

Sleep and sensorimotor integration during early vocal learning in a songbird

Sylvan S. Shank¹ & Daniel Margoliash^{1,2}

Behavioural studies widely implicate sleep in memory consolidation in the learning of a broad range of behaviours^{1–4}. During sleep, brain regions are reactivated^{5,6}, and specific patterns of neural activity are replayed^{7–10}, consistent with patterns observed in previous waking behaviour. Birdsong learning is a paradigmatic model system for skill learning^{11–14}. Song development in juvenile zebra finches (*Taeniopygia guttata*) is characterized by sleep-dependent circadian fluctuations in singing behaviour, with immediate post-sleep deterioration in song structure followed by recovery later in the day¹⁵. In sleeping adult birds, spontaneous bursting activity of forebrain premotor neurons in the robust nucleus of the arcopallium (RA) carries information about daytime singing¹⁶. Here we show that, in juvenile zebra finches, playback during the day of an adult ‘tutor’ song induced profound and tutor-song-specific changes in bursting activity of RA neurons during the following night of sleep. The night-time neuronal changes preceded tutor-song-induced changes in singing, first observed the following day. Interruption of auditory feedback greatly reduced sleep bursting and prevented the tutor-song-specific neuronal remodelling. Thus, night-time neuronal activity is shaped by the interaction of the song model (sensory template) and auditory feedback, with changes in night-time activity preceding the onset of practice associated with vocal learning. We hypothesize that night-time bursting induces adaptive changes in premotor networks during sleep as part of vocal learning. By this hypothesis, adaptive changes driven by replay of sensory information at night and by evaluation of sensory feedback during the day interact to produce the complex circadian patterns seen early in vocal development.

To explore the role of sleep in the early phases of song learning, we characterized the properties of single RA neurons in head-fixed, sleeping juvenile zebra finches during nights before and after the onset of tutor song exposure. Sleep was defined on the basis of behavioural and electroencephalographic criteria (see Supplementary Information)¹⁷. During sleep, RA neurons tended to discharge irregularly or ‘burst’, as seen in distributions of inter-spike intervals (ISIs; Fig. 1a, black curve)¹⁶. Starting on the night after the first day of exposure to the tutor song, there was a sharp increase in the amount of high-frequency spiking activity (Fig. 1a, red curve). Across all birds, we quantified the effect of tutor song exposure as a normalized change in the percentage of ISIs ≤ 40 ms, which showed a significant increase starting on the night after the first day of tutor song exposure and persisted thereafter (Fig. 1b). A significant result was also obtained considering firing rates that were normalized by linearly scaling the ISIs for each cell ($P = 0.02$, repeated measures analysis of variance (ANOVA), $\alpha = 0.05$; Fig. 1b, open circles). This verified that the increase in high-frequency activity was not dependent on changes in mean spike rates after tutoring, but was the result of a specific increase in high-frequency activity including bursting.

Within each bird there was some variation in the amount of high-frequency activity of RA cells on nights after the onset of song learning, but the tendency towards shorter ISIs was apparent in most cells (Fig. 1c).

Emerging RA bursting activity, furthermore, was shaped by the specific tutor song that a bird heard. Nightly mean ISI distributions were calculated for all RA neurons recorded for each bird after tutor song exposure (which showed little difference from night to night; Fig. 2c), and nightly mean distributions were averaged together to generate one mean curve per bird. For the resulting post-exposure curves, within the high-frequency range (ISIs ≤ 40 ms), the shapes—as assessed using Pearson correlation coefficients—were more similar in birds hearing the same tutor song than in birds hearing different tutor songs (see Supplementary Information). This grouping of ISI distribution shapes by tutor song can be visualized by comparing the average ISI distributions for individual birds on nights before tutor song exposure to those on nights after tutor song exposure (Fig. 2a, black and coloured lines, respectively). The differences between groups can be visualized by comparing global average ISI distributions—one for each group of birds hearing a given tutor song (Fig. 2b).

Once a bird was exposed to a tutor song, a prototypical post-exposure ISI distribution shape was quickly obtained and then maintained. To quantify this, we compared (using Pearson correlations) the nightly ISI distributions (≤ 40 ms) for each bird before and after song exposure to the corresponding global mean curve (Fig. 2b), excluding data from the bird being analysed from the global mean distributions. Before tutor song exposure, both the within- and between-group comparisons (Fig. 2d, black and grey dots, respectively) had large variability and were not significantly different from each other on any night ($P = 0.25$ to $P = 0.73$). By the first night after tutor song exposure, ISI distributions had already assumed their post-exposure shape, showing increased Pearson correlations with much lower variability (Fig. 2d, red dots), whereas the across-group correlations did not increase (Fig. 2d, pink dots). These differences were statistically significant starting from the first night ($P = 0.0082$, two-tailed t -test, $\alpha = 0.05$), and continued to show a significant difference throughout the post-exposure period ($P < 0.05$ for 8 out of 11 post-exposure nights; see also Supplementary Fig. 2), emphasizing that stable changes in ISI distributions were rapidly achieved and then maintained in the days after tutor song exposure.

