Biasing the content of hippocampal replay during sleep

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The hippocampus is essential for encoding self-experienced events into memory. During sleep, neural activity in the hippocampus related to a recent experience has been observed to spontaneously reoccur, and this 'replay' has been postulated to be important for memory consolidation. Task-related cues can enhance memory consolidation when presented during a post-training sleep session, and, if memories are consolidated by hippocampal replay, a specific enhancement for this replay should be observed. To test this, we trained rats on an auditory-spatial association task while recording from neuronal ensembles in the hippocampus. We found that, during sleep, a task-related auditory cue biased reactivation events toward replaying the spatial memory associated with that cue. These results indicate that sleep replay can be manipulated by external stimulation and provide further evidence for the role of hippocampal replay in memory consolidation.

The hippocampus is critical for encoding recent episodic experiences into memory[1,2]. After an initial encoding phase, a memory is believed to undergo a process of consolidation in which its representation is stabilized in neocortex, allowing future retrieval to be independent of the hippocampus[3]. The mechanisms underlying memory consolidation are poorly understood, but interactions between the hippocampus and neocortex during sleep have been proposed to be crucial for this process[4–7]. In rodents, neurons in hippocampus and cortex that were active during a previous experience have been observed to spontaneously reactivate during non-REM sleep[8–10]. Similarly in humans, a reactivation of brain activity related to a previous experience has also been observed in the hippocampus during sleep[11].

Although replay of a neural sequence associated with a previous experience is a neural correlate of memory, its causal role in memory consolidation has not yet been demonstrated. The best evidence supporting replay's role in memory consolidation has come from the observation that inactivating the hippocampus during replay events produces learning deficits[12,13]. In these experiments, however, the hippocampus was electrically stimulated while in a hyper-excitable state, suggesting that the learning deficits that were observed could have been a result of the disruption of stored memory traces in the hippocampus rather than the blockade of replay events. An alternative approach for investigating replay's function is to enhance memory consolidation by manipulating which encoded memories are replayed in the hippocampus. Several recent studies in human subjects have paired sensory cues with a hippocampus-dependent memory task and have observed learning improvements when these sensory cues are presented during non-REM sleep between sessions[14–16]. If hippocampal replay drives memory consolidation, presenting a sensory cue during non-REM sleep should bias reactivation events toward replaying the previous experience associated with the cue.

RESULTS

We tested this hypothesis by recording neuronal ensembles[10,17] in the hippocampus (right dorsal CA1) of four rats performing an auditory-spatial association task (**Fig. 1a**). After initiating a trial with a nose poke, the rat heard one of two sounds. For sound R (an upward frequency sweep), rats had to run to the right end of the track to receive a reward, whereas sound L (a downward frequency sweep) indicated reward delivery at the left end of the track (see Online Methods). After rats could perform a significant number of correct trials in a single session ($P < 0.05$, binomial distribution; **Fig. 1b**), we recorded place cell activity[18,19] in the hippocampus during the task (**Fig. 2a**) and a subsequent sleep session (**Fig. 2b**) in which task-related stimuli (sound R and sound L) and control stimuli were played in the background in a random order (see Online Methods). Although the cues were the primary influence on behavior during the tasks, we observed a weak correlation between the rat's position being biased toward the resulting direction traversed (**Fig. 2a**), with a larger correlation observed for error trials (correct trials, $r = 0.09$, $P < 6.5 \times 10^{-6}$, 2,474 trials; error trials, $r = 0.19$, $P < 1.5 \times 10^{-13}$, 1,446 trials). Given that the rats were not performing at 100%, they were guessing on a subset of the trials, and such a correlation could arise if a position bias influenced their decision when guessing.

We recorded a total of 409 neurons (4 rats, 11 sessions), of which 199 units had place fields on the track with a minimum peak firing rate greater than 2 spikes per s and a mean rate less than 5 spikes per s (see Online Methods). Reactivation events (**Fig. 2c**) were detected from large transient increases in multiunit activity (MUA)[17] and analyzed by measuring each place cell's firing rate and reconstructing the content of replay from the entire ensemble of place cells using Bayesian decoding methods (see Online Methods).

If the content of a replay event can be altered by the presentation of an auditory cue during non-REM sleep, we should observe differences between sound R– and sound L–related replay activity. For each place cell, we first computed the rate bias, for which the mean firing rate during non-REM sleep replay events (see Online Methods) occurring after sound L's onset and before the onset of the next acoustic stimulus (between 5.8 and 10.8 s later) was subtracted
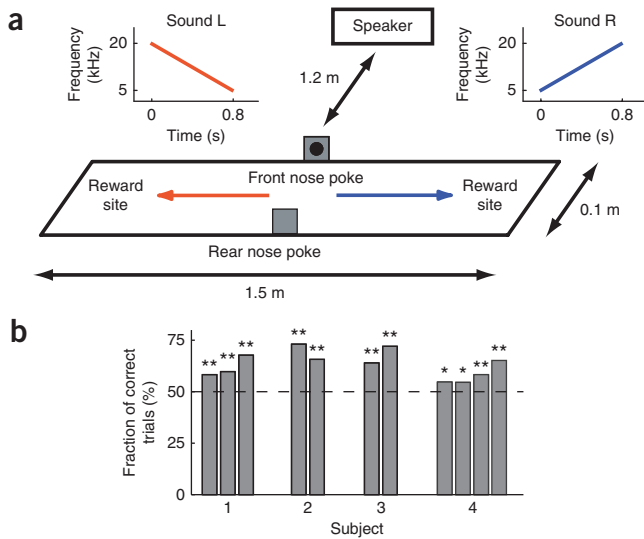
Figure 1 Behavioral task. (**a**) Behavioral task design. After the rat made a nose poke, one of two sounds (sound R or sound L) was played from a speaker 1.2 m from the track center (in front of the nose poke). For a correct trial, the rat had to go the right side of the track for sound R and the left side of the track for sound L. (**b**) Performance on behavioral task. For the 11 recorded sessions in four subjects, task performance was above chance (dashed line) (*$P < 0.05$, **$P < 0.001$, binomial distribution).

