surface. All impedances were under 1 MΩ.

All electrodes were attached to a 6-pin nanoconnector (Ultimate, Orange, CA) which was cemented onto the bird's skull (GripCement, Milford, DE). After recording, birds were terminally anesthetized with sodium pentobarbitol (1.05 mg; Abbot) and perfused with 0.9% saline followed by 2% paraformaldehyde. Electrode placement was confirmed with cresyl violet histology in eight birds. An institutional animal care committee approved all procedures.

Electrophysiology. Experiments were performed in a sound-attenuating chamber (Industrial Acoustics, Bronx, NY), which held a custom-built Plexiglas recording chamber. The nanoconnector on the bird's head was connected to a LinCMOS low noise operational amplifier (OpAmp, TLC27L4B; Texas Instruments, Dallas, TX) which, in turn, was connected to a custom-made 9-channel mercury commutator.

Songs were played back through a wide-band speaker (Madisound Speaker Components, Madison, WI) after 10 kHz low-pass filtering through a 6-pole anti-aliasing filter (FT6; Tucker–Davis, Gainesville, FL). Playback and subject-generated songs were recorded with an omnidirectional microphone (Radio Shack).

Electrode records were amplified with a 4-channel AC amplifier (AM Systems). A 2-pole filter built into this amplifier was used to band-pass filter the HVC recordings between 300 and 20,000 Hz. For EEG data, the filter was set at 1–500 Hz. Data were also 10 kHz low-pass filtered through an FT6 filter.

Electrode and microphone data were digitized at 20 kHz with a 16-bit DAQ board (PCI-MIO-16XE-10; National Instruments, Austin, TX). Data collection software was written by A. Leonardo by using Labview (National Instruments). Songs for playback were digitized at 40 kHz and edited with Matlab (Mathworks, Natick, MA). To ensure that the recording electrodes were indeed in HVC, we measured premotor activity for song in awake birds with these electrodes before monitoring the effects of various manipulations.

We compared waking data with sleep data acquired between 1 a.m. and 5 a.m. For sleep/wake comparisons, 4–5-s songs were presented during 7-s recording trials with 10-s intertrial intervals. Birds were usually presented with 300 sequences. The BOS, the reversed BOS (REV), and a conspecific song (CON) were presented in random order. Data from awake (W) animals were collected with overhead lights on. For comparison, sleep (S) data were obtained within 24 h either before or after the W session. In W sessions, birds were constantly observed to monitor their state of consciousness. Trials during which birds fluffed their

Abbreviations: BOS, bird's own song; CON, conspecific song; EEG, electroencephalography; HVC, high vocal center; REV, reversed BOS; S, sleep; W, wake; PSTH, peristimulus time histogram.

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feathers and closed their eyes were separated from the W trials and analyzed separately.

For the studies of waking with light, 6-s recordings were taken with 3–4 s of BOS playback initiated 1 s after data collection began. A subset of these recordings was "light trials," during which a 300-ms pulse of light was initiated 2.25 or 3.25 s after BOS playback began. The protocol for waking the bird with a pulse of light during BOS playback was designed to familiarize the bird with the auditory stimulus while periodically and randomly waking him. The protocol is represented graphically in Fig. 5*a*. Blocks of trials occurred between 11 p.m. and 3 a.m. Every 25 min, a new block of 125 trials began. Each block of trials lasted 21 min; 50 dark control trials were taken randomly from each of the blocks when no light was presented (blocks 2, 4, 6, and 8). Light was presented randomly in 50 of 125 trials in blocks 3, 5, 7, and 9. Data from block 1 were discarded.

Analysis. Because zebra finches are more active in the light, movement artifacts were a consideration in W sessions. A function written in Matlab was used to select trials or segments of trials from which segments of EEG and HVC electrode data that had very large amplitude artifacts in EEG traces (movement artifacts) had been eliminated. For consistency, this function was applied to both W and S data.