Zebra finches begin singing as early as 25 days of age¹⁸, so our ~ 40 -day-old birds had extensive sensorimotor experience before neural recordings were performed. To explore the influence of singing and auditory feedback on the structure of RA neuronal bursting, we performed two additional experiments. We prevented singing by surgically muting two birds (a third bird, M2, sang in spite of the surgery), and raised a second group of four birds in continuous 100 dB white noise environments to suppress auditory feedback.

¹Department of Psychology, ²Department of Organismal Biology and Anatomy, University of Chicago, Chicago, Illinois 60637, USA.

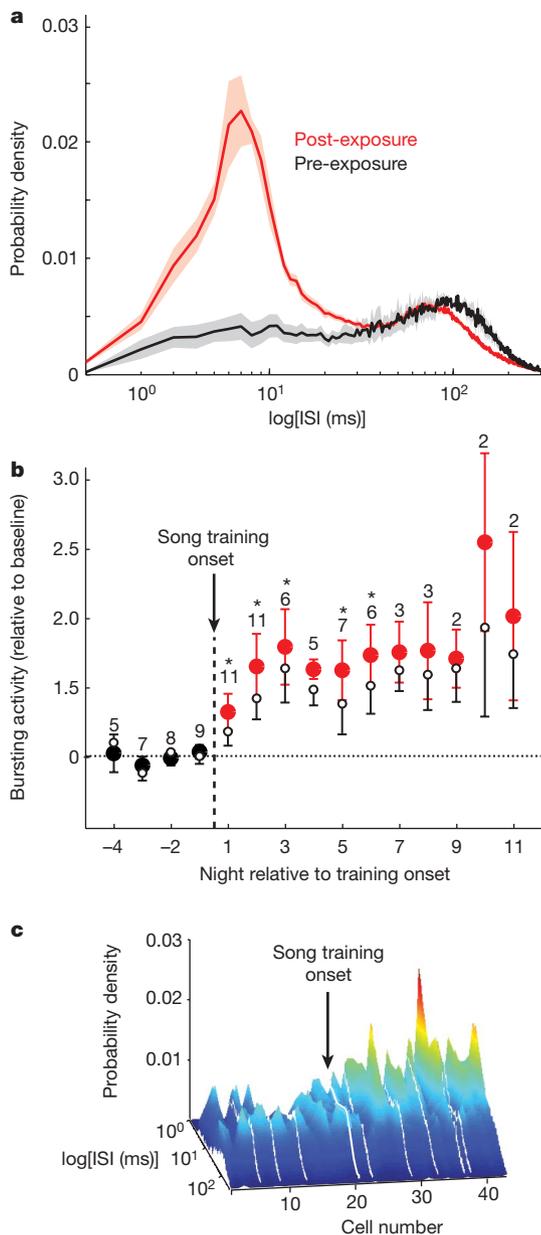


Figure 1 | High-frequency bursting in RA. **a**, ISI distributions for all cells for each night from bird S6 were averaged; each curve (\pm s.e.m.) shows the global average of all nights before tutor song exposure (black; $n = 19$ cells, 3 nights, PHD 43–47) and after exposure (red; $n = 59$ cells, 12 nights, PHD 49–62). **b**, The tutor song triggered an increase in high-frequency bursting (13 birds, $n = 489$ cells, 37.4 ± 56.4 min per cell, 5.6 cells per bird per night). The number of birds contributing to each point is shown above each point. * $P < 0.05$ by sign test comparing mean pre- and post-exposure values within birds, $\alpha = 0.05$. Filled circles: untransformed data (\pm s.e.m.); open circles: spike-rate normalized data ($-$ s.e.m.; see Methods and Supplementary Information). **c**, ISI distributions for all 44 consecutively recorded cells from bird S9. White lines indicate daytime.

All seven birds cued tutor song playback; for birds in the white noise environments, this also briefly eliminated the masking noise. A noise-cancellation technique allowed us to qualitatively assess the amount of singing in white noise conditions (Fig. 3e).

