Phasic basal ganglia activity associated with high-gamma oscillation during sleep in a songbird

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Yanagihara S, Hessler NA. Phasic basal ganglia activity associated with high-gamma oscillation during sleep in a songbird. J Neurophysiol 107: 424–432, 2012. First published October 26, 2011; doi:10.1152/jn.00790.2011.—The basal ganglia is thought to be critical for motor control and learning in mammals. In specific basal ganglia regions, gamma frequency oscillations occur during various behavioral states, including sleeping periods. Given the critical role of sleep in regulating vocal plasticity of songbirds, we examined the presence of such oscillations in the basal ganglia. In the songbird nucleus Area X, epochs of high-gamma frequency (80–160 Hz) oscillation of local field potential during sleep were associated with phasic increases of neural activity. While birds were awake, activity of the same neurons increased specifically when birds were singing. Furthermore, during sleep there was a clear tendency for phase locking of spikes to these oscillations. Such patterned activity in the sleeping songbird basal ganglia could play a role in off-line processing of song system motor networks.

Oscillations of neuronal activity and associated local field potentials (LFPs), at a range of frequencies, have long been considered to be involved in higher cognitive functions such as perception, attention, and memory (Buzsaki and Draguhn 2004; Jensen et al. 2007) as well as motor control (Bevan et al. 2002; MacKay 1997). The presence of such oscillations during specific sleep stages suggests they are important in active brain processes during sleep as well (Steriade 2000). In songbirds, sleep has recently been found to be important in regulating vocal plasticity (Deregnaucourt et al. 2005) and contains periods when neurons in the song motor circuit critical for patterned song output are phasically active (Dave and Margoliash 2000; Hahnloser et al. 2002; Shank and Margoliash 2009). Another distinct song system network, the anterior forebrain pathway (AFP), is critical for vocal plasticity during initial song learning and in maintenance of adult songs (Bottjer et al. 1984; Brainard and Doupe 2000; Kao et al. 2005; Olveczky et al. 2005; Scharff and Nottebohm 1991; Sohrabji et al. 1990). The AFP corresponds to basal ganglia-thalamocortical circuits in mammals (Doupe et al. 2005). Area X is the basal ganglia component of this pathway and contains both striatal- and pallidal-type neurons (Farries and Perkel 2002). Along with neurons in song system motor control nuclei, those in AFP nuclei, including Area X, are selectively active during singing (Goldberg et al. 2010; Goldberg and Fee 2010; Hessler and Doupe 1999a). This AFP activity, which is not required for normal singing output (Bottjer et al. 1984), is critical for regulating potential plasticity during singing in adults (Brainard and Doupe 2000; Kao et al. 2005). Given the importance of oscillations in regulating sleep functions and motor control, we examined their presence in the basal ganglia of zebra finches. We found that in Area X, a critical period of high-frequency gamma oscillation during sleep is strongly associated with phasic neural activity.

Materials and methods

Animals and surgery. All procedures were reviewed and approved by the RIKEN Animal Experiments Committee. General procedures for physiological recording from freely behaving birds were described previously (Yanagihara and Hessler 2006). Adult male zebra finches were used for experiments (161–773 days posthatch, 6 birds). All birds were raised in our breeding colony. During surgery, each bird was anesthetized with isoflurane (1.0–1.3% supplied with air) and placed in a stereotactic apparatus. The bird’s head was immobilized with the lower edge of the upper beak mandible positioned 50° from vertical (down). Each bird was implanted with tetrodes ( nichrome wire, diameter of 12.5 μm; Kanthal Palm Coast) fixed in a lightweight (<1 g) microdrive and targeted to Area X (5.1 mm anterior, 1.7 mm lateral from divergence of the central sinus at the border of the forebrain and cerebellum). Two tetrodes and one reference tetrode (for reference tetrode, 4 wires were electrically connected) were implanted in Area X. Each tetrode wire was electroplated with gold solution (SIFCO) to impedance between 200 and 500 kΩ at 1 kHz. A ground electrode (stainless steel wire, diameter of 200 μm; A-M Systems) was placed on the surface of the brain. The microdrive was secured to the skull with epoxy (Devcon). Recordings were made from the right hemisphere. After surgery, each bird was isolated in a small cage (20 × 20 × 20 cm) inside a sound attenuation box under a 12:12-h light-dark photoperiod. Food and water were available ad libitum.