mean rate bias was significantly different between right-sided and left-sided place fields ($P < 7.8 \times 10^{-5}$, one-way ANOVA; **Fig. 4c**), with a mean positive bias for right-sided place fields (sound R preference) and a mean negative bias for left-sided place fields (sound L preference). We observed a similar significant difference in mean rate bias between right- and left-sided place fields using alternative methods of calculating the rate bias ($P < 5.0 \times 10^{-4}$, one-way ANOVA; **Supplementary Fig. 1**) and each place field's side-of-the-track preference ($P < 1.2 \times 10^{-4}$, one-way ANOVA; **Supplementary Fig. 2**). Three out of four subjects showed a significantly greater sound R bias for right-sided place fields than for left-sided place fields ($P < 0.05$, one-way ANOVA; **Supplementary Fig. 3**).

Although we observed a significant difference in the mean rate bias between left- and right-sided place fields, this analysis does not tell us whether the bias is caused by sound R, sound L or a combination of both sounds. Thus, in addition to playing sound R and sound L while the subject was resting and/or sleeping after the behavioral task, we also played control sounds that were not associated with a particular side of the track during the behavioral task (see Online Methods). We then recalculated the mean rate bias during non-REM

from the corresponding events after sound R. A positive value indicated a sound R bias, that is, a place cell had a higher firing rate during replay events that occurred after sound R than after sound L, whereas a negative value indicated a sound L bias. We observed that the majority of right-sided place fields had a positive rate bias and left-sided place fields more commonly had a negative rate bias (**Fig. 3**; $r = 0.29$, $P < 1.1 \times 10^{-4}$, Pearson correlation; **Fig. 4a**; binomial test: left, $P < 1.9 \times 10^{-4}$; right, $P < 0.003$; **Fig. 4b**). Furthermore, the

Figure 2 Hippocampal activity during behavior and sleep (rat 1, session 2). (**a**) Neuronal ensemble activity during two correct behavioral trials. From top to bottom, (1) the spatial position (green) of the subject on the track, (2) acoustic stimuli (sound R (R) in blue, a tone indicating a correct trial and reward delivery cue (C) in green, and sound L (L) in red), and (3) raster plot of place cell activity. Nose pokes (NP) are indicated by a vertical dashed line. Sound L was played when a nose poke was made and the rat correctly ran to the left side of the track. Having returned and made another nose poke, the rat heard sound R and ran to the right side of the track. In the raster plot, spikes from place cells with place fields on the right side of the track are shown in blue and left-sided place fields are shown in red. Place fields are ordered from top to bottom by their location on the track (right → left side). (**b**) Neuronal ensemble activity during the onset of sleep while the animal was resting in the sleep box after the behavioral task. From top to bottom, (1) the onsets of acoustic stimuli, (2) animal's velocity (green), (3) a raster plot of place cell activity (data are presented as in **a**), (4) MUA, and (5) awake/sleep classification. Note the increase in the timescale from **a** to **b**. (**c**) Example of reactivation events occurring after the onset of sleep (from **b**). Prior to sleep onset, the rat was resting in the sleep chamber. The reactivation event in the green dashed box is shown to the right (data are presented as in **a**). From top to bottom, (1) acoustic stimulus, (2) place cell activity (red = left-sided place fields, blue = right side place fields), (3) MUA, (4) candidate reactivation events, and (5) sleep classification.
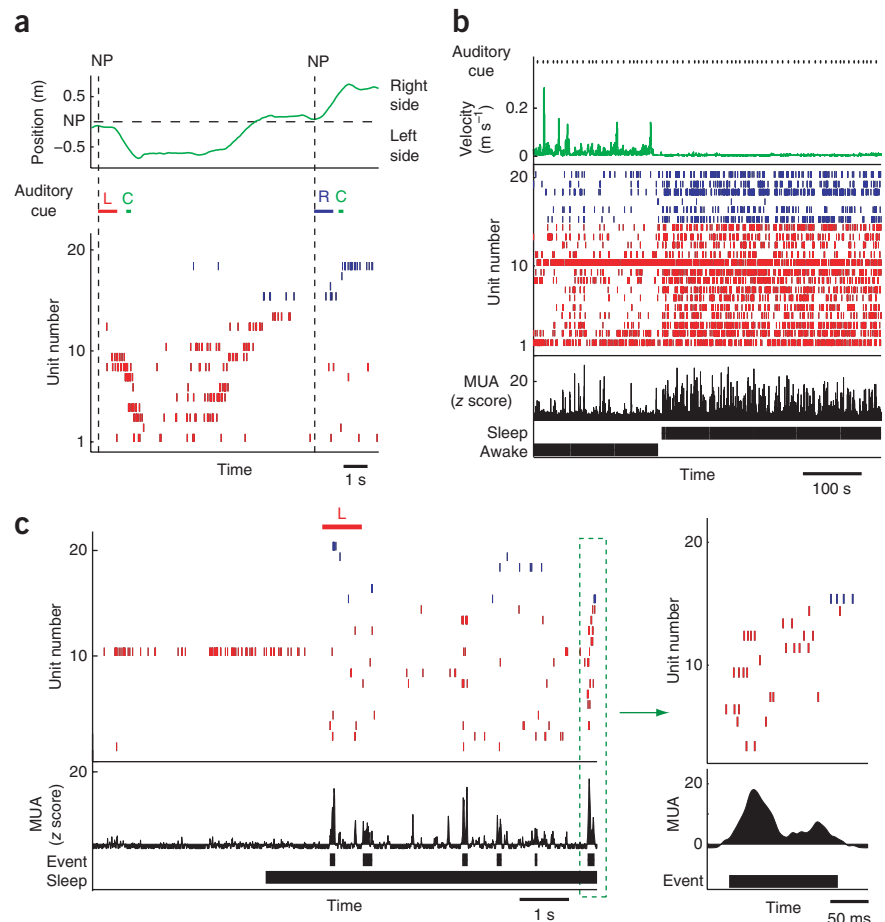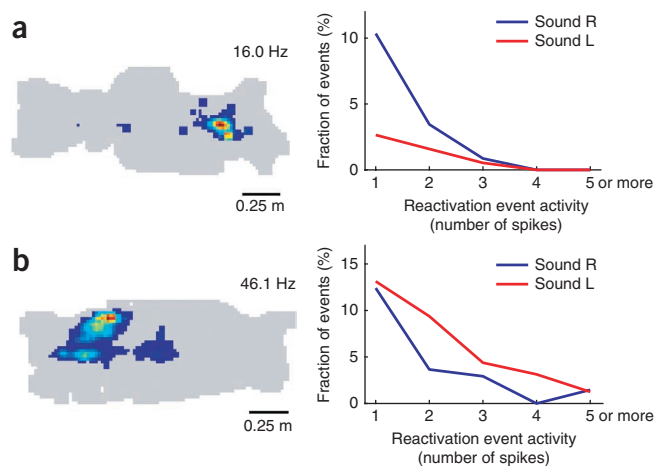
Figure 3 Place cell responses during sleep reactivation events. Place field (left) and plot of place cell activity (right) during reactivation events occurring after sound R (blue) and sound L (red). The maximum firing rate of the place field (left) is indicated in the upper right portion of the plot. Firing rates are plotted using a heat map (blue, minimum; red, maximum). (**a**) Rat 4, session 1, cluster 17. (**b**) Rat 1, session 2, cluster 41.