The absence of auditory feedback had large effects on RA sleep bursting. Even before the onset of tutor song exposure, experimental birds showed notably reduced levels of bursting activity compared to birds with intact auditory feedback. ISI distributions showed suppressed short-duration ISIs for all six feedback-deprived birds (that is, excluding M2; 14 nights, 65 cells; Fig. 3a, lower black line)

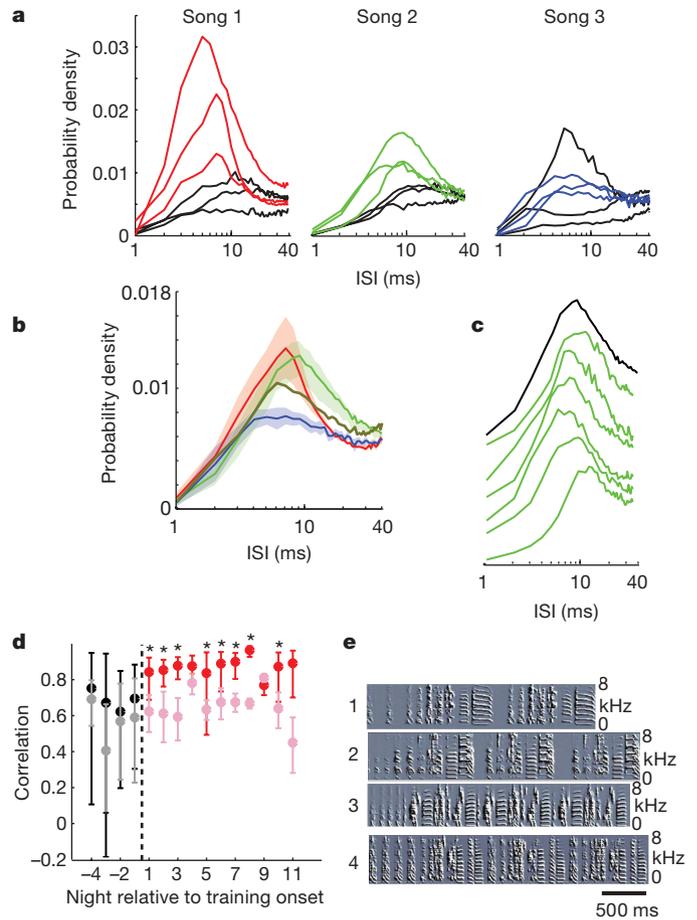


Figure 2 | Tutor songs shape RA bursting. **a**, ISI distributions averaged across all post-exposure nights, one per bird, by tutor song. Black lines, pre-exposure; coloured lines, post-exposure. Song 1, $n = 3$ birds, 59 cells (only a representative 3 out of 7 birds are shown to reduce clutter, see Supplementary Fig. 4 for all birds); song 2, $n = 3$ birds, 85 cells; song 3, $n = 3$ birds, 38 cells. **b**, Global mean (\pm s.e.m.) ISI distributions after tutor song exposure; each curve is the average for each tutor song group. For song 1, two birds with the largest high frequency peaks were removed from the average so that curves have comparable peak heights. The brown line is the single song 4 bird. **c**, Green lines are averages for each of the six post-exposure nights in one bird; the black line is the song 2 global mean (from **b**). **d**, ISI distributions change on exposure to tutor song. Shown are Pearson correlation coefficients comparing nightly curves for a given bird with global post-exposure means (see text). Nightly means (\pm s.d.) comparing birds hearing the same (pre- and post-exposure, black and red dots, respectively) or different (grey and pink dots, respectively) tutor songs are plotted. **e**, Tutor song spectrographs.

compared to birds with normal feedback ($n = 13$ birds, 38 nights, 163 cells; Fig. 3a, upper black line). Considering ISIs ≤ 40 ms, these differences were significant ($P = 0.03$, two-tailed t -test on arcsin-transformed data, $\alpha = 0.05$).

The absence of feedback also disrupted the changes in RA induced by sensory exposure. In feedback-deprived birds, tutor song exposure did not induce an increase in bursting activity (comparing ISIs ≤ 40 ms before to those after exposure, $P = 0.45$, two-tailed t -test on arcsin-transformed data, $\alpha = 0.05$, $n = 6$ birds, 31 nights, 151 cells). Furthermore, on the nights after tutor song exposure, high-frequency bursting was greatly suppressed in feedback-deprived birds ($n = 6$ birds, 18 nights, 98 cells; Fig. 3b, lower red line) compared with normal birds (Fig. 3b, upper red line; $P = 0.04$, two-tailed t -test on arcsin-transformed data, $\alpha = 0.05$).