Electrophysiological recording. After several days of recovery, recording sessions began. To isolate single units, tetrodes were advanced or retracted by manually turning a screw of the microdrive while the bird was restrained. Single-unit activity was monitored from 2.3 to 3.3 mm from the brain surface. Area X was identified by characteristic features of neural activity such as high-frequency and regular spontaneous activity in awake nonsinging state and robust singing-related activity as described in a previous study (Hessler and Doupe 1999a). Single-unit and LFP recordings were made with Multichannel Acquisition Processor or Recorder/16 (Plexon). For single-unit recordings, signals were amplified between 5,000- and 20,000-fold, filtered between 300 Hz and 9 kHz, and digitized at 40 kHz. The spike signals were manually sorted off-line into isolated single units with Offline Sorter (Plexon). Typically, the signal-to-noise ratio of isolated single units was ≥3:1 (see Fig. 3D and Fig. 4D for example). For each unit, the spike width, defined as the half-width of negative deflection, was measured by using an average of 10 consecutive spike waveforms. The median spike width was 0.1 ms [interquartile range (IQR) 0.12, 0.15 ms; n = 31 units], similar to that of Area X units recorded extracellularly from juvenile zebra finches (Goldberg and Fee 2010). Isolation of single units throughout the recording session was confirmed by consistent location of principal
components and clear distinction from noise from early to late during recordings, as well as consistent lack of interspike intervals < 1 ms.

For LFP recordings, the signal was amplified 1,000-fold, filtered between 0.7 Hz and 170 Hz, and digitized at 20 kHz. Once single-unit activity was isolated, recording sessions lasted several hours (median 7.1: IQR 4.8, 8.7 h). During recording sessions, we first collected awake nonsinging and singing (mostly undirected singing, that is, singing while alone) data while lights were turned on (median 2.3; IQR 1.3, 3.7 h). Then lights were extinguished to induce spontaneous sleep (median 4.2; IQR 3.3, 5.0 h). Typically, about 1 h after darkness began, birds had fallen asleep, as determined by visual observation of closed eyes, lack of gross body movement, neck muscle relaxation (head-forward or head-backward position), and ongoing slow wave activity in LFP signals (described in more detail in Behavioral states). The acoustic signal in the sound attenuation box was simultaneously recorded with a small microphone (model C417, AKG) placed inside the sound attenuation box. The acoustic signal was amplified (DMP3, M-AUDIO) and digitized at 20 kHz. Behavior of birds was monitored by video camera and recorded (CinePlex, Plexon).

Behavioral states. Stable sleep was defined as the state in which birds were inactive with a relatively strong low-power EEG signal. Because such stable sleep could be sporadically interrupted by brief slight movements, we chose randomly for analysis epochs of 2 min duration within such stable sleep. Epochs were selected for analysis and quantification while investigators were blind to the corresponding neural activity. These epochs of sleep occurred several hours after lights were extinguished (median 2.7; IQR 2.0–3.5 h). Periods of 2 min were sufficient to quantify characteristic features of LFP and neural activity. The awake nonsinging state was defined as the state in which birds were awake with eyes open and sitting with minimal movement. Because such states tended to be brief in some birds, and in order to compare directly to analysis of stable sleep periods, we used epochs of 2 min duration for analysis of LFP features and neural activity.

