



## Research

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# Replay of innate vocal patterns during night sleep in suboscines

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Activation of forebrain circuitry during sleep has been variably characterized as ‘pre- or replay’ and has been linked to memory consolidation. The evolutionary origins of this mechanism, however, are unknown. Sleep activation of the sensorimotor pathways of learned birdsong is a particularly useful model system because the muscles controlling the vocal organ are activated, revealing syringeal activity patterns for direct comparison with those of daytime vocal activity. Here, we show that suboscine birds, which develop their species-typical songs innately without the elaborate forebrain-thalamic circuitry of the vocal learning taxa, also engage in replay during sleep. In two tyrannid species, the characteristic syringeal activation patterns of the song could also be identified during sleep. Similar to song-learning oscines, the burst structure was more variable during sleep than daytime song production. In kiskadees (*Pitangus sulphuratus*), a second vocalization, which is part of a multi-modal display, was also replayed during sleep along with one component of the visual display. These data show unambiguously that variable ‘replay’ of stereotyped vocal motor programmes is not restricted to programmes confined within forebrain circuitry. The proposed effects on vocal motor programme maintenance are, therefore, building on a pre-existing neural mechanism that predates the evolution of learned vocal motor behaviour.

## 1. Introduction

Although sleep constitutes a period of reduced brain activity and quiescence, specific neural circuitry may be activated at times, and this activity may serve important functions. A well-studied example is neural reactivation resembling daytime patterns in circuitry involved in the formation of motor memory. This so-called replay is thought to aid the consolidation of procedural memory in the acquisition of motor skills [1,2]. Experimental evidence supports this model, as the performance of newly learned tasks is improved by this reactivation [3–7]. Reactivation-related solidification of motor memory has been postulated for a number of different tasks [3,8–11] all of which require the involvement of forebrain and thalamic areas for sensorimotor learning and motor execution.

In addition, reactivation of established and stereotyped motor programmes also occurs during sleep [12–15]. This replay can lead to the execution of the motor instructions at the peripheral muscle systems [14,15]. An important example is the activation of the song production circuitry during sleep in oscine songbirds. In the best-studied species, the zebra finch (*Taeniopygia guttata*), song in adult males is highly stereotyped and consists of 4–8 different syllables, each of which is accompanied by distinct activation patterns of the muscles of the vocal organ, the syrinx [16–19]. Song-like activation during sleep involves contractions of the syringeal muscles without concurrent activation of the

respiratory system, thus no sound is generated [15]. The specific muscle activation patterns facilitated the identification of the silent song sequences during sleep and showed that birds only rarely generate the stereotyped syllable sequence of the daytime song. It is not clear whether and how this form of sleep activation is tied to learning, but it may function in the maintenance of stereotyped motor programmes by enabling error processing during sleep [15,20].

Sensory input during sleep can trigger reactivation events [12,13,20–25]. Thus, irrespective of whether sleep activation occurs in the context of newly acquired motor skills or established motor programmes, elaborate and distributed forebrain circuitry is involved in its motor planning, execution and sensorimotor processing. The involvement of this complex circuitry raises the question about the evolutionary origins of these memory-related mechanisms [26].

During sleep, there is a general lack of mobility, which appears to be promoted by ‘sleep neurons’ comprising a network across sensory and motor pathways throughout the brain [27]. These mechanisms for the prevention of movement are not universal, as some motor activity facilitated by skeletal muscles must occur during sleep. Eye movements and spontaneous body movements occur regularly or occasionally during sleep. Another example relevant for birdsong is the activation of syringeal muscles during the expiratory phase of each respiratory cycle in awake and sleeping songbirds [15]. The mechanisms for blocking movement during sleep must, therefore, be circumvented to facilitate activation of peripheral skeletal muscles, whether this activation is linked to life-sustaining activity, spontaneous movement or replay of existing motor programmes [14,15].

Combining these neural observations, motor memory-related reactivation during sleep may constitute neural processes that have evolved from pre-existing mechanisms. Therefore, it is pertinent to test whether song-like motor behaviour occurs during sleep in suboscine birds, which generate song innately [28,29] and do not possess the elaborate forebrain circuitry for acquisition and generation of learned vocal behaviour [30,31].

## 2. Material and methods

Experiments were performed on four adult great kiskadees (*Pitangus sulphuratus*) and on two adult western wood pewees (*Contopus sordidulus*). Both species belong to the Tyrannidae family. Kiskadees were sexed via polymerase chain reaction amplification of a size-different intron within the highly conserved chromo-helicase-DNA binding protein gene located on the avian sex chromosomes [32]. Three individuals were found to be females, while the sample from the remaining individual could not be amplified for analysis. Pewees were sexed post-mortem by visual inspection of the gonads and determined to be males. Birds were housed in acoustic chambers and subjected to a 14 : 10 h light : dark cycle.

In kiskadees, both the male and female produce song. They sing through the year, but most predominantly during the breeding season (October–March in South America). They are most active and vocal in the early morning after sunrise, and in the afternoon, before sunset, although they continue to sing throughout the day. In the case of western wood pewees, only the males sing during the breeding season [33].