sleep by comparing firing rates during replay events that occurred after either sound R or sound L to the two task-related control sounds (acoustic cues for correct and incorrect trials). Both sound R and sound L independently evoked a significantly different mean rate bias between right- and left-sided place fields, with sound L having a positive bias (sound L > control sounds) on left-sided place fields ($P < 0.04$, one-way ANOVA; **Fig. 4c**) and sound R having a positive bias (sound R > control sounds) on right-sided place fields ($P < 0.02$, one-way ANOVA; **Fig. 4c**). These data indicate that both task-related cues presented during sleep could independently bias hippocampal activity during reactivation events. We found no evidence that acoustic stimulation had an effect on the number of reactivation events that were occurring, as a similar number of replay events occurred for all acoustic stimuli tested (sound R, sound L and control stimuli; Kruskal-Wallis test, $P > 0.05$). This indicates that a task-related cue only biases the content of replay events that are spontaneously occurring during sleep.

Figure 4 Rate bias during sleep reactivation events. Error bars indicate s.e.m. (**a**) Rate bias during sleep replay events of individual place cells with place fields on the left or right side of the nose poke ($n = 171$). Place cells are ordered along the x axis according to the location of their place field's on the track. A positive rate bias indicates that the mean firing rate during replay events occurring after sound R was higher than that after sound L. The vertical dashed line indicates the center of the track (location of the nose poke). (**b**) The number of place cells during sleep replay events with a sound L or sound R bias. Place cells are grouped according to the position of their place field along the track (left or right). Left-sided place cells had significantly more units with a sound L bias than a sound R bias (***$P < 1.9 \times 10^{-4}$, binomial distribution). Opposite to this, right-sided place cells had significantly more units with a sound R bias than a sound L bias (**$P < 0.003$, binomial distribution). (**c**) The mean rate bias for left-sided and right-sided place cells during sleep replay events. In the first column, the mean rate bias compares sound R and sound L. In the second column, the mean rate bias compares sound L and the two task-related control sounds (reward and error cue). In the third column, the mean rate bias compares sound R and the two task-related control sounds (reward and error cue). The mean rate bias was significantly different between place fields on the left side and right side of the track in all three analyses (one-way ANOVA, #$P < 7.8 \times 10^{-5}$, *$P < 0.05$).