Data analysis. All analysis was performed with Matlab (Mathworks). The power spectral density (PSD) for LFP signal was calculated by Welch’s method. Consistent with previous EEG measurements in sleeping zebra finches (Hahloeser et al. 2006; Low et al. 2008; Nick and Konishi 2001; Shank and Margoliash 2009), strong LFP power in the low-frequency range (<10 Hz) was typically observed during sleep (Fig. 1B). Since the PSD of LFP signals during sleep with high-frequency oscillation indicated high power between 80 and 160 Hz (Fig. 1B), the term “high-gamma” is used to describe the high-frequency oscillation of LFP.

Periods containing high-gamma oscillation were identified with similar methods previously used to detect sharp wave/ripple signals (Skaggs et al. 2007). The LFP signal was downsampled to 1 kHz and digitally filtered (80–160 Hz, Butterworth filter). The filtered LFP signal was squared and then smoothed by low-pass filtering (<20 Hz). The threshold for detecting high-gamma oscillation epochs was set to 2 SD above the mean amplitude.

To compare the relative level of high-gamma power during the nonsinging awake period and that during the sleep period with and without detected high-gamma oscillations, high-gamma power was calculated by using 10 representative epochs of 1-s nonsinging awake and sleep periods. Epochs were randomly selected from quiet stationary awake and sleep periods. To avoid obtaining spurious results, we omitted from averages epochs containing large atypical fluctuations of the LFP caused by gross movement such as locomotion or head scratching. These movement artifacts typically consisted of low-frequency nonperiodic fluctuations with peaks at least 10-fold higher than the usual LFP signal and often caused saturation of the LFP signal before digitization.

To determine the relationship between spike timing and oscillation phase, negative peaks of each oscillation cycle were detected in the filtered LFP signal of high-gamma epochs. The filtered LFP signal and spike timing were aligned with the detected negative peaks. For each neuron, the phase histogram (18° bins) of spike timing relative to the negative peaks (phase 0° or 360°) was also constructed as described previously (Tukker et al. 2007). Spiking activity was considered as modulated during the high-gamma oscillation if a Rayleigh test indicated a nonuniform distribution of spike-time phases relative to the oscillation cycle (P < 0.05; Zar 2010).

To quantify the relationship between neural activity and the relative level of high-gamma power of LFP during sleep, Spearman’s rank correlation coefficient between firing rate and mean power of high-gamma band (80–160 Hz) was calculated. For each unit and LFP pair, the firing rate and relative high-gamma power for successive 1-s intervals were obtained, and the correlation values were calculated by using 120 consecutive data points (2 min total). All data are reported as median and IQR (25th–75th percentile), unless noted otherwise.
RESULTS

As previously reported in the zebra finch, EEG signal recorded from the brain surface of sleeping birds contained stronger low-frequency components compared with the awake state (Hahneloser et al. 2006; Low et al. 2008; Nick and Konishi 2001; Shank and Margoliash 2009) (not shown). This tendency was also observed in the LFP recorded in the song system nucleus Area X, but in addition a novel form of high-frequency oscillation was observed in sleeping birds. During sleep, brief epochs of LFP oscillation in the high-gamma range (80–160 Hz; referred to as “high-gamma” oscillation) occurred in Area X (Fig. 1A). We detected such high-gamma oscillation epochs with a similar method used previously for detecting high-frequency field oscillations (Skaggs et al. 2007) (Fig. 1A, bottom; see also MATERIALS AND METHODS). In 2-min samples of stable sleep, 76.5 (IQR 60.5–87.0) epochs of high-gamma oscillation were detected with median durations of 36.9 ms (IQR 34.3–41.3 ms). Thus the fraction of time in high-gamma oscillation during the sleep period was 2.4% (IQR 2.0–2.6; n = 28 sites).