Power spectra for EEG data were calculated and integrated across a range of low frequencies (2–4 Hz). Power in these frequencies increases during avian slow-wave sleep (6). Because birds can show unihemispheric sleep (6), only the right EEG and right HVC or the left EEG and left HVC were recorded. Baseline activity was measured during the 1 s preceding each stimulus presentation. The root mean square (RMS) of the baseline HVC trace was subtracted from the RMS of the HVC trace during stimulus presentation. Were there no measurable response, the RMS subtraction would yield a value of 0. A positive number would indicate an increase in HVC energy during song presentation; a negative number would indicate a decrease. We chose this measure because it appeared that song presentation induced reliable peaks and troughs in HVC activity relative to spontaneous activity. Counting spikes would only have considered the peaks in activity. Because of variability across experimental subjects (see ranges of values in group comparisons presented in *Results*), the Wilcoxon signed rank test with significance defined as $P < 0.05$ was used to compare across birds. "Wilcoxon" is written where used, and median and range are given for these data. For figures, peristimulus time histograms (PSTHs) were constructed by summing the number of spikes for each time point across an equal number of trials for compared data. HVC PSTH data were smoothed with a 20-point moving-window average for Fig. 3 and 1,000-point average for Fig. 5.

Error bars are standard deviation of the mean when mean data are shown, and interquartile ranges when median data are shown. To assess selectivity, we directly compared the RMS_{song}–$RMS_{baseline}$ measurement between BOS and REV or CON stimuli.

Because of the variable and short duration segments of data acquired for the waking-with-light experiment, comparison of RMS values was not practical. "Spikes" were defined as deflections of multiunit voltage records exceeding a threshold. Threshold for event detection of each electrode was four times the mean standard deviation of all S trials. The same threshold was used for baseline and response measurements. For data from a single bird, statistical significance was determined with a two-tailed *t* test and defined as $P < 0.05$. For the waking-with-light experiment, the total of 200 light trials and 200 randomly selected dark control trials (50 per dark control block) were processed with a Matlab function that (*i*) eliminated a large number of trials that had movement artifacts; (*ii*) used a 3-Hz sine wave to convolve with the EEG record for each trial to locate time periods during which the bird was asleep. This method was preferred over the previously described power spectra method for quantification of EEG because it provided better temporal resolution of sleep and wake periods; (*iii*) discarded trials during which the bird was awake during a 750-ms "prelight" window; (*iv*) determined whether the bird was awake for at least 100 ms or asleep during a 750-ms "postlight" window that began at the beginning of the 300-ms light pulse and binned the HVC data accordingly; and (*v*) computed the ratio of the HVC spikes per second occurring during the "postlight" waking window divided by the spikes per second during the prelight window. HVC spikes per second were calculated only for periods in the postlight window during which the EEG was classified "awake" by the Matlab function. The post/pre ratios for trials when the bird was presented with light and awakened were then compared with (*i*) dark control trials during which light was not presented and the bird did not awaken. For these trials, spike rates during time windows equivalent to the prelight and postlight windows were compared; and (*ii*) trials during which light was presented but the bird did not awaken. These data were compared by using the Wilcoxon signed rank test with significance defined as $P < 0.05$.