Birds were captured using mist nets, they were identified using coloured rings and later transported to the laboratory for experimentation. For kiskadees, no longer than two weeks after

capture, birds were released in the same area of the capture and monitored for a few days after release, as described in the permits from the state of Buenos Aires (DI-331-2018-GCDEBA-DFYMAGP). All experiments were conducted according to the regulation of the animal care committees: Institutional Animal Care and Use Committee of the University of Utah (protocol no. 16-03014) and University of Buenos Aires (protocol no. 113, 2019).

We measured the electromyographic (EMG) activity of the obliquus ventralis muscle (ovm) a large syringeal muscle [34], which is situated on the ventral surface of the syrinx. To perform this measurement, we used custom-built bipolar electrodes (25 µm diameter, stainless steel 304, heavy polyimide HML insulated, annealed, California Fine Wire). Pairs of electrodes were inserted in the muscle as previously described [35]. For kiskadee recordings, the signal from these electrodes was filtered (150 Hz high-pass RC filter) and differentially amplified (225×) using a custom-built electronic board, which was mounted on a backpack previously fitted on the birds. In wood pewees, electrode signals were amplified (1000×, a larger amplification to ensure signal integrity, since in this case, the amplifier was farther from the measurement site) and band-pass filtered (Brownlee 440; 100–3000 Hz). The sound was recorded using either a condenser microphone (Takstar SGC568) connected to an audio amplifier (Behringer MIC100) or an Audio-Technica (AT 8356) microphone. All conditioned signals were acquired using a National Instruments acquisition board (NIDAQ-USB-6212) controlled using a MATLAB script or using Avisoft Recorder software at 44.15 kHz sample rate.

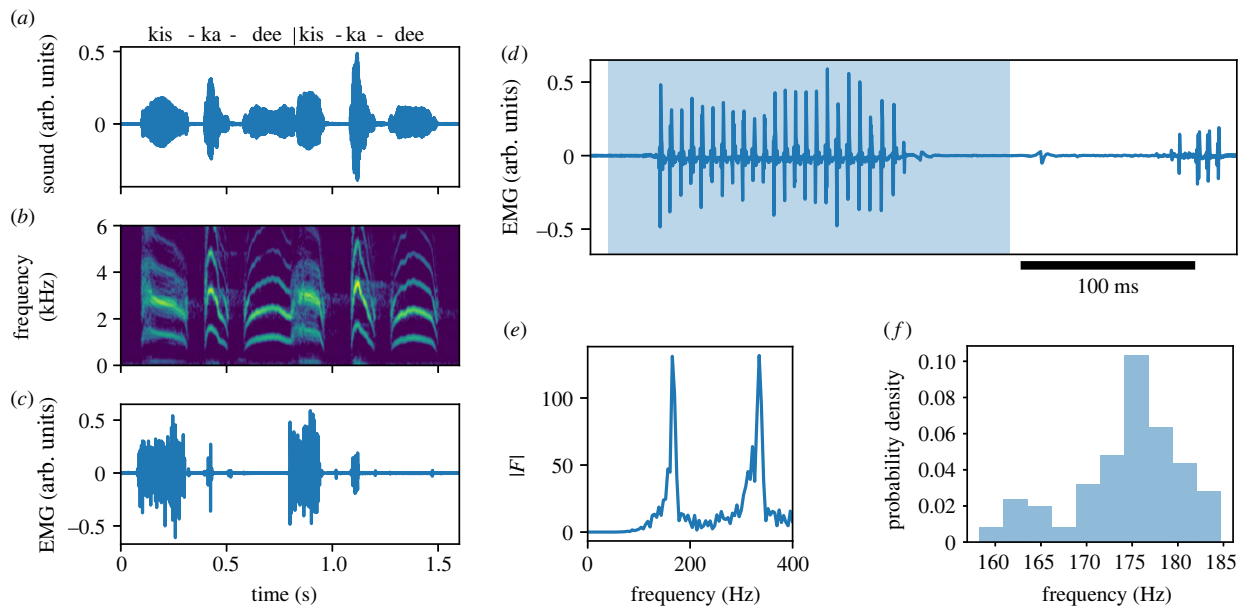
We recorded both signals (sound and EMG) during the day and during the night. EMG signals were then band-pass filtered (100–3000 Hz). Daytime recordings were triggered by a sound level, while night-time recordings were triggered by EMG level (trigger was set at twice the standard deviation of the quiescent signal). In both cases, a 1 s pre-trigger window was recorded.

To control that the birds were actually sleeping we performed additional measurements. In pewees, we measured air sac pressure as previously described [15]. In kiskadees, we were not able to perform both measurements simultaneously, so we decided to record the EMGs while inspecting the bird’s state using an infrared camera (Sony HDR-SR7) to check that their eyes were closed. During the night, and in addition to EMG, in all cases we recorded sound.