To quantify the relative level of such high-gamma power signal in distinct behavioral states, we further calculated the PSD of the LFP. During sleep that contained periods of high-gamma oscillations, the PSD indicated high power between 80 and 160 Hz, with a peak centered around 100 Hz (Fig. 1B; median peak frequency = 95.0, IQR 90.8–98.3 Hz; n = 21 LFP recording sites; recordings that did not exhibit a distinct peak were excluded from this analysis). The strength of such high-gamma oscillation was significantly lower during awake nonsinging periods (Fig. 1C; high-gamma LFP power: awake nonsinging median 5.1 × 10^{-3}, IQR 3.9 × 10^{-5}–6.1 × 10^{-5} mV^{2}/Hz; sleep with high-gamma median = 8.4 × 10^{-5}, IQR 7.1 × 10^{-5}–1.2 × 10^{-5} mV^{2}/Hz; P = 4.72 × 10^{-6}, n = 28 LFP recording sites, Wilcoxon signed-rank test). High-gamma (80–160 Hz) power was calculated by using 10 epochs (1-s duration) of awake nonsinging state and sleep state with high-gamma oscillations.

To determine the relationship between these novel oscillatory periods and neural activity, we simultaneously recorded extracellular single-unit activity and LFP from adjacent sites in the basal ganglia nucleus Area X of adult male zebra finches (n = 31 units, 28 recording sessions). Two types of units in Area X were distinguished by their firing rate during the awake nonsinging state, low-rate (LR) and high-rate (HR) units (Fig. 2; LR units: median 1.0 Hz, IQR 0.6–4.8 Hz (n = 9); HR units: 130.0 Hz, 91.7–153.2 Hz (n = 22); P = 1.72 × 10^{-4}, Mann-Whitney U-test), which can be putatively identified as striatal and pallidal neurons, respectively (Farries and Perkel 2002; Goldberg et al. 2010; Goldberg and Fee 2010; Hessler and Doupe 1999a). Activity of both LR and HR units was strongly modulated during periods of high-gamma oscillation. In the following, we detail both the global and local temporal relationships between high-gamma LFP oscillation and Area X unit activity.

While birds were sleeping, both LR and HR Area X units were phasically active during high-gamma LFP oscillation (Fig. 3A and Fig. 4A). In these representative 6-s samples of sleep, sporadic periods of high-gamma band oscillation are revealed by filtering the raw LFP signal with a band-pass filter. Both individual spikes of LR units (Fig. 3A) and instantaneous spike rate of HR units (Fig. 4A; due to the high firing rate, individual spikes would be obscured on this time scale) appear closely linked to the high-gamma power filtered LFP signal (percentage of detected gamma period: Fig. 3A, 10.9%; Fig. 4A, 3.3%). The shorter time scale relationship between oscillation and unit activity can be seen in the expanded view of Figs. 3B and 4B. For the LR unit in upper traces of Fig. 3B, the timing of epochs of high-gamma oscillation, as detected by the algorithm outlined in Fig. 1A, is indicated by dotted lines. For the HR unit, regular spiking is altered during a high-gamma epoch to a phasic higher rate. In the further expanded view in lower traces of Figs. 3B and 4B a tendency of spikes to occur locked to phase of high-gamma oscillation can be seen. This common tendency of LR and HR units is examined further below.

LR and HR units that were phasically active during high-gamma periods during sleep were also specifically active during singing in awake birds. For the LR unit, the level of activity during singing while awake was similar to the phasic activation during sleep [Fig. 3C; note time scale and rate axes identical to those in Fig. 3A (sleep)]. For the HR unit, the firing rate both increased and paused during singing distinct from the regular firing during the nonsinging period [Fig. 3C; for comparison to Fig. 4B (sleep), note that time scale is expanded 2 times]. In contrast to the sleep periods, high-gamma oscillations were not prominent during singing (high-gamma LFP power during singing, median 5.5 × 10^{-5}, IQR 2.6 × 10^{-5}–6.5 × 10^{-5} mV^{2}/Hz; singing vs. awake nonsinging, P = 0.34; singing vs. sleep with high-gamma, P = 4.9 × 10^{-5}, n = 12 LFP recording sites, Wilcoxon signed-rank test; recordings that contained gross LFP fluctuation due to movement artifacts during singing were excluded for this analysis).