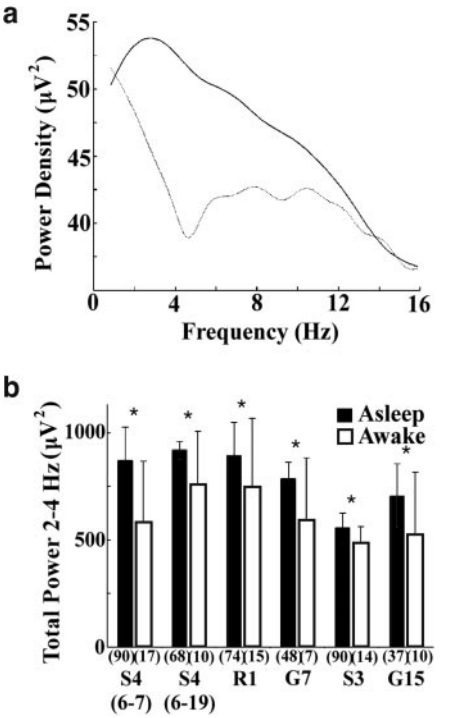

Fig. 1. Electroencephalogram of zebra finch brain indicates sleep/wake state. (*a*) Sample power spectra from sleep (top solid line) and awake (bottom dotted line) EEG records from a single bird (bird S4). (*b*) Total right EEG power from 2–4 Hz for several birds examined in this study. Two dates are shown for bird S4. EEG records of 1.5 s during song playback in both sleep and awake states from each of *n* (in parentheses) of 100 trials that did not have movement artifacts as determined by a Matlab function (see *Methods*) were used to calculate power. Error bars indicate the standard deviation of the mean. Asterisks indicate significance with $P < 0.05$. Group EEG power 2–4 Hz: W (n = 9 birds): 1053.5 μV^2, range: 954.4–1518.6 μV^2; S(9): 1409.3 μV^2, range: 914.1–1841.0 μV^2; $P < 0.006$, Wilcoxon.

Results

We found that slow-wave sleep can be measured with EEG in zebra finches, as with other birds (6): EEG power in lower frequencies increased in sustained darkness and when birds' eyes were closed (Fig. 1*a*). We consistently observed significant

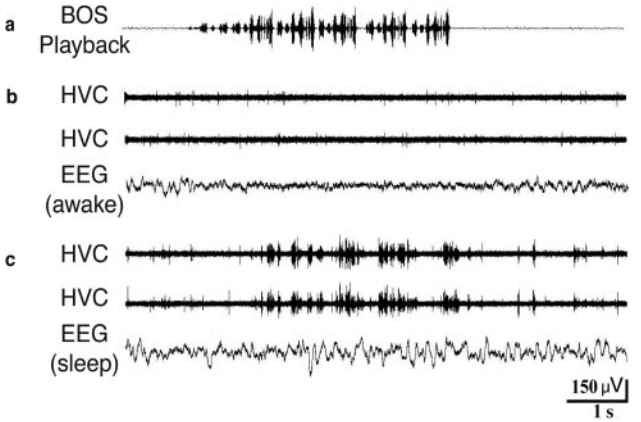

Fig. 2. BOS response in the nucleus HVC is correlated with sleep as measured by EEG (representative data from bird S4). (*a*) BOS stimulus is temporally aligned with electrode data below. (*b*) AWAKE, bird was awake during song and asleep before and after. This playback trial occurred early in the session, when birds tend to awaken during stimulus presentation. Multiunit HVC recordings from two different electrodes (shown in top two panels) showed little response to BOS when bird was awake, as measured by EEG. (*c*) SLEEP, HVC recordings in top two panels exhibit increased firing during BOS when bird was asleep, as indicated by EEG.

increases in low frequency EEG (2–4 Hz) power in adult male zebra finches during apparent sleep (during the day or night) compared with wakefulness (Fig. 1*b*). EEG recordings reliably indicate sleep in zebra finches, allowing assessment of the sleep/wake state. Moreover, we were able to monitor state changes on a millisecond time scale, which was not possible in previous studies (4, 5).

Multiunit extracellular HVC recordings revealed very weak or no response to the bird's own song during waking (Fig. 2*b*), whereas HVC recordings from these same electrodes with the same voltage threshold revealed robust BOS responsiveness during sleep (Fig. 2*c*). HVC electrodes in all birds showed significant increases in BOS response during sleep compared with waking. [RMS_{song}–$RMS_{baseline}$; W (wake/sleep sets across four birds): 0.000, range: −0.218–0.187; S(4): 0.020, range: −0.347–0.771; $P < 0.0001$, Wilcoxon].

Although responses to REV and CON changed to a small extent during sleep [RMS_{song}–$RMS_{baseline}$; REV: W ($n = 4$): 0.000, range: −0.266–0.257; S(4): 0.004, range: −0.732–0.501; N.S., Wilcoxon; CON: W(4): 0.004, range: −0.235–0.222; S(4): 0.000, range: −0.385–0.598; N.S., Wilcoxon], the overall responses to REV and CON seemed less robust than those to BOS (Fig. 3). To assess the selectivity of recorded HVC neurons for BOS compared with REV and CON, we directly compared the RMS_{song}–$RMS_{baseline}$ values for BOS to those for REV and CON. During sleep, we found that this measure was greater for BOS than for REV or CON (values given above; BOS vs. REV: $P < 0.0001$; BOS vs. CON: $P < 0.0001$, Wilcoxon). Thus, HVC neurons respond to BOS more strongly than to REV or CON during sleep.