Subsequent recovery of sensorimotor feedback released RA neurons to rapidly recover to normal patterns. After exposure to 'song 1', but before cessation of the masking noise, the four birds in the white noise environment showed suppressed high-frequency activity compared to

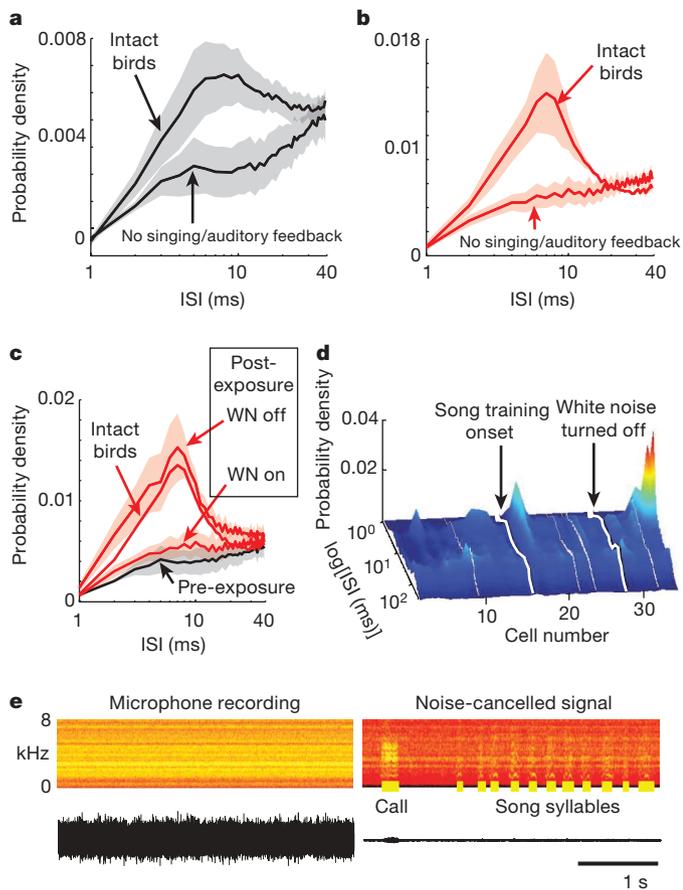


Figure 3 | RA sleep activity in absence of auditory feedback (white noise or muted). **a**, ISI distributions before tutor song exposure, averaged across two muted birds and four birds raised in white noise (lower black line), and averaged (for comparison) across 13 birds with normal feedback (upper black line). **b**, Birds exposed to white noise and those that were muted fail to show tutor-song-specific ISI distributions after song exposure. Lower red line: mean post-exposure distribution from muted birds and those exposed to white noise (5 out of 6 birds heard song 1). Upper red line: mean distribution from intact song 1 birds (same as in Fig. 2c). **c**, Bursting returns when feedback is restored. Birds exposed to white noise ($n = 4$) both pre-exposure (black line) and post-exposure but in white noise (lower red line) show suppression of bursting; bursting recovers after white noise (WN) is turned off (upper red line). Compare with distribution for intact song 1 birds (middle red line). **d**, Profound suppression of bursting for all cells in a bird exposed to white noise. Bursting appears the second night after withdrawal of white noise. White lines represent daytime. **e**, Recording of singing in the presence of 100 dB white noise without and with noise cancellation (left and right, respectively). Right, bold yellow lines mark vocalizations.

birds with normal access to their feedback (comparing ISIs ≤ 40 ms, $P = 0.04$, two-tailed t -test on arcsin-transformed data, $\alpha = 0.05$, $n = 12$ nights, 56 cells), with similar ISI distributions to those observed before tutor song exposure ($n = 8$ nights, 44 cells; Fig. 3c, black curve). The white noise was turned off for a portion of the fourth day of tutor song exposure (turned off for the first three hours, $n = 2$ birds; turned off for the last five hours, $n = 2$ birds) and then turned off completely starting on the fifth day. In two birds that sang robustly during white noise exposure (one released from white noise at the start of the day, the other at the end of the day), during sleep on the night after the fourth day of exposure, the expected tutor-song-specific ISI distributions were observed in the activity of RA neurons for the first time. A third robust singer also showed the expected shift, but only on the following night (Fig. 3d). Singing was suppressed in the fourth bird (white noise (WN)4) throughout the period of white noise exposure and showed a robust increase only by the fourth day after white noise was turned off. Concomitantly, sleep bursting of RA neurons also

remained suppressed for those three additional nights, and then on the night after that first day of singing RA neurons expressed bursting with an ISI distribution appropriate for song 1 ($r = 0.92 + 0.04, -0.08$ within-group on the two nights post-singing onset versus $r = 0.16 + 0.39, -0.46$ for the three nights before singing onset). The mean ISI curve for all four birds ($n = 17$ nights, 84 cells), representing only the data collected after white noise was turned off ($n = 3$ birds) or after white noise was turned off and singing had commenced (for WN4), was typical of birds exposed to song 1 with normal feedback (Fig. 3c, upper red curve).