For all LR and HR units, the level of activity during detected high-gamma periods was significantly higher than that during other sleep periods [with and without high-gamma firing rate: LR: 27.6, 16.1–34.0 Hz vs. 5.2, 2.6–6.4 Hz (n = 9, P = 0.0195), HR: 103.6, 78.0–135.9 Hz vs. 87.2, 53.3–96.8 Hz (Fig. 3B). For LR units the level of activity during singing while awake was similar to the phasic activation during sleep [Fig. 3C; note time scale and rate axes identical to those in Fig. 3A (sleep)]. For the HR unit, the firing rate both increased and paused during singing distinct from the regular firing during the nonsinging period [Fig. 3C; for comparison to Fig. 4B (sleep), note that time scale is expanded 2 times]. In contrast to the sleep periods, high-gamma oscillations were not prominent during singing (high-gamma LFP power during singing, median 5.5 × 10^{-5}, IQR 2.6 × 10^{-5}–6.5 × 10^{-5} mV^{2}/Hz; singing vs. awake nonsinging, P = 0.34; singing vs. sleep with high-gamma, P = 4.9 × 10^{-5}, n = 12 LFP recording sites, Wilcoxon signed-rank test; recordings that contained gross LFP fluctuation due to movement artifacts during singing were excluded for this analysis).
level of modulation from nonsinging periods (nonsinging vs. singing C.V. 0.29, 0.27–0.33 vs. 0.57, 0.50–0.63; n = 4). Such an increased level of modulation also occurred for units in which birds only sang undirected songs (nonsinging vs. singing C.V. 0.34, 0.28–0.48 vs. 0.73, 0.61–0.89; n = 18).

This feature of a higher level of modulation present during singing was also present for high-gamma periods during sleep [directed singing sleep without vs. with high-gamma C.V. 0.35, 0.30–0.51 vs. 0.60, 0.56–0.66 (n = 4); undirected singing sleep without vs. with high-gamma C.V. 0.37, 0.32–0.47 vs. 0.60, 0.53–0.68 (n = 18)]. Thus both high levels of activity and higher modulation of firing rate were features distinguishing singing periods in awake birds and high-gamma from non-high-gamma periods during sleep.

While the level of unit activity was consistently higher during detected “high-gamma” epochs, modulation did not appear to be restricted to such periods. Rather, the level of unit

(n = 22, P = 6.92 × 10⁻⁴; Wilcoxon signed-rank test). When birds were awake, activity of these LR and HR units was selectively modulated during singing [Figs. 2, 3C, and 4C; nonsinging and singing firing rate: LR: 1.0, 0.6–4.8 Hz vs. 19.0, 17.2–51.3 Hz (n = 9, P = 0.004); HR: 130.0, 91.7–153.2 vs. 201.7, 147.4–242.6 (n = 22, P = 2.3 × 10⁻⁴); Wilcoxon signed-rank test] as has been reported (Goldberg and Fee 2010). Especially for HR units, modulation of activity during singing frequently consisted of phasic reductions and increases of firing rate, as can be seen in Fig. 4C. This characteristic was most prominent in distinguishing singing from nonsinging activity for units in which birds sang only directed songs. As in a previous study (Hessler and Doupe 1999b), during directed singing some HR units did not clearly alter their mean firing rate (Fig. 2, directed singing) but consistently increased their

Fig. 4. HR unit activity associated with high-gamma oscillation. A: LFP and spontaneous activity of a representative HR unit during sleep. B: expanded view of the shaded epochs in A. Detected high-gamma epochs are indicated by dotted lines at top. C: activity of the same LR unit during singing. Oscillogram of song, filtered LFP, spike times, and smoothed firing rate are shown from top to bottom. D: unit clusters at initiation of recording and 6 h later. Black dots indicate a sorted single unit. Noise cluster is encompassed with a dotted circle. Inset: representative spike waveforms. Scale bars: 0.2 mV, 1 ms. Vertical scale bars in A–C indicate 0.1 mV.
activity also could increase during periods of high-gamma power below our threshold for detection (see, e.g., Fig. 3B, last 500 ms of trace). To more fully characterize the relationship between LFP power and unit activity, we thus analyzed without distinguishing between discrete “high-gamma” periods. Global features of this relationship can be observed in examples of unit activity and LFP power during extended sleep and awake periods (Fig. 5 and Fig. 6; continuous 2-min epochs). Spiking times for the HR unit are plotted as a grayscale map, since at this time scale and high firing rate a raster plot would appear continuous. For both LR and HR units, during occasional 1-s periods that contained strong high-gamma power, the mean firing rate was increased. In contrast, when birds were awake, such periods of strong high-gamma power were scarce, and the level of neural activity increased only when birds were singing.

