How sleep affects the developmental learning of bird song

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Sleep affects learning and development in humans and other animals, but the role of sleep in developmental learning has never been examined. Here we show the effects of night-sleep on song development in the zebra finch by recording and analysing the entire song ontogeny. During periods of rapid learning we observed a pronounced deterioration in song structure after night-sleep. The song regained structure after intense morning singing. Daily improvement in similarity to the tutored song occurred during the late phase of this morning recovery; little further improvement occurred thereafter. Furthermore, birds that showed stronger post-sleep deterioration during development achieved a better final imitation. The effect diminished with age. Our experiments showed that these oscillations were not a result of sleep inertia or lack of practice, indicating the possible involvement of an active process, perhaps neural song-replay during sleep. We suggest that these oscillations correspond to competing demands of plasticity and consolidation during learning, creating repeated opportunities to reshape previously learned motor skills.

In humans and other animals, sleep facilitates learning and the consolidation of memory[1–11]. Experimental studies have shown reactivation of neuronal populations during post-training sleep[2,11]. For example, in a recent human functional magnetic resonance imaging study, hippocampal areas that were activated during spatial learning were reactivated during sleep, and the amount of this reactivation predicted the improvement in performance during the next day[11]. These and other studies have emphasized the role of sleep in adults, whereas the possible effect of sleep in developmental learning has not been investigated, even though sleep can affect synaptic plasticity and brain development[12]. Furthermore, children with persistent sleep problems are more likely to have behavioural problems[13].

Developmental learning (for example speech acquisition in human infants[14]) takes place during early life and has effects that last the entire lifetime of the individual. Developmental learning is difficult to study because behavioural changes span many time-scales. The challenge is to relate behavioural changes that may occur within hours as well as across daily cycles of wakefulness and sleep to the outcome observed in the adult individual. The study of developmental song learning in birds provides a unique model system for examining this process in detail.

Like humans, songbirds have innate predispositions to imitate complex vocalizations[14,15]. Zebra finch males develop their song between 30 and 90 days after hatching, a time known as the sensitive period for vocal learning[16]. At about day 30 after hatching, young males start producing unstructured sounds (subsong). The onset of vocal learning (after exposure to a song model) is marked by a rapid emergence of structured sounds[17,18]. To learn a song, the bird has to compare these sounds with a memory template of the song model using auditory feedback[19]. Learning is achieved by transforming and differentiating prototype sounds until they resemble the different syllables of the song model[17,18]. Vocal changes occur at both syllabic and syntactical levels[17,18] in conjunction with anatomical and functional changes in the brain[20,21]. Functional changes that are relevant to song learning are driven by changes in behavioural state. For example, neuronal auditory responses to song playbacks are modulated by wakefulness and sleep[22–25]. Furthermore, some neurons of the premotor song nuclei show spontaneous bursting during sleep, similar to the pattern of activity in the awake, singing bird[26,27]. This suggests the possibility of song rehearsal during sleep[28]. However, until now there has been no direct evidence that sleep can affect vocal learning. In this study we recorded the entire song development and examined in detail how vocal learning progresses through cycles of wakefulness and sleep and attempted to relate the transitory effect of sleep episodes to the long-term outcome of developmental learning.

Quantification of vocal changes and vocal learning

To allow continuous measurements we recorded the entire song development and segmented the developing songs into syllable units (about 1 million syllables per bird). We summarized the structure of each syllable by an array of acoustic features. Starting from continuous measurements of features[29], we computed means and variances of each feature across the syllable. These means and variances are the 'syllable features' in our subsequent analysis: duration, mean pitch, mean frequency modulation (FM), mean Wiener entropy (the entropy being a measure of the diversity of power across frequency), mean goodness of pitch, mean continuity (continuity being a measure of spectro-temporal continuity of frequency traces), variance of pitch, variance of entropy, variance of FM, variance of goodness of pitch and variance of continuity. Shortly after the onset of training with song playbacks[17], we observed a rapid emergence of syllable types[30] (clusters; Fig. 1). The development of each cluster was traced semi-automatically[31]. Song developmental learning was then assessed by two different methods. First, we examined changes in means and variances of feature values collapsed across syllables. Vocal changes were measured within each cluster by comparing a sample of 100 syllables produced one day with another sample produced at a different time. We estimated the difference between two samples of songs by averaging vocal changes across all features of all clusters produced by the bird. Second, to estimate vocal learning, we measured similarity to the song model played to the bird, based on a more detailed sliding comparison of feature values in which intra-syllabic temporal relationship between feature values is preserved[29].

Vocal changes during night-sleep

We first trained 12 male zebra finches to imitate a song model from day 43 after hatching. To establish a baseline, we compared differences (absolute percentage of change) across two random samples of 100 songs produced during the same day (Fig. 2a, b, green curve and bars). The difference between those samples is an estimate of the measurement error. We then examined vocal changes from one day to the next, comparing a random sample of 100 songs produced during one day with a random sample produced during the next day. The day-to-day vocal changes were higher than baseline (Wilcoxon, $P < 0.01$) and decreased during song development.