## 3. Results

EMG signals during song production were recorded throughout the day in two kiskadees. The song of the great kiskadee consists of three syllables, composing a motif. This motif (as in the case of zebra finches) can be repeated a variable number of times. In figure 1*a,b*, we display an example of a song, in which the three-syllable ‘kis-ka-dee’ is repeated two times. This song structure is stereotyped for each individual, but there is context-dependent variability in the number of repetitions and in the duration and timing of each syllable. Some individuals may also skip the first syllable. The song is accompanied by characteristic patterns of strong EMG activation in the ovm during the first two syllables (figure 1*a–c*).

The ovm muscle has been shown to be involved in the control and amplification of an amplitude modulation present in the first syllable of the kiskadee song [36], via a burst-like pattern of activation. This muscle also presents a minor activation during the second syllable of the song and is not activated during the third syllable. There are other syringeal muscles, but their activation patterns and roles in sound production are still unknown. Kiskadees use the air



**Figure 1.** Song and EMG activity of the kiskadee. (a) Recorded sound of two consecutive songs. (b) Spectrogram of the sound. (c) EMG activity of the ovm muscle. (d) Detail of the EMG activity during the production of the first two syllables of kiskadee song. (e) Frequency spectrum of the shaded segment of the signal in (d), corresponding to the first syllable of the song. (f) Distribution of the mean EMG burst frequencies for the activity during song production in the great kiskadee. The bimodality in the frequency distribution of bursts from daytime vocal behaviour arises from the two types of vocalizations that involve the ovm. Typically, the burst frequency produced during the first syllable of the song was  $170 \pm 8$  Hz, while bursts produced during the isolated 'kis' syllables have a frequency of  $176 \pm 4$  Hz. This, together with the fact that the number of events is not balanced between the two vocalizations ( $n = 25$  songs,  $m = 70$  isolated 'kis' syllables), explains the asymmetry in the distribution. (Online version in colour.)

sac pressure to control the fundamental frequency and timing of their vocalizations.

A detailed view of the EMG signal during the first syllable shows burst-like activation with a characteristic burst frequency (figure 1*d,e*). This activity is involved in sound amplitude modulation and gives rise to a characteristic spectral enrichment of the sound [37]. The 'kis' can also be produced in a call-like manner by itself. To quantify the burst frequency in the EMG accompanying the first syllable, we computed the power spectra and obtained the frequency of its first peak. An example is displayed for the shaded region in figure 1*d* (corresponding to the first syllable of the song on figure 1*a-c*), with its spectrum depicted in figure 1*e*. The burst frequency distribution for 95 vocalizations produced by two birds is shown in figure 1*f*. This dataset was manually curated to include only vocalization-related patterns of activity observed during sound production, and it consisted of isolated 'kis' syllables ( $n = 75$ ) and song ( $n = 20$ ) events. During the day, we did not observe vocalization-like patterns of activity without sound production, either during waking hours or during daytime sleep (12 h were measured).

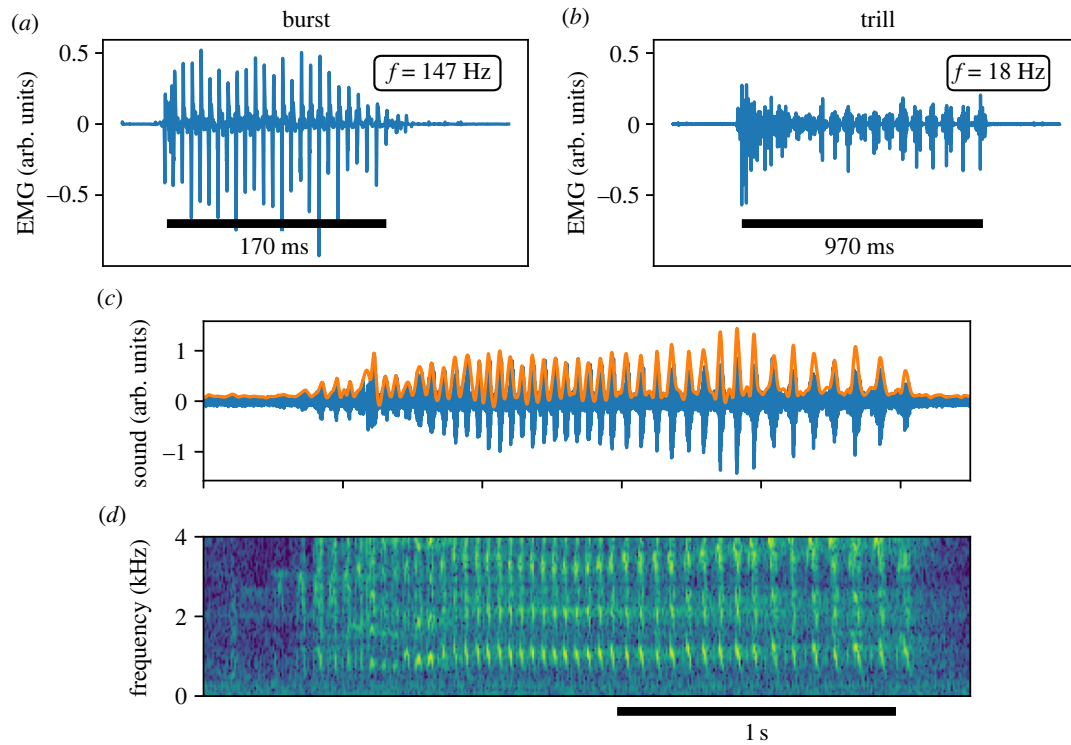
In four kiskadees, we recorded EMG activity for at least two nights. We found that two different patterns of strong EMG activation occurred during sleep. During these patterns of activation, no sound was produced. We also checked that the bird's eyes were closed in the video recordings, whenever possible (i.e. when the bird was facing the camera). First, we found song-like activity, consisting of bursts (figure 2*a*—note the similarity to the EMG activity shown in figure 1*d*).