The relationship between the level of high-gamma frequency LFP and unit activity was quantified by measured Spearman’s rank correlation coefficient, based on 120 consecutive 1-s samples during sleep (as shown in Fig. 5A and Fig. 6A, center and right). For representative LR and HR units (Fig. 7, A and B, respectively), relatively high-gamma periods were clearly associated with stronger unit activity. Note that the wide range of measured high-gamma power within 1-s intervals partially reflects variability in the duration of detected high-gamma occurrences within them. Correlations between unit activity and relative high-gamma power were significant for every recorded unit (Fig. 7C; median correlation value = 0.59, IQR 0.48–0.68; n = 31/31, P < 0.05).

Beyond the overall correlation of firing rate with periods of high-gamma frequency LFP signal, most Area X units tended to fire during a particular phase of the high-gamma oscillations. This tendency is illustrated for the example LR and HR units in Fig. 8, A and C, respectively. In both, multiple 20-ms epochs of band-pass filtered LFP waveform are aligned to the negative peak of the oscillation cycle, along with rasters for spikes that occurred concurrently. The spike rasters show that there were consistent temporal relationships between spikes and the LFP waveform: a higher probability of occurring out of phase with each negative peak. Confirming this tendency, the phase distributions of spikes relative to oscillation cycles were significantly nonuniform and centered around the positive phase of the LFP (Fig. 8B, P = 8.54 × 10\(^{-15}\); Fig. 8D, P = 1.35 × 10\(^{-14}\); Rayleigh test; Zar 2010). A similar analysis of phase distribution was performed on all units and found that timing of most LR units (n = 6/9) and half of the HR units (n = 12/22) was significantly dependent on the phase of ongoing LFP oscillation (Fig. 8E; for each unit, 2 cycles of polar phase distribution as in Fig. 8, B and D, are plotted as a grayscale map). While all significantly modulated HR units were aligned to the positive phase of LFP (median phase = 218.1°, IQR 200.0–235.0°; n = 12), there was a clear distinction between sets of LR units aligned to the positive (median = 195.4°; n = 3) and to the negative phase (median = 65.3°; n = 3).

**DISCUSSION**

Here we demonstrate that during sleep the occurrence of high-gamma oscillations in the LFP is associated with phasic firing of basal ganglia neurons that are also active during singing. This phasic activation appears similar to that previously reported for song system motor control nuclei during sleep and could be involved in the AFP’s function in adult vocal plasticity.
Gamma frequency oscillations in avian basal ganglia involved in singing motor plasticity may be related to similar oscillations in mammals. While much focus has been placed on their important role in higher brain functions such as attention and consciousness (Buzsaki and Draguhn 2004; Jensen et al. 2007), oscillations also have important functions in motor control and learning (Bevan et al. 2002; MacKay 1997). Gamma oscillations frequently occur in mammalian basal ganglia during particular behavioral states (Berke 2009; Kalenkoscher et al. 2010; Tort et al. 2008; van der Meer and Redish 2009). Along with better-characterized lower-frequency oscillations, gamma oscillations also occur spontaneously during sleep and anesthesia (Le Van Quyen et al. 2010; Molle et al. 2004; Sharott et al. 2009; Steriade 2000). Furthermore, it has long been known that the presence or absence of oscillations of various frequencies can be associated with dysfunctional basal ganglia networks and motor disorders as in Parkinson’s disease (Hammond et al. 2007; Mallet et al. 2008).