We then examined vocal changes that occurred during night-sleep by comparing the last 100 songs produced during the evening of one day with the first 100 songs produced in the morning of the next day (red curve). Strikingly, the evening-to-next-morning changes were higher than the day-to-day changes ($P < 0.01$). Similar results were obtained in a bird trained by a live tutor (Fig. 2b, striped bars). In adult birds (1 year old) we still observed that the day-to-day variations were larger than the within-day baseline, confirming retention of some plasticity[32,33]. However, transitory changes in the morning were not observed (see below).

Because vocal changes after 12 h of night-sleep were larger than the overall changes that occurred during 24 h, vocal changes must be non-monotonic; that is, they must oscillate during a daily cycle. Indeed, we observed strong daily oscillations in syllable features, most pronouncedly in variance features that capture the richness of acoustic structure within a syllable (Supplementary Table 1). Figure 3 shows how values of Wiener entropy variance (EV) increased from day to day during development (Fig. 3a, b). Strong daily oscillations were observed shortly after training started (Fig. 3c, d), and decreased thereafter (Fig. 3e, f). Note that the decrease in EV after night-sleep was opposite to the overall trend of increase. Pooling all the variance features showed that during early syllable development (Fig. 3h, red curve) the diversity in syllable features was low in the morning, indicating a decrease in intra-syllabic structure after the night (repeated-measures analysis of variance (ANOVA), $P < 0.001$). Syllable structure recovered with 2–3 h of intense morning singing. Suspending training with song playbacks in the morning did not affect the recovery of song structure ($n = 5$, Wilcoxon, not significant (n.s.)). Daily oscillations in song structure were much weaker during late syllable development (blue curve), and undetectable in 1-year-old birds (green curve). Birds trained when adult, starting from day 90 ($n = 8$), exhibited substantial vocal changes but showed very weak post-sleep changes. We compared post-sleep EV changes across age groups, selecting syllables that showed similar magnitudes of change (±s.e.m.) from one day to the next ($5.1 \pm 0.3\%$ versus $5.3 \pm 0.5\%$ EV increase per day in birds trained from day 43 and in birds trained from day 90, respectively). We found strong oscillations, with a post-sleep decrease in EV ($-16.7 \pm 1.5\%$) in birds trained from day 43, and a very weak decrease ($-2.4 \pm 2\%$) in birds trained from day 90 (Mann–Whitney, $P < 0.01$; Supplementary Fig. 1 and Supplementary Data).

The decrease in syllable structure in the morning may suggest that vocal changes during the day progress with the overall developmental trend, whereas vocal changes after night-sleep oppose the developmental trend. To test this hypothesis we assigned a sign to vocal changes during night-sleep, by reference to the overall developmental trend: positive if in the same direction, negative if opposite. For example, if syllable mean pitch decreased during