Second, another qualitatively different type of EMG pattern occurred at night, which was typically much longer than a song-like event (about 5 $\times$ ) and is characterized by a slow modulation of the EMG activity (15–20 Hz). Although our birds did not produce a vocalization corresponding to this EMG activity during the day, based on its temporal

characteristics and the role of the ovm in sound amplitude modulation, we infer that it is consistent with a 'trill' vocalization produced by kiskadees in the context of territorial disputes (figure 2*c,d*). This vocalization is part of a multimodal display during which the bird beats its wings and raises its head feathers to display its bright yellow crown (see electronic supplementary material, movie S1). To systematically find all events of song-like activity during the night, we first identified intervals of the relevant activity. This was done by evaluating the envelope of the EMG signal and searching for segments in which it exceeded a threshold. To calculate the EMG envelope, we calculated the signal peaks considering a minimum separation of 0.02 s and then performed a cubic interpolation between the peaks.

The considered threshold was 0.04. This threshold was selected for two reasons. First, song EMG activity was found to have peak values an order of magnitude greater than this value. Second, by analysing one hundred 30 s segments of EMG activity, in which the birds were not singing, we were able to estimate the range of resting EMG activity. We found an average standard deviation  $\sigma = 0.006$  ( $\sigma \leq 0.02$  for all cases). We used this low threshold in the first stage to avoid missing any relevant events, and to focus on the subsequent classification (either as noise or song-like activity) of the events.

Recently, it was shown that the EMG activity of the ovm consists mainly of bursts of a characteristic frequency, which are produced during the first syllable of the song [37]. The step-wise extraction is exemplified in figure 3 for the data of one of the birds. In order to find song-like replays during sleep, for each segment over the threshold we computed its autocorrelation and the most prominent autocorrelation peak (other than the one at zero-time lag). The inverse of the time lag corresponding to this peak is then the fundamental frequency of the segment. The prominence of the peak, a measure of its harmonicity, was calculated as the height difference between



**Figure 2.** (a,b) Examples of two replays produced during sleep. (a) Song-like activity, corresponding to the activity of the first syllable of the song (note the similarity with figure 1d). (b) Trill-like activity: long intervals (0.7–1 s) of activity with a slow (10–25 Hz) modulation in amplitude were observed, consistent with the production of a trill. (c,d) Trill produced during daytime. (c) Sound amplitude (blue line) and envelope (orange line) (d) Spectrogram of the sound. (Online version in colour.)

the maxima and its closest minima. In figure 3a, we show a typical song-like pattern of activity. Figure 3b shows its autocorrelation as a function of time lag. From this result, we extracted the most prominent peak (black dot in figure 3b, located near 7 ms) and its prominence (vertical black bar). A full analysis for all the data of one bird is displayed in figure 3c. Each dot corresponds to a segment of above threshold activity, with its size proportional to the prominence of the autocorrelation peak. In this figure, we can see that, while most of the segments aggregate to the left (indicating the absence of a harmonic component), some data points cluster to the right (high autocorrelation), at a specific frequency range (approx. 140 Hz), and with a relatively high value of prominence. The dashed vertical line indicates the minimum autocorrelation value that we considered significant.

To calculate a threshold autocorrelation, we first computed the average logarithmic prominence of all events above each possible threshold. This curve shows two different regimes (electronic supplementary material, figure S1). For low threshold values, it grows rapidly due to the decreasing number of low-prominence points for increasing thresholds. For high threshold values, it still grows, but at a slower rate. We used this curve to select the autocorrelation threshold as the transition point between the two growth rates. To estimate this point, we computed linear regressions for each regime (see orange and green lines in electronic supplementary material, figure S1) and used their intersection point as the threshold.

After establishing a threshold, we manually verified each data point above it to determine its nature. In electronic supplementary material, figure S2, we present three examples of events that lie above the autocorrelation threshold but are qualitatively different. While the three cases presented show