Further study can clarify how this phenomenon is related to a variety of previously characterized LFP processes. A number of studies in various brain areas reported similar brief occurrences of high-frequency oscillation, with frequency of ~100 Hz. As this range is near the upper limit of common definitions of gamma frequency oscillation, we have used the term “high-gamma” oscillation, as have a number of previous studies (Canolty et al. 2006; Edwards et al. 2005; Le Van Quyen et al. 2010; Ray et al. 2008). A similar phenomenon first observed in hippocampus, “ripples,” can occur at similar frequencies, but they are associated with large-amplitude “sharp waves,” which we did not observe, and their range tends to extend much higher than those we present here (100–200 Hz; Grenier et al. 2001; Klausberger et al. 2003; O’Neill et al. 2010). Although details of the temporal pattern of such gamma occurrences are not typically presented, episodes of high-gamma frequency oscillations appear in other systems also to have

Fig. 6. Time course of HR unit activity and high-gamma LFP power. A: left: firing rate of HR unit during 2-min recording period in sleep state, indicated by a grayscale map. Center and right: mean firing rates and corresponding high-gamma LFP powers of each 1 s, respectively. B: same HR unit activity and high-gamma LFP power during awake state. Data are from the HR unit shown in Fig. 4.

Fig. 7. Firing rate of Area X units was positively correlated with high-gamma power of LFP. A: relationship between high-gamma power (80–160 Hz) of LFP and firing rate of a single LR unit during sleep. Firing rate and relative LFP power in consecutive 1-s windows (n = 120) are significantly correlated (Spearman’s correlation coefficient, r = 0.79, P = 1.36 × 10⁻²⁶). Data are from the recording session shown in Fig. 5. B: relationship between high-gamma power and firing rate of a single HR unit during sleep. A significant positive correlation was found (r = 0.76, P = 2.20 × 10⁻²⁶). Data are from the recording session shown in Fig. 6. C: distribution of Spearman’s correlation coefficient values for all Area X unit and LFP pairs (n = 31). All values are statistically significant (P < 0.05).
Fig. 8. Spike timing of Area X units was phase locked to the high-gamma LFP oscillation. A: relationship between high-gamma oscillation and spike timing of LR unit. LFP and spike times are aligned with the detected negative peaks of high-gamma oscillations (n = 274). Filtered LFP (grayscale), mean filtered LFP trace, raster plot, and firing probability are shown from top to bottom. B: phase distribution of the same LR unit relative to the high-gamma oscillation. Mean phase = 195.4°, P = 8.54 × 10⁻¹³, Rayleigh test. Data are from the LR unit shown in Fig. 3. C: relationship between high-gamma oscillation and spike timing of HR unit. D: phase distribution of the same HR unit relative to high-gamma oscillation. Mean phase = 197.2°, P = 1.35 × 10⁻¹³, Rayleigh test. Data are from the HR unit shown in Fig. 4. E: phase distribution of all Area X units relative to the high-gamma phase. Each row corresponds to data from a single unit. Unit types are indicated by the black (LR) and white (HR) bars. Statistical results by Rayleigh test (P < 0.05) are shown on left. N.S., not significant. Data in B (LR unit) and D (HR unit) are indicated by asterisks. The firing probabilities of each unit are indicated as grayscale (scale bar: 0.03 to 0.2), Two high-gamma cycles are shown for clarity.