Analysis of the fine structure of vocal development further demonstrated the tight temporal correlation between song development and night-time bursting. We examined the entropy variance—a measure of song complexity that shows an upward developmental trend tracking song learning¹⁵—for all song segments each bird sang each day. By this measure, songs gained structure in a saltatory manner on the second day of tutor song exposure ($P = 0.006$, repeated measures ANOVA, $\alpha = 0.05$; Fig. 4a)¹⁹. The increase in entropy variance on the second day of training was not present with the first vocalizations of that day. Instead, the increase seen on the second day was achieved across singing in the early part of the day. On days before tutor song exposure and on the first day of exposure, there was no significant increase in entropy variance across morning singing (Fig. 4b, c, see Supplementary Information for statistical analysis). However, starting on the second day of exposure and on the days thereafter, birds showed a clear increase in entropy variance across the early part of the day (Fig. 4b, c), similar to what has been reported previously^{15,20}, and this was significant (see Supplementary Information). Because birds vocalized on the first day of song tutoring before and after they cued tutor song playback, however, this could confound the implications of changes in night-time RA bursting if the birds modified their songs on the first day but only after tutor song exposure, and this was not reflected in mean changes for that day. Analysis of singing at the end of the day, analysis of the most complex songs and analysis of singing immediately after tutoring, however, all failed to support this alternative explanation (Supplementary Information).

In birdsong learning, memories of conspecific songs are acquired early in development and act as a referent to guide subsequent auditory-feedback-dependent sensorimotor learning^{12–14,21–24}. In an influential account of this process, the acquired memory of song was envisioned to act as a ‘sensory template’, directly matching auditory feedback arising from singing with the sensory representation of the memorised song guiding changes in vocal output^{13,25}. We found that the effects of tutor song exposure were immediate, profound and distributed—expressed as rapid, fundamental and long-lasting changes in song-specific high-frequency spiking activity in a fore-brain nucleus one synapse from the motor nucleus innervating the syrinx. The isolation rearing our birds experienced before tutor song exposure is likely to have enhanced the magnitude of these effects beyond what would otherwise be seen in normally raised birds²⁶. Nevertheless, the timing and song-type specificity of the changes are consistent with the action of a song (sensory) memory influencing the functional organization of a motor circuit²⁷, although a causal link has yet to be established. The rapidity of changes in RA sleep activity and its subsequent stability bolsters the idea that the sensory representation was quickly established, opening a ‘gate’ or enabling a dormant circuit, and that song development proceeded with the sensory representation expressed at night guiding changes in daytime premotor patterns. This motivates a new hypothesis: sensory memories can act indirectly (offline) on sensorimotor performance by means of spontaneous activity.

We speculate that during sleep, changes in the RA network (‘consolidation’) reflect updating of sensory memories. Night-time changes would establish a new configuration in the RA network. The following morning, plastic responses released by singing subsequent to night-time reconfiguration might first drive the network away from stability before it recovers stability with additional iterations; this could explain