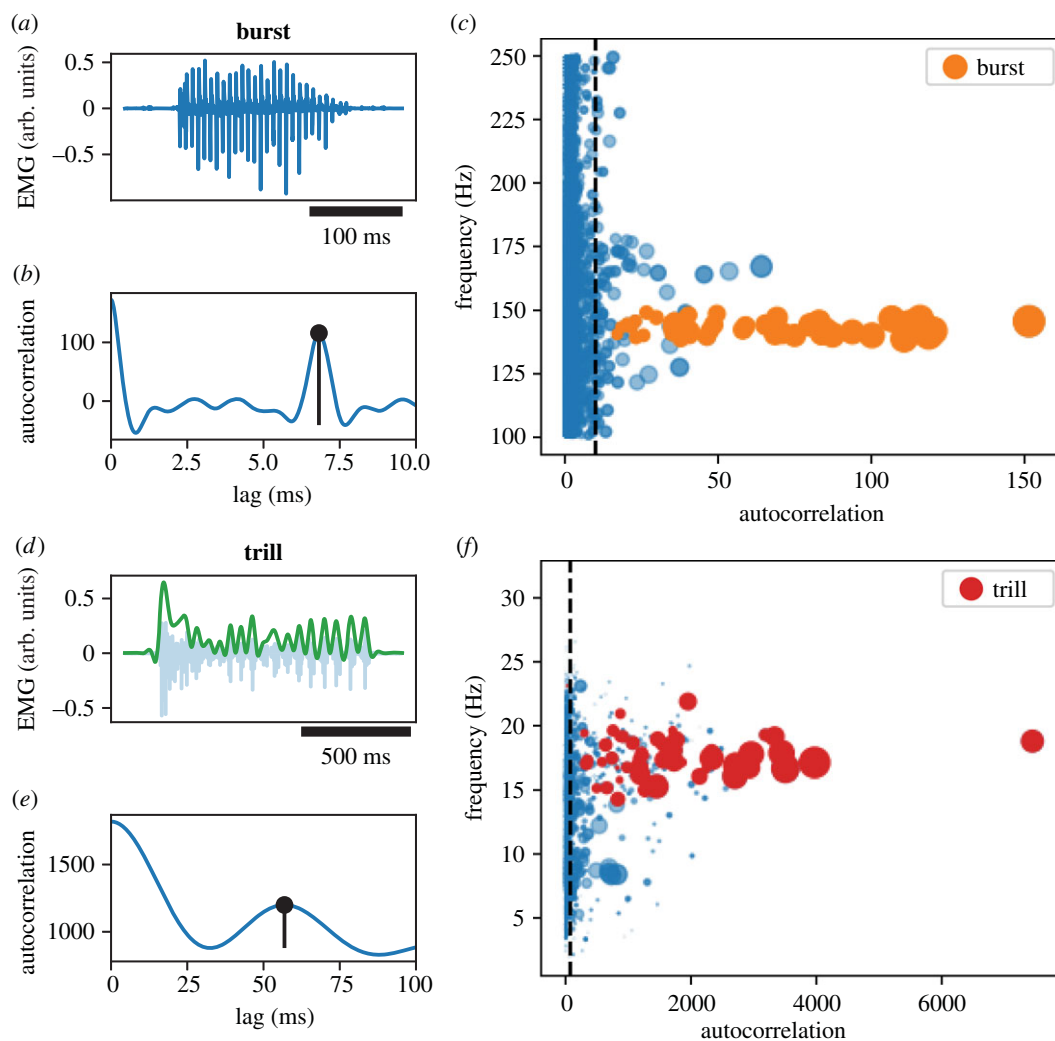
similar autocorrelation maxima, prominence and frequency, only the first one was classified as song-like activity. To determine if they were in fact song-like activity, we again used the autocorrelation function. In the case of song-like activity, which has a very defined frequency, the autocorrelation function is smooth and close to zero between the two maxima. In the other cases (both in trill-like activity and in other events, such as EMG activity accompanying defaecation, or movement artefacts caused by the pecking of the surgery suture) the autocorrelation has many maxima and minima, and changes sign multiple times. Neither the duration of the event nor its frequency was used as an aid for this classification.

The events that were confirmed as song-like activity are represented in figure 3c by orange dots, while blue dots indicate other types of events (note the absence of song-like events near and above the threshold).

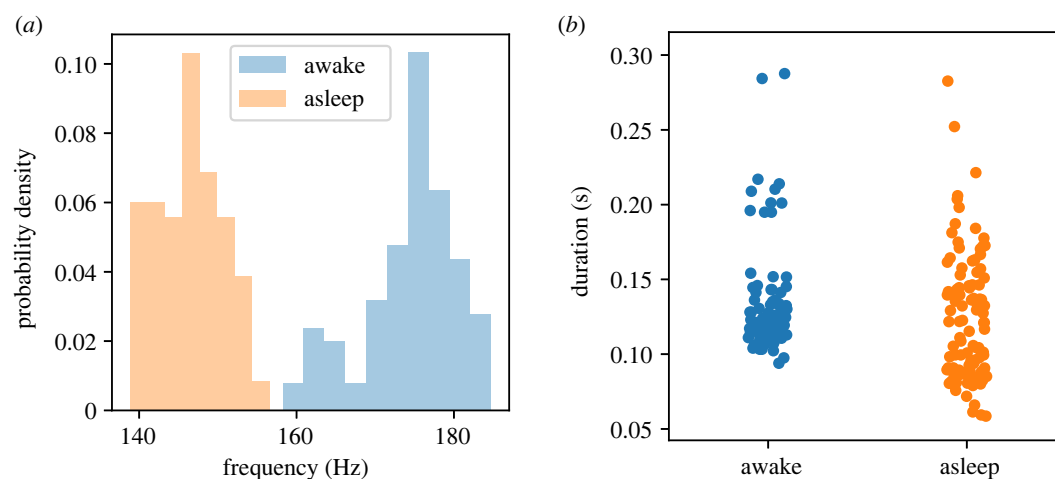
To find events of trill-like activity we proceeded in a similar manner, but since we were interested in events with a much slower modulation, we computed the autocorrelation of the signal's envelope. The results of these analyses are displayed in figure 3d–f. In this case, the classification of over the threshold events was aided by the autocorrelation function of the signal's envelope.