HVC response to BOS changed within milliseconds as one physiological state replaced the other. Abrupt changes in the EEG occurred when the bird fell asleep, awoke spontaneously, or was awakened by the experimenter. Concomitant with these changes, the BOS response of HVC increased (with sleep) or decreased (with waking). Fig. 4 shows examples from two birds. Temporally aligned panels (*a*–*c*) show HVC response to BOS (*a*) during a trial in which the bird remained asleep (*b*) and a trial during which the bird spontaneously awakened, as indicated by the loss of low frequency power in the EEG after a movement artifact (*c*). The second prominent burst of HVC activity seen in

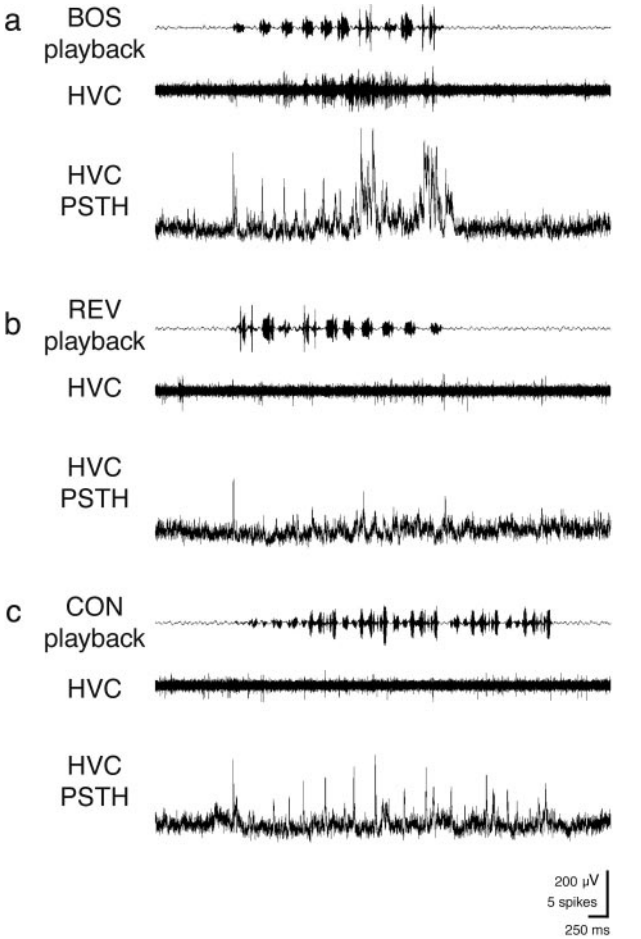

Fig. 3. During sleep, increased firing during stimuli is selective for BOS. (*a*) Response recorded with HVC electrode is specific for BOS playback. PSTHs show group data across 100 trials. (*b*) Weak response recorded from same HVC electrodes during reverse BOS. (*c*) Response during playback of a conspecific song was also weak.

b did not occur in *c*, when the bird was awake. The time elapsed between HVC exhibiting a robust response and responding weakly, if at all, was less than 50 ms. Another example is shown in the temporally aligned panels (*d*–*g*). BOS (*d*) elicited a pattern of robust bursting in HVC during a trial in which the bird remained asleep (*e*). Bursting response gradually increased in a rare trial during which the bird went to sleep (*f*). HVC bursting abruptly ceased concurrent with a loss in EEG low frequency power after the experimenter tapped the sound-proof box (*g*, tap at arrow).