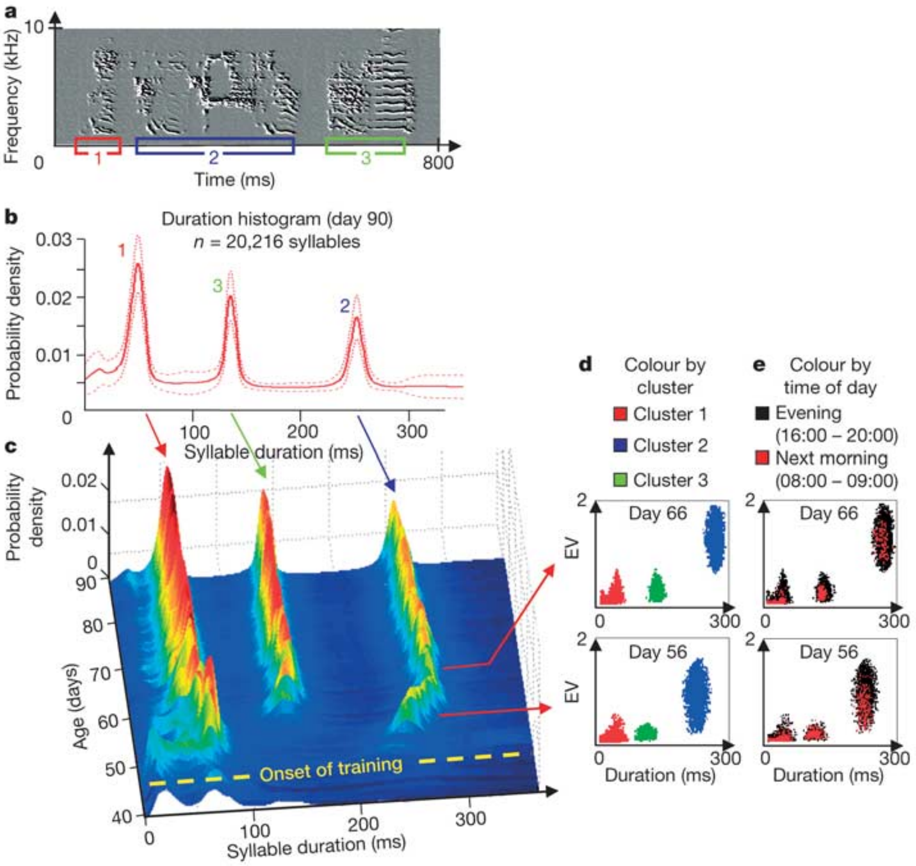

Figure 1 Tracing vocal changes. **a**, Spectral derivatives[29] of adult song motif with three syllables. **b**, Smoothed histogram of syllable durations[46] in an adult bird (±95% confidence interval). Each peak corresponds to a song-syllable type of distinct duration. **c**, To trace the development of syllable types we plot developmental maps in which each row represents the duration histogram of syllables during one day. Syllable types (ridges) emerged shortly after training. **d**, Plotting two-dimensional distribution (duration versus Wiener EV) shows syllable types as clusters (unclustered syllables are not shown). Changes in the position of clusters reveal vocal changes. **e**, Colour-coding circadian time shows vocal changes after night-sleep.

night-sleep but increased during development, we assigned change during night-sleep as negative. Note that if vocal changes during night-sleep were due to random perturbations, the net change across many samples should approach zero. As shown in Fig. 2c, analysis across the 12 birds (including all features of all syllables) confirmed that the net effect of vocal changes after night-sleep was negative. Furthermore, deterioration was observed in both mean and variance feature values (Supplementary Table 1) and was maximal from day 50 to day 55. To separate the effect of training from that of chronological age, we repeated the experiment in birds trained from day 60 (Fig. 2d, $n = 6$). As shown, delaying the onset of training delayed the effect (see Supplementary Data and Supplementary Fig. 2). Isolated birds ($n = 6$) that were kept in the same conditions but were not trained did not show a significant trend (Fig. 2e, f). The magnitude of post-sleep deterioration differed significantly across the groups: highest when training started early, lower when training started later, and lowest in the untrained birds (Fig. 2f, median test, $P < 0.05$). Overall, song was less structured and more primitive after night-sleep but only during days of rapid learning.