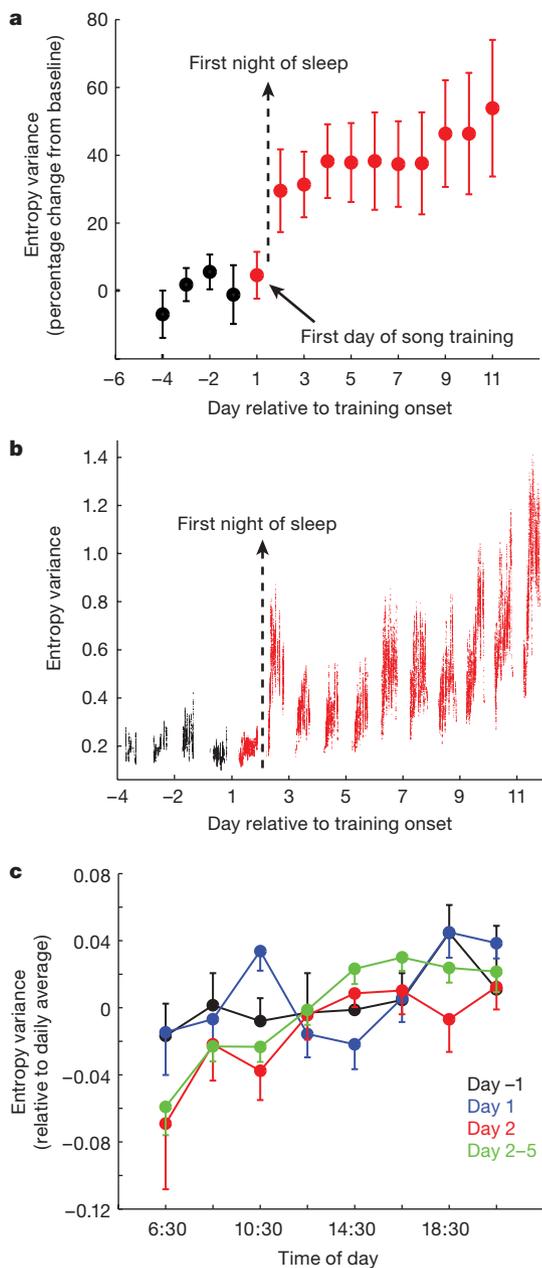


Figure 4 | Entropy variance changes after a night of sleep. **a**, Mean daily entropy variance for bird vocalizations. Data for each bird were normalized by dividing within-day means by the mean of the four nights preceding exposure. **b**, Entropy variance of all sounds produced by one bird across 15 days, smoothed by 40 data points within each day. The entropy variance increase across the second day of training is achieved after morning singing. **c**, Entropy variance daily trends, averaged across all (non-manipulated) birds in two-hour bins (\pm s.e.m.) for the day before training onset (black), the first day of training (blue), the second day of training (red) and the average of the second to the fifth day of training (green).

the non-monotonic changes in singing observed each morning during development¹⁵. A larger magnitude of non-monotonic singing behaviour—which is positively correlated with the eventual degree of song copying¹⁵—would result from stronger or more accurate sensory influences on premotor networks. Because the differences between sensory (night time) and motor (daytime) network configurations decrease during development (improved sensorimotor integration), the magnitude of the circadian singing patterns would decrease, as is actually observed¹⁵. The plastic changes that we have described are released by the confluence of two sensory signals: the model and feedback. We further speculate this could occur at the level of RA itself,

which receives inputs from the descending motor pathway and from the cortico-basal ganglia pathway.

Spontaneous activity guides early development of the nervous system. Here, high-frequency burst-mode firing, a common feature of vertebrate forebrain neurons, is structured by sleep-mediated learning mechanisms, emerging strikingly late in development as the organism experiences appropriate environmental cues. It remains to be seen whether bursting sleep activity represents the activation of sensory traces in other forms of skill learning, and perhaps more generally. Structured spontaneous discharge is observed broadly in the forebrain during sleep²⁸, and has been associated with recall^{29,30}.

METHODS SUMMARY

Birds were bred in-house in sound-isolation boxes, and were female-raised to isolate them from adult male song beginning on or before post-hatch-day (PHD) 15. Males ($n = 21$) were isolated in individual sound-isolation boxes between PHD 30 and PHD 35, and received a preparatory surgery between PHD 37 and PHD 40. Song was recorded continuously starting on or before the first day post-surgery until at least PHD 90, and neural recordings began two to three days later. Birds were permitted to self-elicited song playback by pulling a string in the cage after 3.3 ± 1.6 nights of neuronal recordings.

Bursting increase. We quantified the increase in high-frequency neuronal activity as a normalized change in the percentage of the ISI distribution ≤ 40 ms, which captures short intervals associated with bursting in these juvenile birds and the difference in bursting before and after song exposure (Fig. 1a). Nightly values were calculated by first taking the mean ISI distribution across cells for a given night for a given bird. The proportion of the mean distribution ≤ 40 ms was found and nightly values were normalized within birds by the mean from the four nights preceding tutor song exposure onset (Fig. 1b). We also performed the same calculation but first applying a linear transformation of ISIs so that the mean spike rate was the same for all cells before and after tutor song exposure, finding a similar increase in bursting after song exposure (Fig. 1b, open circles).

Correlation coefficients. For statistical analysis of Pearson correlation coefficients, Fisher's r -to- z transformation was performed on the correlation values. Where the transformed data are plotted (Fig. 2d), the r values have asymmetrical standard deviations because the transformation yields values between -1 and 1 .

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 11 June; accepted 31 October 2008.

Published online 14 December 2008.