Sound-evoked responses have been observed in the hippocampus[20]. We identified 17 of 199 neurons that made candidate sound-evoked responses during the behavioral task, defined as having a response to sound R that was significantly different (one-way ANOVA, $P < 0.05$) from that to sound L and a firing rate during either sound R's or sound L's presentation that was significantly different (one-way ANOVA, $P < 0.05$) from the discharge rate calculated over the preceding one second of activity (**Supplementary Fig. 4**). To minimize the influence of a neuron's place field on our analysis, we directly compared correct and incorrect trials to the same side of the track (for left and right trials), and did this separately for trials initiated from the front and rear nose poke (candidate auditory-evoked responses: front nose poke, run left = 5 neurons, run right = 6 neurons; rear nose poke, run left = 2 neurons, run right = 5 neurons) (**Supplementary Fig. 5a–d**). One neuron had a candidate sound-evoked response from both the front nose poke (run right) and the rear nose poke (run left). Although some of these responses appeared to be the results of subtle differences in the animal's behavior that correlated with sound R and sound L trials, this classification of candidate sound-evoked responses conservatively estimates the maximum number of neurons that could have sound-evoked responses in our experiment. To explore the possibility that our observed cue-evoked biasing of place cell firing during sleep reactivation events was simply a consequence of correlated sound-evoked responses in the behavioral apparatus and the sleep chamber, we recalculated the rate bias after excluding all 17 neurons that had candidate sound-evoked responses and observed a similar result (left-sided mean rate bias = −0.47, right-sided mean rate bias = 0.62, one-way ANOVA, $P < 8.3 \times 10^{-4}$). We also calculated the number of units with candidate sound-evoked responses while the subject was awake in the sleep box (after the behavioral session). Only two neurons met our criteria for a candidate sound-evoked response, and neither of these neurons had candidate sound-evoked responses during the behavioral task. Furthermore, we did not observe a significant correlation in the sound-evoked activity bias (sound R − sound L) between the two environments (behavioral apparatus and sleep chamber) while the animal was awake (Pearson correlation; front nose poke: right trials versus sleep box, $r = 0.08$, $P = 0.26$; left trials versus sleep box, $r = 0.06$, $P = 0.42$; rear nose poke: right trials versus
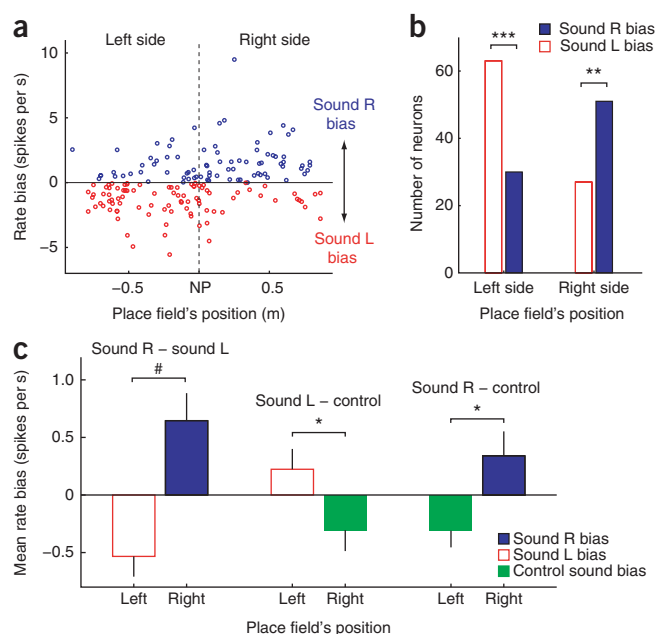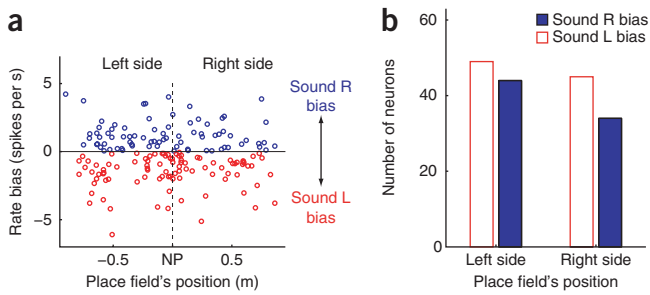
Figure 5 Rate bias during awake reactivation events. (**a**) Rate bias of individual place cells during awake reactivation events ($n = 172$). Data are presented as in **Figure 4a**. Pearson correlation coefficient: $r = -0.03$, $P = 0.74$. (**b**) Number of neurons with sound R and sound L biases (binomial test; left-sided place fields, $P = 0.27$; right-sided place fields, $P = 0.87$).

sleep box, $r = -0.13$, $P = 0.08$; left trials versus sleep box, $r = -0.03$, $P = 0.69$; **Supplementary Fig. 5e,f**). To address any potential bias arising from longer latency sound-evoked responses, we calculated the correlation between the sound-evoked activity bias (sound R – sound L) in the two environments while the animal was awake using different analysis windows (0–1 s, 0–5 s and 0–10 s), starting after the offset of the acoustic stimulus. We did not observe a significant correlation in any of these cases (0–1 s, $r = -0.03$, $P = 0.63$; 0–5 s, $r = 0.01$, $P = 0.85$; 0–10 s, $r = -0.01$, $P = 0.87$). These results indicate that direct sound-evoked responses are context dependent[20] and that any sound-evoked response occurring during behavior will differ from that evoked in the sleep chamber.

In human subjects, the enhancement of memory consolidation by task-related cues is specific to non-REM sleep; no enhancement is observed during REM sleep or when the subject is awake[14,15]. In fact, presentation of task-related cues while a subject is awake can be detrimental for memory consolidation if interference is caused by a second behavioral task, potentially a byproduct of reconsolidation[16]. Thus, we computed the mean rate bias using replay events that occurred while the rat was awake in its sleep box. We did not observe a significant difference between the rate bias for right- and left-sided place cells ($P = 0.82$, one-way ANOVA; **Fig. 5** and **Supplementary Fig. 6**) during awake reactivation events. These data indicate that, in rats, the sound-evoked bias in the replay activity of place cells occurs during non-REM sleep, but not in the awake state, which is consistent with previous data in human subjects.