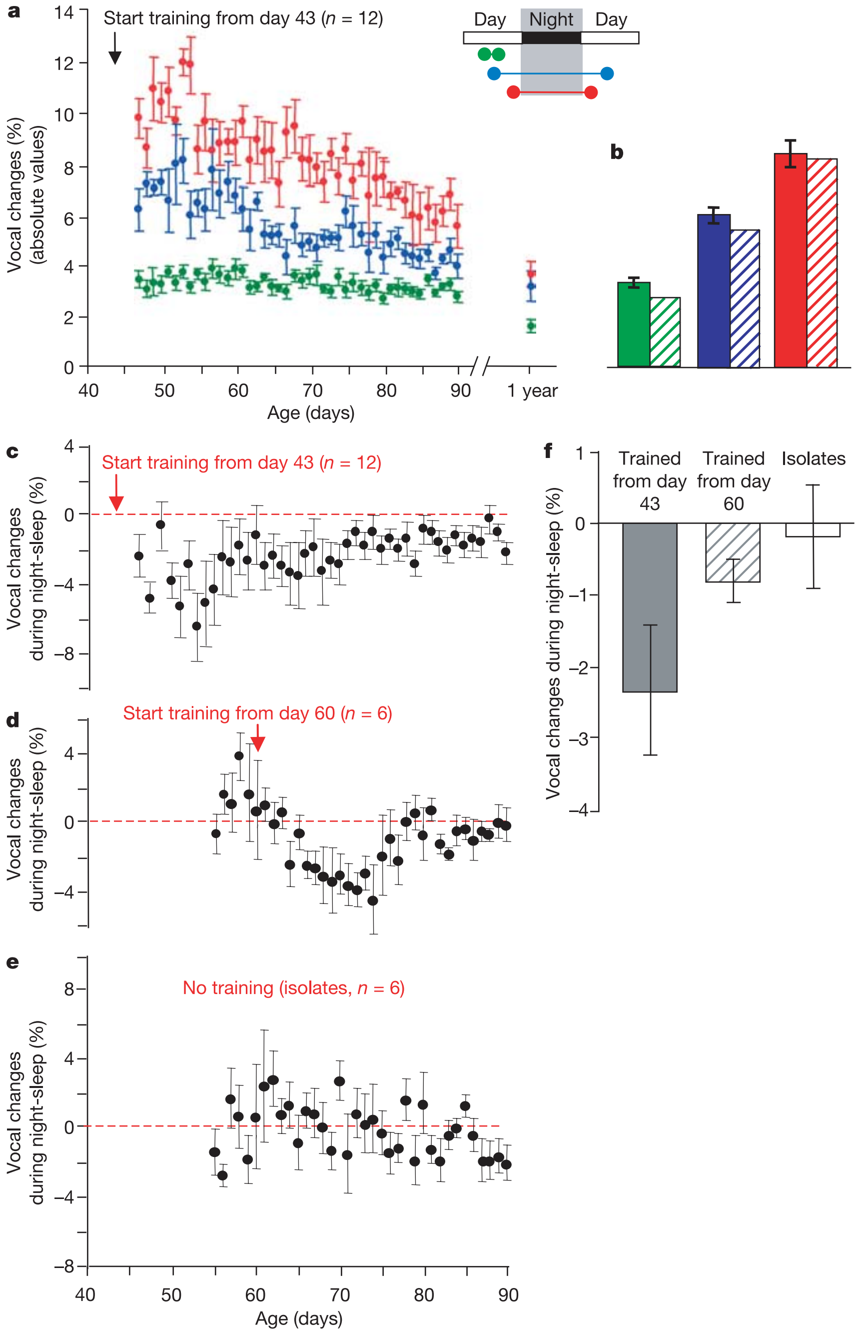

Figure 2 Vocal changes during night-sleep. **a**, Vocal changes (absolute values; medians ± s.e.m.) observed during song development in 12 birds trained from day 43. Green, vocal changes across random samples obtained during the same day (baseline); blue, vocal changes from one day to the next; red, vocal changes from one evening to the next morning (during night-sleep). **b**, The same data, with medians across song development (full bars) and in a live tutored bird (striped bars). **c**–**e**, Vocal changes during night-sleep with reference to the overall developmental trend, in birds trained from day 43 (**c**), in birds trained from day 60 (**d**) and in untrained (isolated) birds (**e**). **f**, The same data as in **c**–**e**, showing medians across song development (median test, $P < 0.05$ across groups and post hoc between birds trained from day 43 and isolates; results are medians; error bars are s.e.m.).

Post-sleep deterioration and learning

We now turn to measurements of vocal learning and examine the eventual similarity to the model song (measured on day 90) against the magnitude of night-sleep deterioration. Similarity analysis is based on comparing detailed temporal structure across sliding windows of two sounds, yielding similarities as a percentage measure[29]. The overall magnitude of post-sleep deterioration during development is positively correlated with the eventual similarity to the model song (Fig. 4a, Spearman $\rho = 0.6$, $P = 0.038$; note, however, that correlation does not prove causality—see further analysis in Supplementary Data). In contrast, the net change in feature values during the entire learning period did