After event detection, all song-like replays were analysed in the same manner as daytime songs to calculate their frequency and duration. We found that in both quantifications night-time activity differed from song activity. The frequency distributions for song-like activity during sleep and during song production show that sleep replays are characterized by a significantly decreased characteristic frequency (figure 4a, Welch's *t*-test,  $p < 0.001$ ). While daytime bursts have a frequency of  $174 \pm 6$  Hz, sleep replays have a frequency of  $146 \pm 4$  Hz, representing a 16% decrease in mean

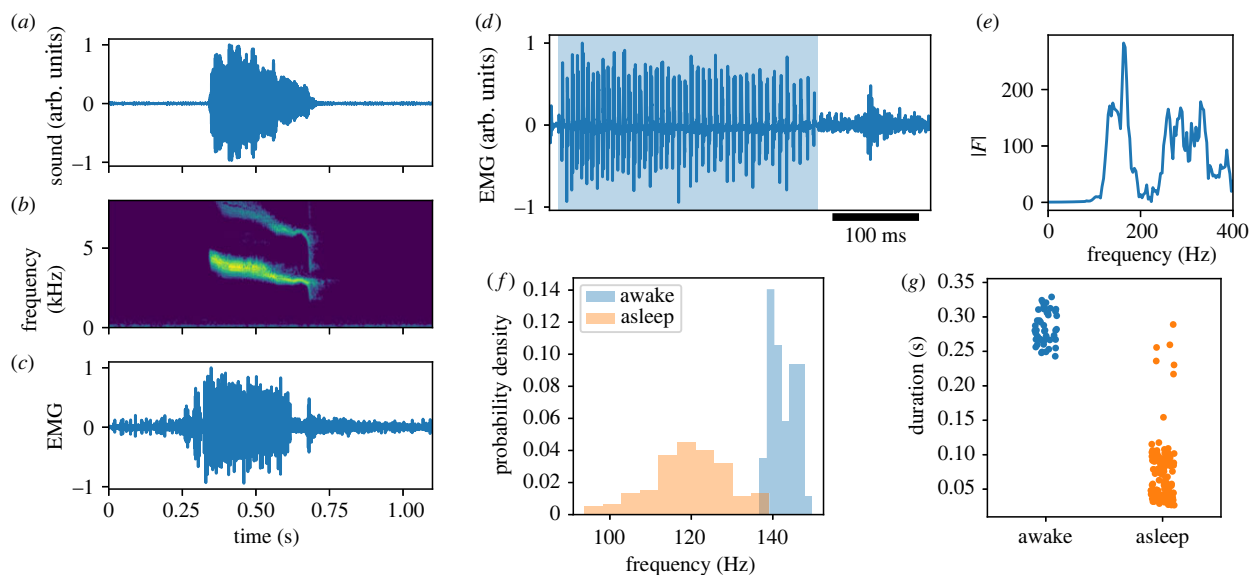




**Figure 3.** Detection of activity events in kiskadees. (a) An example of a song-like replay during sleep. (b) Autocorrelation of the signal as a function of lag. The black dot indicates the most prominent peak, and the vertical black bar its prominence. The inverse of the lag of maximal autocorrelation is the burst frequency. (c) Frequency and maximal autocorrelation for all events of one bird. Point size is proportional to the prominence of the autocorrelation peak. Orange dots indicate those manually identified as burst-like activity. The vertical dashed line indicates the autocorrelation threshold (see electronic supplementary material, figure S1) considered for manual classification. (d) An example of a trill-like replay during sleep. (e) Autocorrelation of the signal's envelope as a function of lag. The black dot indicates the most prominent peak, and the vertical black bar, its prominence. The inverse of the lag of maximal autocorrelation is the frequency. (f) Frequency and maximal autocorrelation for all events of one bird. Point size is proportional to the prominence of the autocorrelation peak. Red dots indicate events manually identified as trill-like activity. Note how events identified as trill-like replays are characterized by a relatively higher autocorrelation, a frequency of 14–20 Hz and a high prominence of the autocorrelation maxima (encoded as marker size). We proceeded in a similar manner to select the threshold (dashed vertical line) as in the previous case. (Online version in colour.)



**Figure 4.** Comparison of awake and asleep song activity. (a) Distribution of EMG frequency for both singing activity (blue) and night-time replays (orange). (b) Duration of EMG bursts produced during awake singing (left, blue), and replayed during sleep (right, orange). Night-time replays show greater variability in duration. (Online version in colour.)