We next investigated the temporal dynamics of the observed bias in non-REM sleep replay events occurring after the onset of a task-related auditory cue. The analysis window starting at the onset of the task-related auditory cue and ending with the onset of the next cue was divided into two equal time windows: early (0–5.4 s after stimulus onset) and late (5.4–10.8 s after stimulus onset). The difference in mean rate bias between right-sided and left-sided place fields remained significant for both the early and late portion of this analysis window (one-way ANOVA; early, $P < 0.007$; late, $P < 7.4 \times 10^{-4}$; **Fig. 6a**). However, after the onset of the next cue, the previous task-related auditory cue no longer biased the content of reactivation events (one-way ANOVA, $P = 0.87$; **Fig. 6a**). These data indicate that, although the cue itself only lasts for 800 ms, the evoked reactivation bias persists for up to 10 s after the offset of the auditory cue (sound R and sound L); however, after the presentation of a second acoustic stimulus, this bias is reset.

We next examined the temporal dynamics of the observed bias in non-REM sleep replay events over the entire sleep session in each experiment, comparing the early versus late portion of the total time spent asleep (across one or more sleep/wake cycles; see Online Methods). For both the early and late sleep, right-sided place fields had a positive rate bias (sound R preference) and left-sided place fields had a negative rate bias (sound L preference) (**Fig. 6b**). However, the auditory cue was more effective at biasing the content of reactivation in the beginning of the sleep session, as only during the earlier portion of sleep (first half) was the mean rate bias significantly different between right-sided and left-sided place fields (one-way ANOVA, $P < 1.7 \times 10^{-5}$).

Thus far we have examined how task-related sounds bias the firing rates of individual place cells during replay events. The content of replay is determined by the structured activity from an ensemble of place cells during a reactivation event, and as such it is important to determine whether the decoded content of a replay event is actually biased by task-related sounds. To do this, we employed a Bayesian method of decoding the rat's spatial position (**Fig. 7a**) on the basis of neural ensemble activity[17,21]. By computing the conditional probability of each place cell's response given the rat's position on the track while it performed the behavioral task, we were able to use Bayes' rule to measure the estimated likelihood of the rat's virtual position on the track on the basis of neural ensemble activity during a non-REM sleep replay event (**Fig. 7b** and Online Methods). Bias in the mean-decoded position on the track during a replay event was computed using a probability-weighted average (see Online Methods). We observed that replay events occurring after sound R were more likely to be decoded as right sided, whereas replay events occurring after sound L were more likely to be decoded as left sided (sound R, $P < 0.05$; sound L, $P < 0.002$; binomial distribution; **Fig. 7c**). Similarly, if we calculated the mean decoded position bias on the track across all replay events, we found a positive bias for sound R replay events (right-sided bias) and a negative bias