- Karni, A., Tanne, D., Rubenstein, B. S., Askenasy, J. J. & Sagiv, D. Dependence on REM sleep of overnight improvement of a perceptual skill. *Science* **265**, 679–682 (1994).
- Fenn, K. M., Nusbaum, H. C. & Margoliash, D. Consolidation during sleep of perceptual learning of spoken language. *Nature* **425**, 614–616 (2003).
- Wagner, U., Gais, S., Haider, H., Verleger, R. & Born, J. Sleep inspires insight. *Nature* **427**, 352–355 (2004).
- Walker, M. P. & Stickgold, R. Sleep, memory, and plasticity. *Annu. Rev. Psychol.* **57**, 139–166 (2006).
- Peigneux, P. *et al.* Are spatial memories strengthened in the human hippocampus during slow wave sleep? *Neuron* **44**, 535–545 (2004).
- Rasch, B., Buchel, C., Gais, S. & Born, J. Odor cues during slow-wave sleep prompt declarative memory consolidation. *Science* **315**, 1426–1429 (2007).
- Ji, D. & Wilson, M. A. Coordinated memory replay in the visual cortex and hippocampus during sleep. *Nature Neurosci.* **10**, 100–107 (2007).
- Qin, Y. L., McNaughton, B. L., Skaggs, W. E. & Barnes, C. A. Memory reprocessing in corticocortical and hippocampocortical neuronal ensembles. *Phil. Trans. R. Soc. Lond. B* **352**, 1525–1533 (1997).
- Nadasdy, Z., Hirase, H., Czurko, A., Csicsvari, J. & Buzsáki, G. Replay and time compression of recurring spike sequences in the hippocampus. *J. Neurosci.* **19**, 9497–9507 (1999).
- Hennevin, E., Huetz, C. & Edeline, J. M. Neural representations during sleep: from sensory processing to memory traces. *Neurobiol. Learn. Mem.* **87**, 416–440 (2007).
- Marler, P. & Tamura, M. Culturally transmitted patterns of vocal behavior in sparrows. *Science* **146**, 1483–1486 (1964).
- Marler, P. A comparative approach to vocal learning: song development in white-crowned sparrows. *J. Comp. Physiol. Psychol.* **71**, 1–25 (1970).
- Konishi, M. The role of auditory feedback in the control of vocalization in the white-crowned sparrow. *Z. Tierpsychol.* **22**, 770–783 (1965).
- Zeigler, H. P. & Marler, P. R. (eds) *Behavioral Neurobiology of Birdsong* (Ann. NY Acad. Sci., 2004).

15. Derégnaucourt, S., Mitra, P. P., Feher, O., Pytte, C. & Tchernichovski, O. How sleep affects the developmental learning of bird song. *Nature* **433**, 710–716 (2005).
16. Dave, A. S. & Margoliash, D. Song replay during sleep and computational rules for sensorimotor vocal learning. *Science* **290**, 812–816 (2000).
17. Low, P. S., Shank, S. S., Sejnowski, T. J. & Margoliash, D. Mammalian-like features of sleep structure in zebra finches. *Proc. Natl Acad. Sci. USA* **105**, 9081–9086 (2008).
18. Roper, A. & Zann, R. The onset of song learning and song tutor selection in fledgling zebra finches. *Ethology* **112**, 458–470 (2006).
19. Tchernichovski, O., Mitra, P. P., Lints, T. & Nottebohm, F. Dynamics of the vocal imitation process: how a zebra finch learns its song. *Science* **291**, 2564–2569 (2001).
20. Crandall, S. R., Adam, M., Kinnischtzke, A. K. & Nick, T. A. HVC neural sleep activity increases with development and parallels nightly changes in song behavior. *J. Neurophysiol.* **98**, 232–240 (2007).
21. Marler, P. & Peters, S. Sparrows learn adult song and more from memory. *Science* **213**, 780–782 (1981).
22. Hultsch, H. & Todt, D. Memorization and reproduction of songs in nightingales (*Luscinia megarhynchos*): evidence for package formation. *J. Comp. Physiol. A* **165**, 197–203 (1989).
23. Funabiki, Y. & Konishi, M. Long memory in song learning by zebra finches. *J. Neurosci.* **23**, 6928–6935 (2003).
24. Leonardo, A. & Konishi, M. Decrystallization of adult birdsong by perturbation of auditory feedback. *Nature* **399**, 466–470 (1999).
25. Konishi, M. in *Perception and Experience* (eds Walk, R. D. & Pick, H. L. J.) 105–118 (Plenum, 1978).
26. Adret, P. & Margoliash, D. Metabolic and neural activity in the song system nucleus robustus archistriatalis: effect of age and gender. *J. Comp. Neurol.* **454**, 409–423 (2002).
27. Nick, T. A. & Konishi, M. Neural song preference during vocal learning in the zebra finch depends on age and state. *J. Neurobiol.* **62**, 231–242 (2005).
28. Evarts, E. V., Bental, E., Bihari, B. & Huttenlocher, P. R. Spontaneous discharge of single neurons during sleep and waking. *Science* **135**, 726–728 (1962).
29. Pastalkova, E., Itskov, V., Amarasingham, A. & Buzsáki, G. Internally generated cell assembly sequences in the rat hippocampus. *Science* **321**, 1322–1327 (2008).
30. Gelbard-Sagiv, H., Mukamel, R., Harel, M., Malach, R. & Fried, I. Internally generated reactivation of single neurons in human hippocampus during free recall. *Science* **322**, 96–101 (2008).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank M. Fukushima for extensive discussions, and J.-M. Ramirez, H. C. Nusbaum, S. M. Sherman and M. Konishi for comments on the manuscript. A. S. Dave and M.F. designed and implemented the white noise recording/cancellation environment.