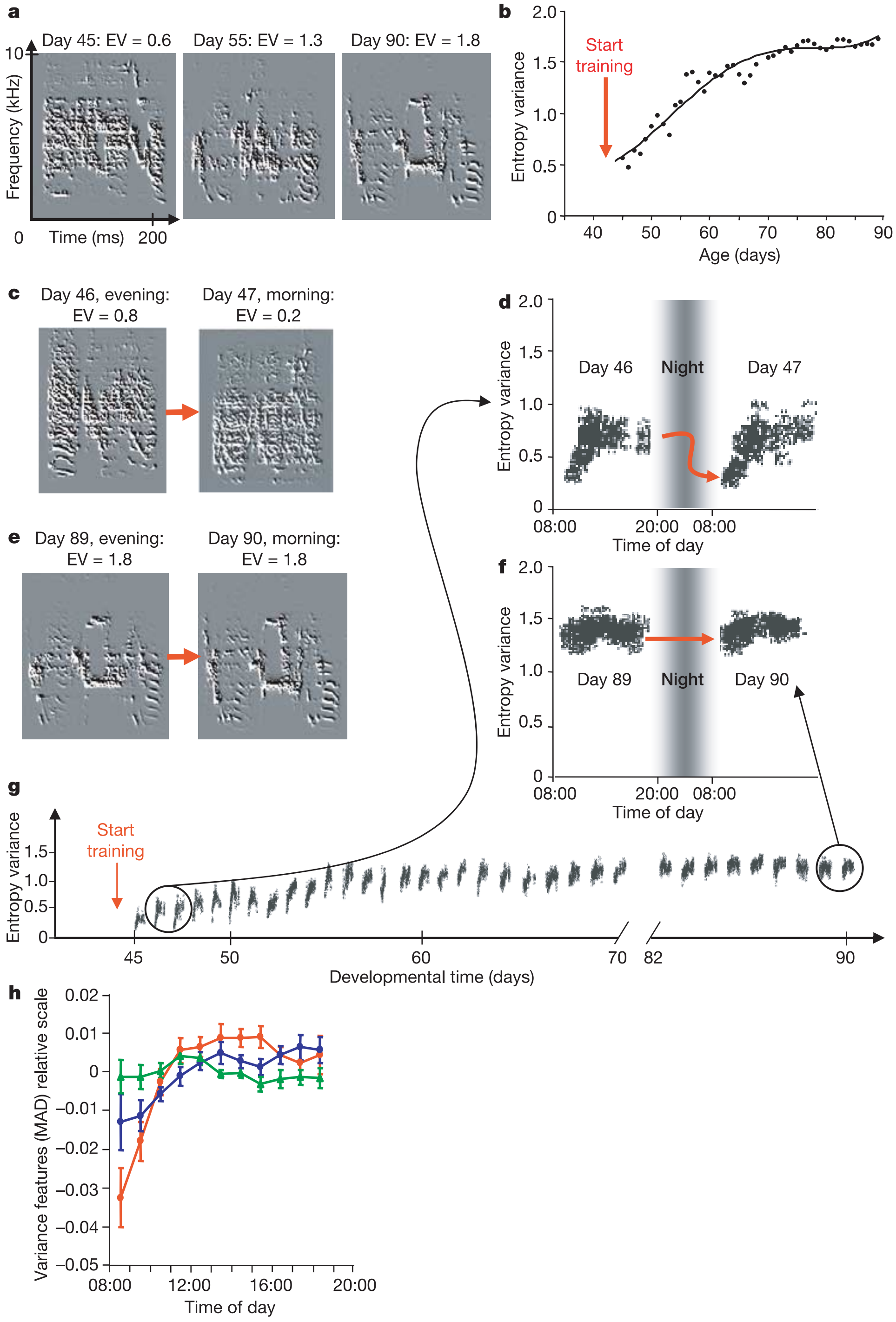

Figure 3 Recovery of syllable structure during the morning. **a**, Three examples of increasing syllable structure during development. **b**, Developmental change in the structure of the same syllable as captured by Wiener EV. **c**, Changes in the structure of that syllable during night-sleep. **d**–**f**, Tracking EV values continuously shows a decrease in EV values after the night-sleep of day 46 (**d**) but not after the night-sleep of day 89 (**e**, **f**). **g**, Tracing EV values continuously during development shows oscillations between days 45 and 60. EV values have been smoothed with a running median (period = 40 data points). **h**, Collapsing together all variance features across birds shows the time course of syllable structure recovery during the day. Red curve, early syllable development (days 50–60); blue curve, late syllable development (days 85–90); green curve, 1-year-old birds. Features units were transformed to median absolute deviation from the mean (MAD). Mean daily MAD values are shifted to zero, to enhance the display of hourly changes in syllable structure. Error bars are s.e.m.

not correlate with better imitation (Spearman $\rho = 0.25$, $P = 0.443$), further confirming that the initial acoustic distance from the song model does not predict song learning[17]. In sum, the magnitude of daily oscillations in song structure, but not the total developmental change, seems to be a predictor of accurate song learning. A similar trend was observed in birds trained from day 90 (Supplementary Fig. 1).

We propose that the morning period of less structured (and perhaps more plastic) sounds gives the bird an opportunity to explore its vocal abilities and improve imitation. To examine the dynamic relations between daily changes in song structure and the progression of vocal learning, we selected the most complex syllable of each bird's song and computed similarity to the model syllable continuously (for each syllable produced) from day 55 to day 70 after hatching. As expected, similarity values dropped during night-sleep (repeated-measures ANOVA, $P < 0.001$) and then increased to a plateau (Fig. 4b). To measure a bird's overall progress towards imitating the tutor song, we defined a measure called 'record similarity', the quality of a bird's best success so far in copying the tutor song (Fig. 4b, c). For robustness, we computed the 95th centile of the similarities within a 1-h moving window. The record similarity at a given time is defined as the best 95th centile achieved so far. Improvements in record similarity are computed with reference to developmental time, not to the previous hour, thus excluding improvements that are due solely to recovery (Fig. 4b, c).

Strong improvements in record similarity were achieved during the third hour of the photophase (Fig. 4d), overlapping with the late phase (third hour) of the recovery of song structure (Fig. 3h, red curve). Little improvement was achieved during the second half of the day. This pattern was not a consequence of different rates of singing across the day, because dividing the increases in record similarity by the singing rate (Fig. 4e) gave similar results, with large increases in record similarity from 3 to 6 h and very low increases during the second half of the day. Once syllable structure reached an asymptote at midday, record similarity did not increase much further, despite continued singing.

Causes of post-sleep deterioration

The underlying cause of the observed effects might be sleep, but there are alternative hypotheses. First, sleep inertia, a transitional state of lowered arousal[34], and possibly altered states of the vocal apparatus ('morning voice'), could explain the deterioration in song structure. Alternatively, vocal changes might be caused by circadian changes in hormonal state. To test both hypotheses, we prevented the bird from singing for 2 h during one morning ($n = 6$, range: 50–57 days) by taking the cage from the sound box into the laboratory[35]. The bird did not attempt to sing but exhibited usual activities including eating and calling. When singing resumed (3–4 h after lights were turned on) we would have expected to see at