Figure 6 Temporal dynamics of sound-evoked reactivation bias. Error bars indicate s.e.m. (**a**) Mean rate bias for events occurring at different time points relative to the onset of the acoustic stimulus. Events were divided into three groups on the basis of when the events occurred, relative to the sound. For the first group (early), events occurred between 0–5.4 s after the onset of the sound (one-way ANOVA, *$P < 0.007$). For the second group (late), events occurred between 5.4–10.8 s after the onset of the sound, but before the onset of the next acoustic stimulus (one-way ANOVA, **$P < 7.4 \times 10^{-4}$). For the third group, events occurred after the onset of the next acoustic stimulus (one-way ANOVA, $P = 0.87$). (**b**) In each experiment, the sleep session consisting of one or more sleep/wake cycles was concatenated and divided into two periods, first half (early) and second half (late) of the total time spent asleep. The mean rate bias was calculated using all reactivation events in the early and late sleep periods. A significant mean rate bias difference between right- and left-sided place fields was observed during the early period of sleep (one-way ANOVA, ***$P < 1.7 \times 10^{-5}$).
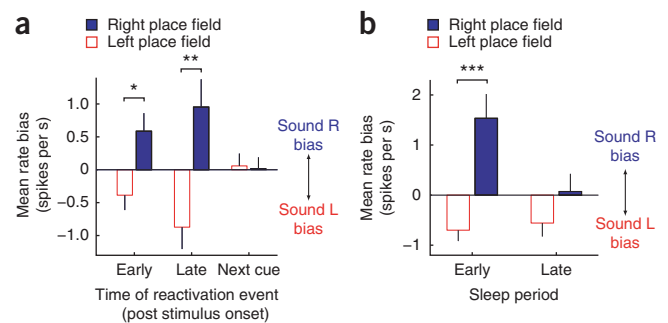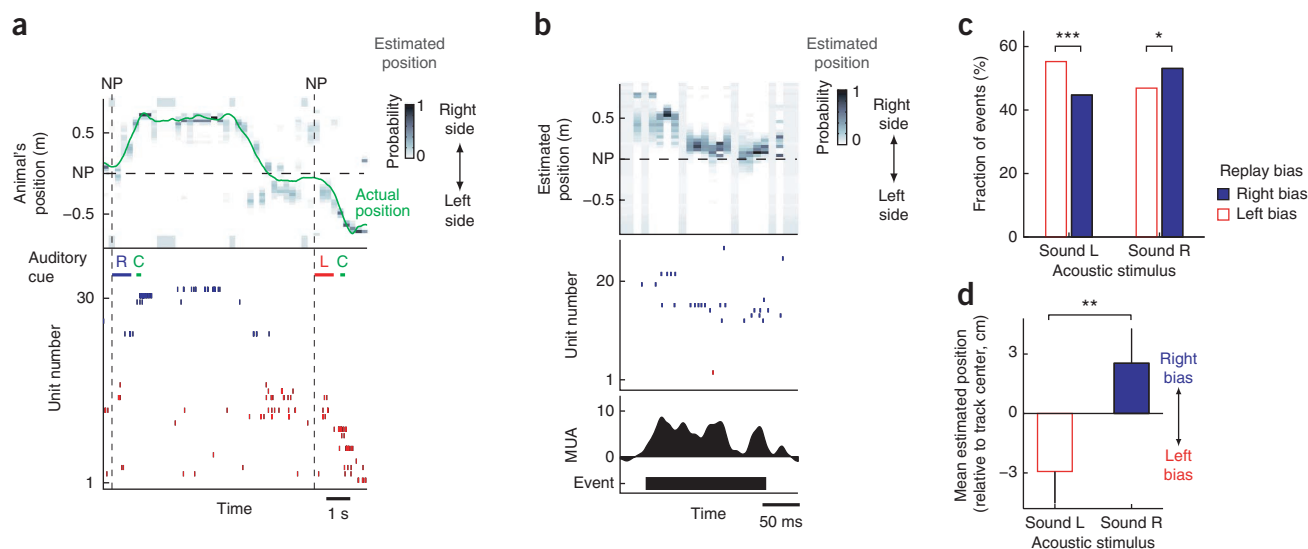
Figure 7 Bias in Bayesian decoded position during sleep reactivation events. (**a**) Example of Bayesian decoding during two behavioral trials. From top to bottom, (1) the estimated spatial position (Bayesian reconstruction) and actual position (green) of the subject on the track, (2) acoustic stimuli (sound R (R) in blue, a tone indicating a correct trial and reward delivery cue (C) in green, and sound L (L) in red), and (3) raster plot of place cell activity. Nose pokes are indicated by a vertical dashed line. All place fields with peak firing rates greater then 0.5 spikes per s are displayed in the raster plot and were subsequently used for the reconstruction. Sound R was played when a nose poke was made and the rat correctly ran to the right side of the track. Having returned and made another nose poke, the rat heard sound L and ran to the left side of the track. In the raster plot, spikes from place cells with place fields on the right side of the track are shown in blue and left-sided place fields are shown in red. Place fields are ordered from top to bottom by their location on the track (right → left side). (**b**) Example of Bayesian decoding of a reactivation event during sleep (rat 2, session 1). From top to bottom, (1) the estimated spatial position of the replay (Bayesian reconstruction), (2) place cell activity (red = left-sided place fields, blue = right-sided place fields), (3) MUA, and (4) reactivation event detection. (**c**) Fraction of replay events occurring after sound R and sound L with a mean decoded position with a right- or left-sided bias. Sound L had significantly more replay events with a left-sided bias (***P < 0.002, binomial distribution), whereas sound R was significantly biased toward the right side (*P < 0.05, binomial distribution). (**d**) Mean estimated position during replay events occurring after sound L and sound R. Sound R replay events had a positive bias (mean position is on the right side of the track), whereas sound L replay events had a negative bias (mean position is on the left side of the track). The difference between the mean estimate position for sound L and sound R replay events was statistically significant (**P < 0.006, Wilcoxon rank sum test). The mean estimated track position is relative to the center of the track (negative values, left side; positive values, right side). From the track center (location of nose poke), the track extends 75 cm to each side. Error bars indicate s.e.m.

for sound L replay events (left-sided bias) (*P* < 0.006, Wilcoxon rank sum test; **Fig. 7d**). These results indicate that, in addition to firing rates of individual place cells being modulated by task-related sounds, the content of a reactivation event is biased toward replaying the portion of the track associated with the task-related cue.

DISCUSSION

We found that hippocampal replay activity can be manipulated by presenting a task-related auditory cue during sleep, such that the content of replay is biased toward the previous experience associated with that cue. This effect was observed at the level of individual place cell responses, as well as in the overall content of replay represented by the entire neuronal ensemble. These data support recent findings in human subjects that memory consolidation is improved by presenting task-related cues during non-REM sleep after a hippocampus-dependent memory task[14,15] and indicate that a cue-evoked bias in replay content is a potential mechanism for the improvement in memory consolidation. Furthermore, our data help to establish a causal relationship between sleep replay and memory consolidation.

Although a sound-evoked bias of replay events was observed during non-REM sleep, this effect was not observed during replay events occurring while the rat was awake. These data match similar findings in humans, where memory consolidation was enhanced by presenting task-related cues during non-REM sleep (but not when awake

or in REM sleep). In laboratory rodents, replay has been observed in an awake (immobile) state[22–24] as well as during both REM[25] and non-REM sleep[9,10]. We do not know whether replay serves the same function in all of these different behavioral states. Because awake replay occurs on a compressed timescale, similar to replay during non-REM sleep (roughly tenfold faster than the real speed observed during behavior), these two forms of replay are more likely to be functionally alike, in contrast with REM replay (which happens on a timescale similar to the previous behavioral episode). However, given that cue-biased memory consolidation and replay are only observed in non-REM sleep, awake replay and non-REM sleep replay may still differ functionally. Whether REM sleep replay is also susceptible to bias by external stimuli remains to be explored.