Author Contributions S.S.S. performed the experimental work.

Author Information Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to D.M. (dan@bigbird.uchicago.edu).

METHODS

Birds were maintained on a reverse light schedule (light, 6:30 p.m.–10:30 a.m.; light:dark, 16h:8h) to permit experiments during daytime hours. Around PHD 38, subjects received a single surgery, including implantation of a head-restraint pin and dural EEG electrodes, and, for some birds, muting.

Neural recordings began two to three days after the surgery, in the same boxes that housed the birds. Birds were wrapped in a cloth and the head was immobilized, connectors were attached to EEG electrode leads, and a high-impedance electrode was lowered into the brain above RA. The box was then closed for the duration of the eight-hour night. Birds were illuminated with infrared light, and EEG signals and video were recorded for the duration of the night while single-cell recordings in RA were obtained. Birds experienced normal levels of rapid eye movement (REM) and non-REM sleep as that which has been reported in songbirds previously^{17,31}, and brief periods of wakefulness. RA was identified stereotaxically and by the readily identifiable activity patterns of RA neurons.

After 3.3 ± 1.6 nights in the recording apparatus (range 1–6 nights), when sufficient pre-exposure data had been obtained (2.6 ± 1.1 nights of successful recordings; range 1–5 nights), birds were allowed to self-elicited playback of a song model by pulling a string, until PHD 90. Birds were limited to ten renditions of the tutor song during each morning and afternoon training session. Neuronal recordings were collected over 5–16 nights (except one bird for which we only succeeded in collecting data for nights –2, –1 and 1 relative to song exposure).

Because clearly identifiable repeated syllables do not begin to emerge until several days after tutor song exposure onset¹⁵, rather than try to cluster song elements in proto-syllables, we examined the entropy variance of all song elements produced to assess daily trends before and after the onset of tutor song exposure. Data collection and analysis used Sound Analysis Pro, Matlab, and in-house software.

Surgical muting. For some birds, a muting procedure³² was performed at the time of the surgery. A small opening was made into the interclavicular air sac. A small fenestra was made in the trachea just rostral to the syrinx and in the bronchi immediately caudal to the syrinx within the interclavicular air sac. The external opening was sutured closed. This procedure probably eliminates the ability to generate pressure across the syrinx. Birds M1 and M3 were successfully muted but bird M2 continued to vocalize immediately after the surgery (presumably, the vocal tract holes sealed). Both muted birds eventually recovered the ability to sing. On days 9 and 11 post-surgery, M1 and M3 began making very sparse, soft vocalizations composed of simple harmonic stacks of various length. Over the following few weeks, birds developed normal plastic song and both eventually achieved a final copy that showed learning in the range of the non-devocalized birds.

White noise cancellation. White noise was played continuously, during the subjective day, starting on the day before the first night of neural recordings. To assess whether birds were singing, we used an active noise cancellation technique³³. In brief, probe auditory signals were played and recorded to estimate the transfer function of the recording chamber. Using the known input to the speaker and the transfer function, the white noise signal broadcast from the speaker was subtracted from the overall signal reaching the microphone, allowing us to observe song (albeit with low fidelity). On the basis of this technique we observed that birds WN1, WN2 and WN3 sang frequently while exposed to continuous white noise (and they also sang immediately after the white noise was turned off). Bird WN4 failed to sing in white noise and also only recovered singing four days after white noise exposure was terminated.

Song similarity measures. The similarity of a bird's song to its tutor song was computed as described previously^{15,19,34} using Sound Analysis Pro software. In brief, means and variances of acoustic features, principally duration, pitch, Weiner entropy and mean frequency, were calculated for each syllable produced by each bird. Similarity between a given song and the tutor song a bird heard was computed by a quantitative comparison of this array of acoustic features.

31. Rattenborg, N. C. *et al.* Migratory sleeplessness in the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). *PLoS Biol.* **2**, E212 (2004).
32. Pytte, C. L. & Suthers, R. A. A bird's own song contributes to conspecific song perception. *Neuroreport* **10**, 1773–1778 (1999).
33. Leonardo, A. Experimental test of the birdsong error-correction model. *Proc. Natl Acad. Sci. USA* **101**, 16935–16940 (2004).
34. Tchernichovski, O., Nottebohm, F., Ho, C. E., Pesaran, B. & Mitra, P. P. A procedure for an automated measurement of song similarity. *Anim. Behav.* **59**, 1167–1176 (2000).