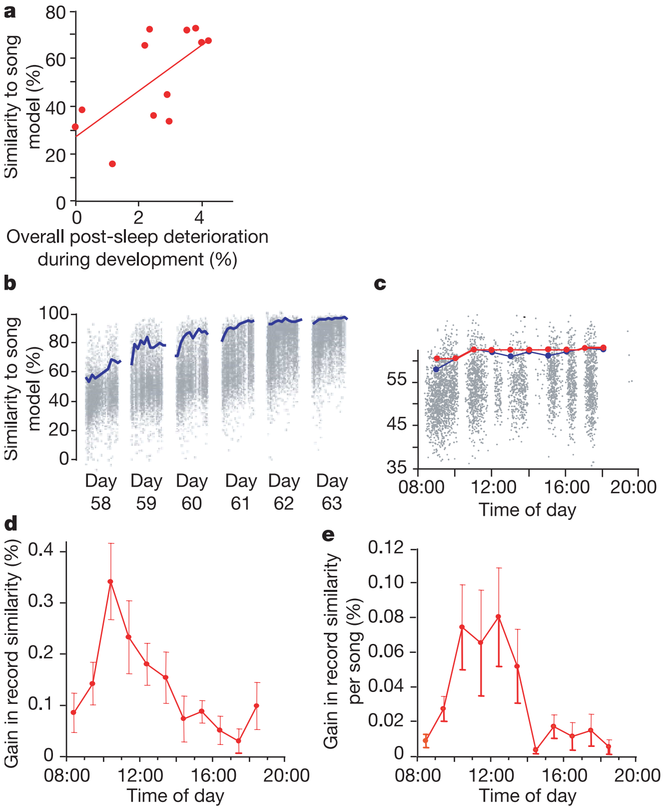

Figure 4 Progression of song learning. **a**, Relation between magnitude of post-sleep deterioration and similarity to the song model. **b**, An illustration of similarity measurements during six consecutive days. Grey dots represent the raw similarity scores and the blue curve shows the 95th centile. **c**, An illustration of record similarity measurements during one day. Red segments illustrate record similarity values and the blue curve shows the 95th centile. **d**, Gain in record similarity to the song model during the day across birds (means ± s.e.m.). Similarity scores were computed continuously from day 55 to day 70 in 12 birds. **e**, As in **d**, but divided by the number of songs produced in each 1-h time bin (means ± s.e.m.).

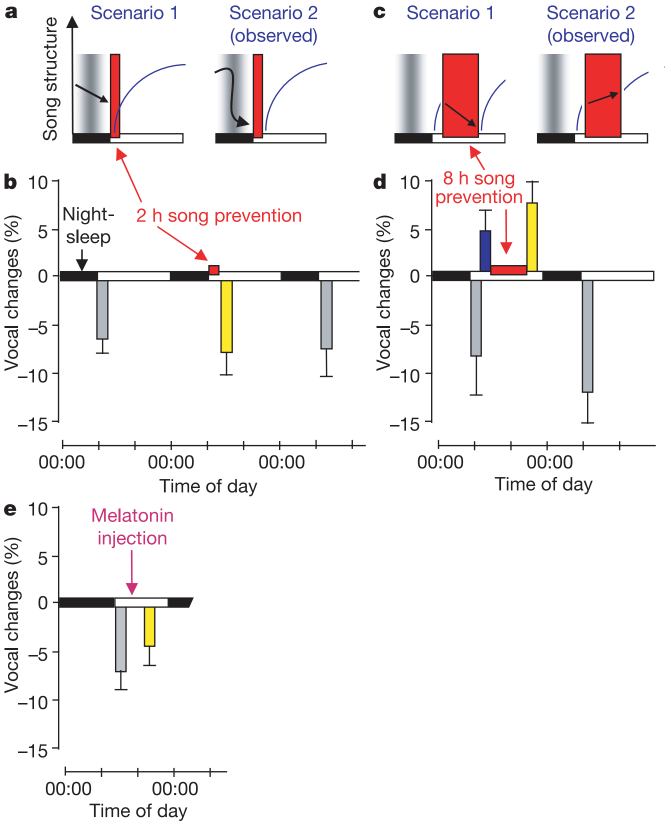

Figure 5 Vocal changes after song prevention and sleep manipulation. **a**, Recovery of song structure could be circadian (scenario 1) or it might require singing activity (scenario 2). **b**, Vocal changes (in reference to the developmental trend) were measured after night-sleep plus 2 h of song prevention (yellow bar, mean ± s.e.m.). Results were similar to those of post-sleep deterioration alone (grey bars, n.s., $n = 6$ birds). **c**, If deterioration of song structure during sleep were due to lack of practice, 8 h of song prevention should cause deterioration (scenario 1) or the bird might continue the morning recovery when singing resumed (scenario 2). **d**, Vocal changes during 8 h of song prevention (yellow bar) were similar to morning changes (from 08:00 to 10:00, blue bar, n.s.) and differed from vocal changes during night-sleep (grey bars, $P < 0.02$, $n = 9$ birds). **e**, Vocal changes after melatonin-induced sleep ($n = 6$ birds). Results in **b**–**e** are means and s.e.m.

least some recovery of song structure if the cause had been sleep inertia or circadian hormonal state (Fig. 5a), but song deterioration remained similar to that observed the day before (Fig. 5b, Wilcoxon, n.s.). Thus, post-sleep deterioration is not simply a circadian phenomenon and cannot be explained by sleep inertia.