How does a task-related sound affect what the hippocampus replays? During non-REM sleep, replay occurs during frames, short periods of elevated activity in the cortex and hippocampus that are related to cortical up-states[9]. Although both cortex and the hippocampus replay similar content in a coordinated manner, replay appears to be initiated by the hippocampus[9]. However, frame activity shows the opposite trend, with a cortical frame starting roughly 50 ms before the corresponding hippocampal frame. Thus, although replay of sequential event memory may be driven by the hippocampus, the selection of which memories are replayed by the hippocampus could still be biased by cortical activity occurring before a replay event. Bi-directional interactions between the hippocampus and neocortex

have been proposed to be important for the storage and maintenance of episodic memories in computational models of hippocampal replay[26]. Furthermore, external manipulation of cortical activity via stimulation or pharmacology has been used before to improve[27] or disrupt[28] memory consolidation of a hippocampus-dependent task. Likewise, given that auditory cortex still responds to sound during non-REM sleep[29,30], cortical responses evoked by task-related sounds could also potentially bias hippocampal activity.

We observed that the cue-evoked replay bias decreased in the second half of the sleep session (**Fig. 6b**). Task-related cues may become less effective at biasing replay as a result of either the latency from sleep onset or the number of preceding cue-biased reactivations. If multiple events need to be consolidated, events not associated with the task-related cue (and biased against in early sleep) may take priority at later stages of the sleep cycle. Repeatedly reactivating the same neural ensemble could result in its depotentiation, acting as a homeostatic mechanism that decreases the neural ensemble's future involvement in replay in the sleep cycle.

The content of hippocampal replay activity during the first hour of sleep more closely matches the preceding behavioral episode, as compared with replay after the first hour of sleep[10]. Our data further extend these findings, indicating that there is a limited capacity for biasing replay with task-related cues, and it is most effective in early sleep or during a short nap. Human studies using task-related cues during sleep to improve hippocampus-dependent learning have also targeted early sleep[14–16]. The consolidation of declarative memories is thought to depend more on the early period of sleep[31], at least partly as a result of non-REM sleep being more prominent earlier in the night.

Our data suggest that a cue-evoked bias in the content of a reactivation event is maintained for up to at least 10 s, or until another acoustic stimulus is played. Although this bias may be stored locally in auditory cortex, other brain regions connected to hippocampus and auditory cortex (for example, entorhinal cortex) could also potentially store this bias using persistent activity[32]. Task-related cues presented during sleep can also be used to improve procedural learning[33], suggesting that cue-evoked replay bias may also occur for memories that are not dependent on the hippocampus. How interactions between cortex and hippocampus select the memory replayed during a reactivation event is an open question, but the use of external stimulation to bias memory consolidation provides a model system for further investigation.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Supplementary information is available in the online version of the paper.

AUTHOR CONTRIBUTIONS

D.B. designed the experiment, and collected and analyzed the data. D.B. and M.A.W. co-wrote the manuscript. M.A.W. supervised the experiment.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

All procedures were approved by the Committee on Animal Care of Massachusetts Institute of Technology and followed US National Institutes of Health guidelines.

Behavioral task. Four Long-Evans rats (400–500 g) were initially trained to nose poke to receive a food pellet reward (45 mg, Bio-Serv). Following this, the behavioral task was modified so that, after initiating a trial with a nose poke, subjects heard one of two sounds. For sound R (upward frequency sweep, 5–20 kHz, 800 ms), subjects had to run to the right end of the linear track, whereas for sound L (downward frequency sweep, 20–5 kHz, 800 ms), subjects had to run to the left end of the linear track. On correct trials, subjects heard a brief tone (6 kHz, 200 ms) and received a food pellet, and on error trials, subjects heard a brief white noise burst (500 ms) and received a 10-s time out. If the subject did not make a choice (right or left) within 20 s, the trial was treated as an error trial. The behavioral apparatus was custom made and automated using custom-made software (MATLAB), a USB-based digital I/O module (Measurement Computing), infrared sensors (Sharp GP2D15), reward delivery (ENV-203M, Med-Associates) and acoustic stimulation (JobSite A45-X2 2-Channel Stereo Amplifier, M-Audio Audiophile 192 64-Bit PCI Audio Interface). Sounds were delivered at 70 dB SPL from a speaker (Sony SS-FRF7ED) 1.2 m in front of the subject. The task was performed in a quiet room. The track was 1.5 m long and 0.1 m wide, with two nose poke sensors positioned near the center (one nose poke sensor on each side of the track). The nose poke closer to the speaker is referred to as the front nose poke (**Fig. 1a**). Both nose poke sensors could be used to initiate a trial; however, if the subject used the two nose poke sensors unevenly, the more frequently used nose poke sensor was remotely inactivated, so that the subject would be forced to use the other nose poke sensor. Two nose poke sensors were used so that the task would reinforce a spatial association with sound R and sound L, rather than a motor response (head turn). The mean amount of time (±s.d.) to perform 200 trials across all experiments was 51 min (±13 min). The number of sessions to reach learning criterion was 4 sessions for rat 1, 7 sessions for rat 2, 20 sessions for rat 3 and 14 sessions for rat 4.

Microdrive array and data collection. After learning the task and performing a statistically significant number of correct trials in a single session ($P < 0.05$, binomial distribution), subjects were implanted with a microdrive array containing 16–18 independently moveable tetrodes. Tetrodes were constructed by twisting together four 13 μm in diameter nichrome wires (RediOhm-800, Kanthal), with each microwire electroplated with gold for an impedance of 250–400 kΩ. Microdrive arrays were implanted under isoflurane anesthesia and positioned over the right dorsal hippocampus (2.5–3 mm lateral and 4 mm posterior to Bregma). Tetrodes were lowered slowly over 2–3 weeks until they reached the pyramidal cell layer of CA1. Prior to recording, subjects were retrained until at least two consecutive behavioral sessions had a statistically significant number of correct trials. Reference tetrodes were lowered into the white matter above dorsal CA1, and a skull screw above the left cerebellum served as a ground. The local field potential was filtered between 1 and 475 Hz and recorded at a sampling rate of 3,125 Hz. Video tracking (sampling rate of 30 Hz) of the subject's head orientation and position was performed using an overhead camera to record the movement of two infrared diodes connected to the implanted microdrive array.