**Figure 5.** Analysis of the western wood pewee. (a) Song of the western wood pewee. (b) Spectrogram of the song. (c) EMG activity of the ovm muscle during song production. (d) Detail of the EMG activity during song production. (e) Frequency spectrum of the shaded segment of the signal in (d). (f) Distribution of the EMG frequencies for the song-like activity replayed during sleep and during singing for the western wood peewees. Burst frequency is significantly lower during sleep replays. (g) Duration of EMG bursts produced during awake singing (left, blue), and replayed during sleep (right, orange). Night-time replays are significantly shorter. (Online version in colour.)

frequency. Furthermore, all the sleep replay events found ( $n = 105$  from four birds) were of a lower frequency than any of those produced during daytime song ( $n = 95$ ; maximum  $f_{\text{night}} = 155$  Hz; minimum  $f_{\text{day}} = 158$  Hz).

EMG burst duration was more stereotyped and consistent during daytime song production than during night-time replay (figure 4b, Levene test,  $p < 0.001$ ). Daytime bursts are clustered in three groups, which correspond to the specific types of vocalizations included in the dataset. The longest duration bursts stem from two atypical vocalizations shortly after the surgery. The middle cluster was produced exclusively by one of the birds which generated a longer first syllable during the first song of a song bout. During sleep, not only the dispersion of the burst durations increased (from 0.03 s to 0.06 s), but also bursts of shorter duration were produced (down to 0.06 s, while the shortest daytime burst had a duration of 0.09 s).

Another difference was found when we analysed the production of the activity corresponding to the second syllable of the song. During the day, the EMG activity was produced either during a call or during the first syllable of the song, in which case it is followed by the activity of the second syllable (after a time gap  $\Delta t = 180 \pm 40$  ms, see the short period of activity after the initial burst in figure 1c) During song production, the motif can be played once, or repeated, in which case the activity of the second syllable is followed (after a gap of  $\Delta t = 360 \pm 20$  ms,  $n = 10$  events) once again by a first syllable burst. During the replays, we found that, whenever the second syllable activity was produced ( $n = 10$ ), it was followed by a first syllable activity. By quantifying the time difference between these two events we found that it was significantly shorter than the observed time difference during song production ( $\Delta t = 150 \pm 90$  ms), suggesting that during the night-time activation the third syllable of the song may have been omitted, something that we did not observe in any case during daytime song production.

We then compared the night-time EMG burst frequency of the presumed trills to the trill rate of sound recordings obtained

in the field. We quantified the amplitude modulation frequency of the trill by computing the envelope of the sound and determining modulation frequency as the inverse of the lag of maximum autocorrelation of the envelope. The trill rate ( $15 \pm 4$  Hz;  $n = 16$ , from two birds) matched the night-time EMG burst frequency very closely ( $15 \pm 4$  Hz;  $n = 93$  from four birds). Since the ovm activation displays a dynamics in the same frequency as the modulation of the sound amplitude during the production of a trill, we infer that this EMG activity represents its silent replay. These patterns were also qualitatively different from any other caused by movement artefacts (caused by flight, eating, drinking or pecking of the surgery suture, see electronic supplementary material, figure S2).

Interestingly, the recordings of the infrared camera showed that the production of trill-like replays during sleep is consistently accompanied by a partial behavioural display, in which the birds raised their crest feathers to expose their yellow crown (see electronic supplementary material, movies S2 and S3). This behaviour accompanied every trill-like EMG activation event produced during the night.

The song of the wood pewee (figure 5a) is accompanied by strong, burst-like activation of the ovm (figure 5c). We did not observe any event of EMG activation without sound production during the day (120 h were analysed). During the four nights of recording in each individual, song-like EMG activity occurred during sleep. To control that birds were sleeping we measured the respiratory rate for sequences of 10 breaths. While the rate during wake hours was ( $2.12 \pm 0.4$ ) Hz ( $n = 180$ , range 1.74 – 2.66 Hz), the rate during the night was ( $1.0 \pm 0.1$ ) Hz, ( $n = 380$ , range 0.85 – 1.38 Hz). The rate during song-like activation was ( $1.0 \pm 0.1$ ) Hz, ( $n = 60$ , range 0.82 – 1.1 Hz). Night-time activation events varied in burst frequency and duration from daytime song. As in the kiskadee, bursting frequency decreased by 15% (Welch's  $t$ -test,  $p < 0.001$ ) from song in the daytime ( $142 \pm 3$  Hz) to night-time events ( $120 \pm 9$  Hz) (figure 5f). As in kiskadees, we also found a significant difference in the duration of the song-like activation. However, while in kiskadees it differed in its dispersion, in

this species, bursting activity produced during night-time events ( $0.07 \pm 0.04$  s) was significantly shorter (Welch's *t*-test  $p < 0.001$ ) than the EMG burst produced during song production ( $0.28 \pm 0.02$  s) (figure 5g).