The strong phase locking of Area X units to the high-gamma oscillation raises a critical issue of interpretation: What is the causal relationship between the two? A similar relationship between spike timing and gamma oscillation phase has frequently been observed in mammalian systems (Fries et al. 2007; Sharott et al. 2009; Tukker et al. 2007). As in these systems, the gamma oscillation we observed could reflect local interactions of inhibitory networks. Although we could not identify neuronal types directly with our extracellular recording, unit activity patterns suggest that HR and LR units are "pallidal"-like and "striatal"-like Area X neurons (Farries and Perkel 2002; Goldberg et al. 2010; Goldberg and Fee 2010). Like models that produce spontaneous gamma oscillations (Bartos et al. 2007; Sohal and Huguenard 2005; White et al. 1998), local networks in Area X may be primed for gamma oscillation, as both LR and HR units could be inhibitory, with local projections within Area X (both LR and HR) as well as efferent to the thalamus (HR). As in other systems, the activity of the units we recorded could contribute to generation of the simultaneous high-gamma LFP. Given the consistent phase relationship of HR units we recorded (across different animals), it may be that many pallidal-type units in Area X fire nearly synchronously during these occasional high-gamma LFP events. Interestingly, the LR units, potential inhibitory inputs to HR units, could have either similar or opposite phase relationships with high-gamma oscillations. An alternate or additional possibility is that the field oscillation could directly modulate membrane properties of local neurons and regulate their firing dynamics (Anastassiou et al. 2011).

An intriguing potential source of the phasic activity associated with high-gamma oscillations is the strong input to Area X from the song system motor control nucleus HVC. During singing, HVC provides patterned input both to the motor-related nucleus RA (Hahnloser et al. 2002; Yu and Margoliash 1996) and to Area X (Kozhevnikov and Fee 2007; Prather et al. 2008), as the first stage of the AFP. This activity propagates through the AFP to LMAN, which then projects to the motor network in RA. A striking feature of the song system is its occasional phasic activity during sleep, when individual neurons fire in a temporal pattern as they do during singing (Dave and Margoliash 2000; Hahnloser et al. 2002). Such reactivation, similar to that reported in many mammalian brain systems (O’Neill et al. 2010; Pennartz et al. 2004; Wilson and McNaughton 1994), has thus far been characterized mainly for the motor control nuclei HVC and RA. During reactivation, neurons in HVC that project to RA and Area X occasionally burst concurrently (Hahnloser et al. 2006). This input could drive the phasic activity that we observed during sleep in Area X. In general, the results of this study are consistent with the suggestion of Hahnloser et al. (Hahnloser et al. 2006) that during sleep reactivation events song system networks are activated in a manner similar to that during singing.

Phasic reactivation during sleep in song system nuclei has been proposed to serve in off-line motor system processing during the initial learning period of juveniles, and possibly also in maintaining singing quality of adults (Margoliash 2005; Margoliash and Schmidt 2010). The AFP, which directly modulates the level of plasticity in motor networks (Brainard and Doupe 2000; Kao et al. 2005), may also play a critical role in off-line modulation of singing. The present results present a potential link between AFP-modulated plasticity and sleep. The gamma oscillation and phasic neural activation could represent brief periods when anterior forebrain plasticity networks could interact with activated singing motor networks, allowing potential for modulation. Further study will be required to determine whether the phasic activity associated with
high-gamma oscillation we observed is related to reactivation of song system motor nuclei, by simultaneous recording from Area X and HVC or RA.

This system has notable potential for investigation of the behavioral function of high-frequency brain oscillations, as song system nuclei, including Area X, are specialized for the readily quantifiable behavior of singing. If this activity during sleep is involved in maintenance of singing, altering normal levels of high-gamma oscillation or phasic neural activation by local alteration of electrical field or stimulation (Cardin et al. 2009; Frohlich and McCormick 2010; Girardeau et al. 2009) could disrupt normal song maintenance in adult birds. Alternatively, perturbation of normally stable singing in adults could be accompanied by disruption of phasic high-gamma oscillation. Such results could strengthen this system as a model for investigating the role of mammalian basal ganglia oscillations in motor control and their disruption in disorders such as Parkinson’s disease (Hutchison et al.).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: S.Y. conception and design of research; S.Y. performed experiments; S.Y. and N.A.H. analyzed data; S.Y. and N.A.H. interpreted results of experiments; S.Y. and N.A.H. prepared figures; S.Y. and N.A.H. drafted manuscript; S.Y. and N.A.H. edited and revised manuscript; S.Y. and N.A.H. approved final version of manuscript.

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