Second, it could be that maintaining song structure during days of rapid learning requires intense practice, and that the lack of singing practice during sleep causes deterioration in motor performance. To test this hypothesis, we raised birds under a light regime of 16 h light and 8 h of darkness (16 h:8 h LD) to allow matching of the 8-h night-sleep with an 8-h song prevention interval during the photophase ($n = 9$, range 50–57 days). We monitored song development on-line to detect the time of rapid learning, and confirmed that post-sleep deterioration took place after 8 h of darkness. In the morning we allowed the bird to partly recover its song structure for 2 h. Then we prevented singing for 8 h; finally we allowed the bird to restart singing during the evening of the same day. When singing resumed, we would have expected to see deterioration if lack of practice had an effect, and continued improvement otherwise (as would be expected when comparing the second to the third hour of singing in the morning; Fig. 5c). As shown, vocal changes continued in a positive direction after the 8 h of song prevention (Fig. 5d). The difference between the effects of night-sleep and those of song prevention was statistically significant (Wilcoxon, $P = 0.02$).

Finally, we tested for causal relations between sleep and deterioration of song structure by inducing sleep during the day. Melatonin can induce sleep in zebra finches, and electrophysiological recordings suggest that song replay occurs readily in this induced sleep state[27]. We allowed each bird ($n = 6$, age 54–63 days) to recover its song structure for 4 h after night-sleep and then injected 3 μg of melatonin[27]. All birds fell asleep within 15 min, and slept for 2–3 h during 4 ± 0.5 h of vocal rest. When singing resumed we observed song deterioration in all birds, comparable to that observed after night-sleep (Fig. 5e, Wilcoxon, n.s.).

Vocal changes during sleep could relate to synaptic[21] or cellular[36,37] remodelling that might occur during sleep. Regardless of the specific mechanism, our results indicate the involvement of an active process, perhaps neural song-replay during sleep. How might the bird's 'internal singing'[26,27] during sleep give rise to the observed plasticity? If the lack of auditory feedback during replay during sleep has a similar effect to that of abolishing[32,33,38] or perturbing[39] auditory feedback, we would expect to see drifts in song structure after sleep replay. We analysed post-deafening deterioration in eight adult birds and found that the deterioration of intra-syllabic temporal structure was similar to that observed after night-sleep in young birds (Fig. 6). Therefore, song replay along with lack of auditory feedback could by itself explain the decrease in song structure after prolonged sleep. If feedback-guided singing never resumes (that is, after deafening), this effect is detrimental, but in the intact bird, transitory drifts in song structure could promote learning.

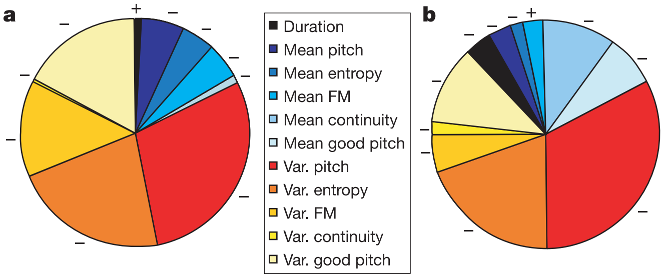

Figure 6 Comparison of post-sleep deterioration and post-deafening deterioration. **a**, Post-sleep changes in birds trained from day 43. **b**, Post-deafening changes (2–4 weeks after deafening). Each slice indicates the mean relative contribution of each feature to the overall effect. The sign indicates whether the feature value increased or decreased. Var, variance.

Discussion

Many procedural and declarative learning skills are enhanced after sleep[1–11] with no apparent oscillations in performance. Oscillations in performance have been reported in the behaviour of several species, but possible relations to sleep were not investigated. For example, bumblebees can learn to approach and probe unfamiliar flowers faster as they gain experience during a foraging day, but these skills deteriorate strongly overnight[40]. Another example is the development of voluntary movement in vertebrates. Movement patterns appear gradually in the newborn infant, progressing from simple to complex, but after prolonged immobility, movements become simple and primitive[41] ('warm up' phenomenon). A similar effect was observed in the development of exploratory behaviour in the juvenile rat[42].

Our study bridges these past observations by demonstrating a sleep-associated oscillation in performance that has a role in developmental learning. A potential reason why such a phenomenon has not been reported before is that the effect is specific to (or stronger in) developmental learning. A second reason for this might be the requirement for exhaustive, long-term behavioural measurements spanning many days to establish the effect.

We advance some speculations about the utility of these sleep-related oscillations in performance. Our speculations are based on the observation that a larger oscillation is associated with better final imitation. The oscillations could reflect a compromise between plasticity and consolidation of structure: periodic increases in plasticity might allow improvement of similarity to the song model through the correction of inappropriately consolidated structure.

Finally, it is worth noting that certain optimization algorithms such as simulated tempering[43] use non-monotonic trajectories of parameters. The ideas of simulated annealing[44] and tempering[43] come from metallurgical processes such as the reduction of brittleness of steel through tempering, which involves a cyclical process of reheating and cooling. ☐

Methods

Animal care

All experiments were performed in accordance with guidelines of the National Institutes of Health and have been reviewed and approved by the Institutional Animal Care and Use Committee of the City College of New York and the Wesleyan University.