Post-behavior sleep session. After performing at least 200 trials with a significant number of correct trials ($P < 0.05$, binomial distribution), rats were placed in a remote location in a quiet room, and allowed to rest and/or sleep for 2–2.5 h. Five different sounds were played to the rat: sound R (upward frequency sweep, 5–20 kHz, 800 ms), sound L (downward frequency sweep, 20–5 kHz, 800 ms), an auditory cue for correct trials (6-kHz tone, 200 ms), an auditory cue for error trials (white noise, 500 ms) and a complex tone not associated with the behavioral task ($f_0 = 1$ kHz, harmonics spanning 5–20 kHz, 800 ms). The last three stimuli were used as control sounds. Sounds were randomly ordered and played with a randomized interstimulus interval ranging between 5–10 s (uniform distribution). Acoustic stimuli were played at a sound level of 50 dB SPL.

Reactivation event detection. MUA was measured using a smoothed histogram (1-ms bins, Gaussian kernel, σ = 15 ms) to calculate the firing rate of all units (not necessarily isolated) with spikes that had a peak amplitude greater than 100 μV,

across all tetrodes. Candidate reactivation events had a minimum z score of 2 throughout the duration of the event, and a minimum peak z score of 4. Multiple events less than 50 ms apart were grouped together into a single event. All reactivation events had to be at least 50 ms in duration. MUA was highly correlated with ripple power but could be measured with greater temporal precision[17]. A total of 409 neurons were recorded in our experiments, with 199 neurons passing our criteria of place fields with peak firing rates greater than 2 spikes per s and mean rates less than 5 spikes per s (to avoid interneurons). Neurons with place fields centered at the nose poke and/or neurons unresponsive during all analyzed reactivation events were excluded from our analysis of rate bias. As a result, our analysis of rate bias was performed on 171 of 199 neurons (sleep replay) and 172 of 199 neurons (awake replay analysis).

Reactivation data was analyzed for post-behavior sessions with at least 30 reactivation events during awake periods and sleep periods for each acoustic stimulus (sound R, sound L, control sounds), occurring after a behavioral session where the subject performed at least 200 trials, with a significant number of correct trials ($P < 0.05$, binomial distribution). A total of 11 behavioral sessions matched these criteria.

Classification of sleep. Non-REM sleep was defined by time periods with high frame activity (upper quartile)[10] containing intermittent high MUA (z score of 4 or greater) and at least 25% of places cells active in a 2-s window, and minimal movement (lower quartile) detected using video tracking. REM sleep was defined by the ratio of the spectral power density in the theta band (6–10 Hz) to the overall power being greater than 0.4 (ref. 34). We obtained similar REM sleep detection using an alternative REM sleep detection algorithm (theta/delta ratio > 6)[35]. The mean ± s.d. total time spent sleeping across experiments was 47 ± 16 min. The mean ± s.d. length of each sleep episode (across experiments) was 12 ± 5 min.

The early and late non-REM sleep periods were determined by concatenating all non-REM sleep periods; early (late) sleep was the first (second) half of this sleep session.

Data analysis. Place fields were computed using velocity-filtered data (>15 cm s^{-1}). Each place cell's position on the track was computed by a firing rate–weighted mean of the place field. We also used two alternative measurements of the place field's position on the track:

$$\sum \frac{R_i}{\mu} \tag{1}$$

$$\frac{\Sigma R_i - \Sigma L_i}{\Sigma R_i + \Sigma L_i} \tag{2}$$

where R_i represents the firing rates of place field on right side of track (ith spatial bin), L_i represents the firing rates of place field on left side of track and μ represents the mean firing rate of place field.

The rate bias (sound R – sound L) was computed by taking the mean firing rate of a place cell during all replay events occurring after the onset of sound R (for up to 10.8 s or the onset of the next sound) and subtracting from this value the mean firing rate of the same place cell during all replay events occurring after sound L. The mean firing rate during replay was the number of spikes divided by the median replay duration (for the session).

To estimate the subject's position during both behavior and replay events from hippocampal ensemble activity, we employed a Bayesian reconstruction algorithm[17,21] using the following equation:

$$P(x|n) = CP(x) \left(\prod_{i=1}^{N} f_i(x)^{n_i} \right) \exp\left(-\tau \sum_{i=1}^{N} f_i(x) \right)$$

where C is a normalization constant, x is the subject's position, $f_i(x)$ is the firing rate of the ith place field at a given location x, and n is the number of spikes in the time window τ.

The maximum likelihood of the subject's position was calculated during the behavioral task using a 250-ms temporal window and during replay using a 10-ms temporal window. The track bias in the reconstructed replay event was calculated by taking a probability weighted average of all reconstructed positions.

Place fields with peak firing rates greater than 0.5 spikes per s and mean firing rates of less than 5 spikes per s (to exclude interneurons) were used in this analysis. We also required a minimum of five place fields on each side of the nose poke for reconstruction. One experiment (subject 3, experiment 2) was excluded from the group analysis for failing to pass this requirement.

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