To further examine the temporal dynamics of gating during state changes, we designed an experiment to awaken the bird randomly during the night while BOS was played back. BOS was played back every 10 s throughout a 4-h period. In a relatively small number of trials, we pulsed a halogen lamp for 300 ms at a specific point during BOS playback (either 2.25 or 3.25 after song initiation; protocol shown in Fig. 5*a*). This woke the bird during some trials. A computer function sorted trials according to whether the bird woke (W) or not (S). We found that the ratio of the number of HVC spikes occurring after the light to those occurring before the light significantly decreased if the bird woke [raw and PSTH data presented from one bird on one night in Fig. 5 *b*–*d*; group data (six birds, Fig. 5*e*): W (322 trials): 0.47, range: 0.0–25.01; S (395 trials): 1.03, range: 0.0–27.5; $P < 0.0001$, Wilcoxon]. The post/pre ratio during waking with light was also

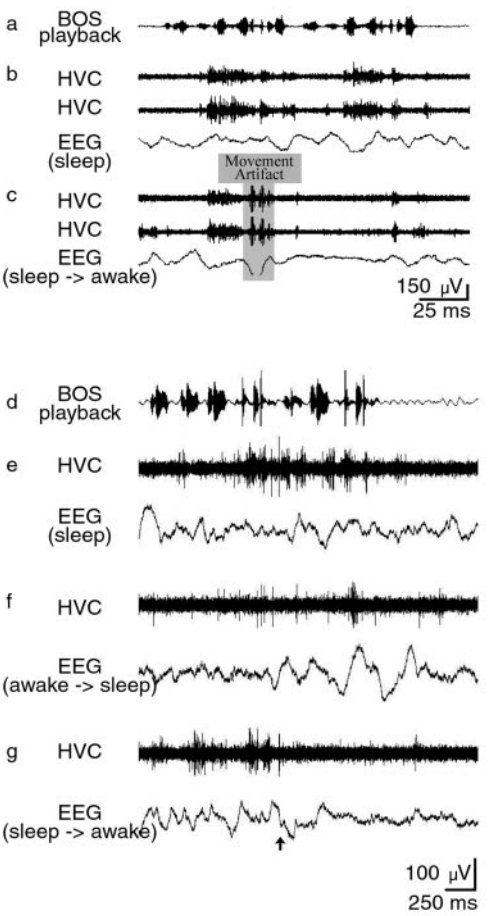

Fig. 4. Abrupt changes in the EEG and HVC BOS response occur on spontaneous waking. (*a*) BOS playback stimulus is temporally aligned with electrode recordings *b* and *c* below. (*b*) A typical trial throughout which the bird remained asleep as indicated by the EEG (bottom trace) and by behavioral observation (daytime; lights were on). BOS response is robust in recordings from two HVC electrodes (top two traces). (*c*) A trial during which the bird awakened briefly as indicated by flattening of the EEG (bottom trace) after a movement artifact (indicated by the gray bar). HVC BOS response ceases in conjunction with a pronounced decrease of EEG power in low frequencies. Data from bird S4. (*d–g*) Representative data from another bird (O4). (*d*) BOS playback stimulus is temporally aligned with electrode recordings below. (*e*) A typical trial throughout which the bird remained asleep (daytime; lights were on). (*f*) During this trial, the bird fell asleep. Increases in EEG slow-wave power and in HVC auditory response were seen. (*g*) During this trial, the experimenter tapped the sound box (at arrow). Bird awakened, as seen in the EEG, and lost HVC auditory response.