Experimental design

We used 50 zebra finches (*Taenyopygia guttata*) from the City College of New York breeding colony. Colony management and experimental design have been described previously[17]. All birds were kept in isolation from days 30 to 90 after hatching.

Recordings from deafened birds were obtained from eight birds from the breeding colony of the Wesleyan University. These birds were kept in a large aviary with their parents and siblings of both sexes until days 70–90, when they were removed and housed together in groups of two to five.

Experimental groups

Training from day 43 to day 90: 12 birds from 8 families were trained with song playbacks[17], starting from day 43 after hatching. We used three different songs (four birds per song model). Birds were raised from hatching under an artificial photoperiod of 12 h:12 h LD.

Training from day 60 to day 90: six birds from three different families were trained from day 60 with the three song models used in the first group (12 h:12 h LD).

Training from day 90 to day 120: eight birds from seven different families were trained from day 90 with the three song models used in the first group (12 h:12 h LD). Birds stayed in acoustic isolation until day 120.

No training: six birds from four different families were kept in similar sound boxes from day 30 to day 90 but were not trained (12 h:12 h LD).

Singing prevention: in 15 birds from 10 families, photoperiod was shifted from 12 h:12 h LD (days 0–30) to 16 h:8 h LD thereafter. Birds were trained from day 43 to day 90 (five birds per song model). We prevented singing once in each bird by taking the bird (with its cage) out of the training box and placing it in the laboratory under observation[35]. We found that the young bird did not attempt to sing when moved to a new location but performed activities such as perch jumping, eating and frequent long and short calls, suggesting that the stress level was not excessive. We detected vocal changes by tracking song development on-line with the DVD-maps module of Sound Analysis Pro[31] (http://ofer.sci.ccny.cuny.edu). Singing was prevented on a single day when rapid vocal changes were observed (between day 50 and day 57). In six birds we prevented singing during the first 2 h of the photophase, and in nine birds we prevented singing for 8 h, from the third to the tenth hour of the photophase (the same duration as the night in this group). In practice, however, singing resumed only about 2 h after our manipulation (2 h song prevention, 99 ± 24 min (mean ± s.e.m.), $n = 6$; 8 h song prevention, 121.6 ± 26.4 min, $n = 9$).

Tutoring prevention: to test the effect of training regimen, we halted training with song playbacks for 2 h during a single day of training (chosen randomly between day 50 and day 55) in five birds trained from day 43 to day 90.

Live tutoring: a young bird was raised and isolated as described previously (12 h:12 h LD). On day 43, an adult male (more than 1 year old, without any affiliation with the pupil) was introduced into its cage and both males stayed together until day 90. To obtain sound data from the young bird, we had to develop a custom source separation algorithm. We had to resolve the problem of source separation because the bird performed a close imitation of its tutor.

Melatonin-induced sleep: six birds were trained from day 43 to day 90 (one song model; 12 h:12 h LD). We confirmed deterioration after night-sleep during that morning (age varied between 54 and 63 days after hatching), and on the fourth hour of the photophase we injected 3 μg of melatonin (Calbiochem) in phosphate-buffered saline (0.3 ml) intraperitoneally. Behavioural state was measured by continuous video recording (see 'Observations on sleep' below).

Deafened birds: eight birds were deafened by bilateral cochlear removal[19] between 123 and 140 days after hatching. Songs were recorded 2–4 weeks after deafening.

Observations on sleep

The zebra finch is a diurnal species that does not sing in darkness. Infrared video recording confirmed that juvenile birds (aged 50–60 days, $n = 5$) presented characteristic postures of sleep[26,28] (that is, head under wing and deep slow breathing) during most of the night. The same behavioural criteria were used to assess melatonin-induced sleep.

Song recording, storage and initial analysis

Using Sound Analysis Pro[31] we recorded the entire song development in all birds, with the exception of occasional system crashes that caused the loss of up to 10% of the raw data. Song activity was detected automatically and saved (16 bits, sampling frequency 44.1 kHz) continuously throughout the experiment. We recorded and analysed 2.2 terabytes of song, stored as wave files in a DeskForce II tera-storage raid system and on DVDs. Songs were analysed with the batch module of Sound Analysis Pro, and results (for example, syllable features) were stored in mySQL 4.0 tables (http://mySQL.com). Final stages of analysis were performed with Microsoft Excel and MATLAB (The Mathworks, Natick, MA).

Spectral analysis and computation of acoustic features

We performed multitaper spectral analysis[45] to compute spectral derivatives and acoustic features as documented in the Sound Analysis Pro user manual. Subsequent analysis was based on the six acoustic features computed on each spectral frame: amplitude, pitch, entropy, FM, continuity and goodness of pitch[29].

Methods of segmentation into syllables, computing syllable features, clustering syllables and tracing the evolution of clusters, estimating vocal changes across time scales, similarity measurements and hypothesis testing are presented in Supplementary Methods with appropriate references.

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