#### 4. Discussion

The song-like activation patterns of syringeal muscles during sleep in the two suboscine species extend our records of motor reactivation to motor behaviour, which (i) is innate (i.e. not learned), (ii) might occur in males and females, (iii) includes vocalizations that are not considered song and (iv) is executed by circuitry residing in the mid- and hindbrain, rather than the forebrain [31]. Suboscines in the family Tyrannidae develop song innately without the need for acoustic models or auditory feedback [28,29]. Unlike oscine songbirds, they do not exhibit the clearly delineated forebrain and thalamic circuitry necessary for learning from tutor models and for generating learned vocal behaviour [30], although some connections from the arcopallium to mid- and hindbrain vocal and respiratory areas appear to exist [38,39]. It is thought that the vocal motor programme arises in the mesencephalic dorsomedial nucleus of the intercollicularis (DM, equivalent to the mammalian site for vocal control, the periaqueductal grey, PAG). Electrical stimulation in this region can elicit unlearned, species-specific vocalizations [40–42]. Thus, unlike in oscines, the execution of the song motor programme does not require contributions from the forebrain. Telencephalic and hypothalamic input to the DM must play a role in initiating song production [31], but it is not required for the control of normal, species-typical vocalizations.

The reproduction of song-like activation of the syringeal muscles in both suboscine species is similar to that seen in the zebra finch in several regards [15]. Its occurrence is distributed throughout the night, and its timing in the zebra finch suggests that it occurs during slow-wave episodes in NREM sleep [43]. This is consistent with the finding that auditory responses in the sensorimotor nucleus HVC preferentially occur during this sleep state [44]. A further parallel is lower stereotypy in the reactivation patterns, which results in different durations of the burst trains and different burst frequencies. In oscine birds, sleep has been shown to be involved in the development of learned vocal behaviour [8,45]. In species of this group, sleep consists of both rapid-eye-movement (REM) and non-REM sleep [46,47] states that are comparable to those observed in mammals [48]. These sleep patterns have been found in many species [49], but to the best of our knowledge, specific data for suboscines are not available. Whereas our indirect evidence (IR videos and respiratory rate) indicates that birds were asleep during the song-like activation events, we could not determine whether these events occurred during REM or non-REM phases.

In addition, the kiskadee replay of the motor programme of another vocalization that is not considered to be song is remarkable. In the case of the kiskadee trill, even part of the visual component of the multi-modal display is replayed during sleep. This adds a motor programme for a complex multi-modal display to the known cases of replay events. As with the suppression of the respiratory contribution in the vocal display component to prevent sound production, the wing movements of the visual display are not executed, while head feathers are raised to display the yellow crest. In both cases,

display components that might alert potential predators to the sleeping bird are suppressed during the replay of displays.

The burst repetition rate during sleep tends to be slower during song in kiskadees and wood pewees, but not during the trill in kiskadees. The latter observation makes it less likely that the slower rhythm is caused by lower brain temperature during sleep [50,51]. Alternatively, or in addition, intrinsic sleep-induced changes in circuit dynamics may differentially affect the rhythms. Melatonin may play a critical role in these timing changes. The slowing of the rhythm and the shorter duration of song-like activation patterns compared to daytime song agree well with observations following reduced melatonin production in the vocal learning zebra finch and the non-learning Japanese quail (*Coturnix japonica*). Song and crowing were shorter and had a faster rhythm when night-time melatonin production was prevented either through exposure to constant light or pinealectomy [52]. In birds with innate vocal behaviour, melatonin receptors are present in the intercollicular vocal nucleus [53], suggesting that melatonin could directly affect the rhythm of vocalizations where the vocal motor execution presumably originates. Although the songs of the two species in this study are comparatively simple, the changed timing between second syllables and the following first syllable of the next song suggests that deviations from stereotyped song sequence also occur, as was observed in the zebra finch [15].

The parallels to song-like reactivation in the oscine zebra finch indicate that the observed activity in suboscines also constitutes 'replay' of song and even multi-modal displays. The fact that suboscines show these remarkable similarities warrants questions about the evolution of motor replay during sleep. It is currently unclear whether tyrannid suboscines secondarily lost the vocal learning ability or whether vocal learning may have arisen independently multiple times in the clade including parrots and passerines [38,54]. In either case, the fact that the midbrain centre for unlearned vocal behaviour is activated during sleep suggests an ancestral mechanism that may have predated vocal learning. The possible functions of this reactivation in innate vocal behaviour remain to be investigated. Among the exciting possibilities are a neutral hypothesis, peripheral motor system maintenance and/or general consolidation of motor programme circuitry [15]. The latter mechanism fits nicely with the variable reactivation patterns of the stereotyped daytime song in oscines [15] and may, therefore, be a precursor to the more sophisticated telencephalic sensorimotor processes for learned vocal behaviour.

**Ethics.** All experiments were conducted according to the regulation of the animal care committees: Institutional Animal Care and Use Committee of the University of Utah (protocol no. 16-03014) and University of Buenos Aires (protocol no. 113, 2019). Kiskadees were released in the same area of capture and monitored for a few days after release, as described in the permits from the state of Buenos Aires (DI-331-2018-GCDEBA-DFYMAGP).

**Data accessibility.** Code and processed data available from the repository <http://doi.org/10.5281/zenodo.4599099> [55].

**Competing interests.** We declare we have no competing interests.

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