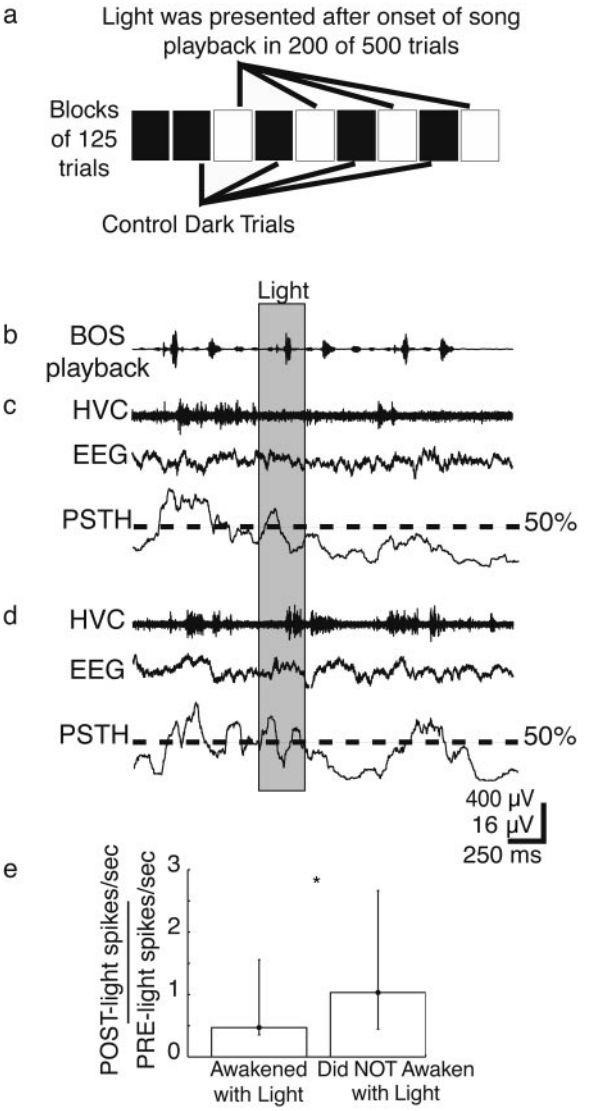

Fig. 5. Further analysis of the waking result. (*a*) Protocol for the waking-with-light experiments. Light was presented during BOS playback in 50 randomly selected trials in each of the 125-trial experimental blocks, indicated by the white squares. BOS playback occurred without light presentation during 125 trials of each control dark block, indicated by the black squares. (*b*) BOS playback stimulus is temporally aligned with electrode recordings below. (*c*) A trial during which the bird awakened to light presentation. The EEG lost some low-frequency components before regaining them later in the trial. Firing in HVC was decreased after the light stimulus awakened the bird. The poststimulus PSTH, which groups data across multiple trials during which the bird awakened, was decreased relative to that presented in *d*. A dotted line indicating 50% of the maximum PSTH value is drawn to facilitate comparison of PSTH values across time. (*d*) A trial during which the bird slept through the light. The EEG was slow-wave, and HVC firing continued throughout the trial. After the light, firing across multiple trials, as indicated by the poststimulus PSTH, peaked higher than that presented in *c*. (*e*) The group data for all birds and trials in the light study. Columns represent medians. Error bars are interquartile ranges. These data are significant by using a Wilcoxon signed rank test, $P < 0.0001$.

significantly less than a similar ratio calculated during trials in which the bird was kept in the dark during BOS playback [50 trials were randomly selected from each dark control block; DarkS (473 trials): 0.8665, range: 0.0–86.0; $P < 0.0001$, Wilcoxon]. Thus, a robust decrease in HVC firing that is correlated with waking occurs within 750 ms of light onset.

Discussion

The response of HVC neurons to the bird's own song occurred only during sleep and ceased on waking. A previous single-unit study that reported a similar phenomenon in robustus archistriatalis did not use an objective criterion for sleep (5). Such a criterion is important because the presence and absence of song-specific responses may not be directly related to sleep/wake states but to other variables such as the circadian clock. Our study provides EEG-based evidence that auditory gating in HVC is associated with sleep. The occurrence of auditory gating in both HVC and robustus archistriatalis indicates that the latter is not the primary site of gating. We do not know, however, whether HVC is the primary site because it receives input from several sources. We must discriminate between the mechanisms and signals for gating. The signals may be coming from nucleus

UVA in the thalamus because electrical stimulation of this area abolishes auditory responses in HVC in anesthetized birds (7). Whatever the gating mechanisms may be, they must respond quickly because our data indicate that the gate closes within less than a second after the EEG changes to the wake mode.

The function of the auditory response in sleep that we observed remains to be elucidated. A previous study has suggested that the activity of the song system during sleep is a form of replay of learned information (5). This begs the question of why auditory input can also trigger similar activity. Moreover, the information that is putatively "replayed" is not a new memory but rather the bird's crystallized, or stable, adult song. Thus, it is likely that if the vocal control system replays song, then the replay functions to maintain the memory of the song in the adult. Studies of song-system sleep activity during periods of vocal plasticity such as (*i*) juvenile birds in the process of memorizing tutor song or (*ii*) adult birds in the process of losing their song through decrystallization (8), will illuminate the consolidation and maintenance function of song-system activity during sleep.

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