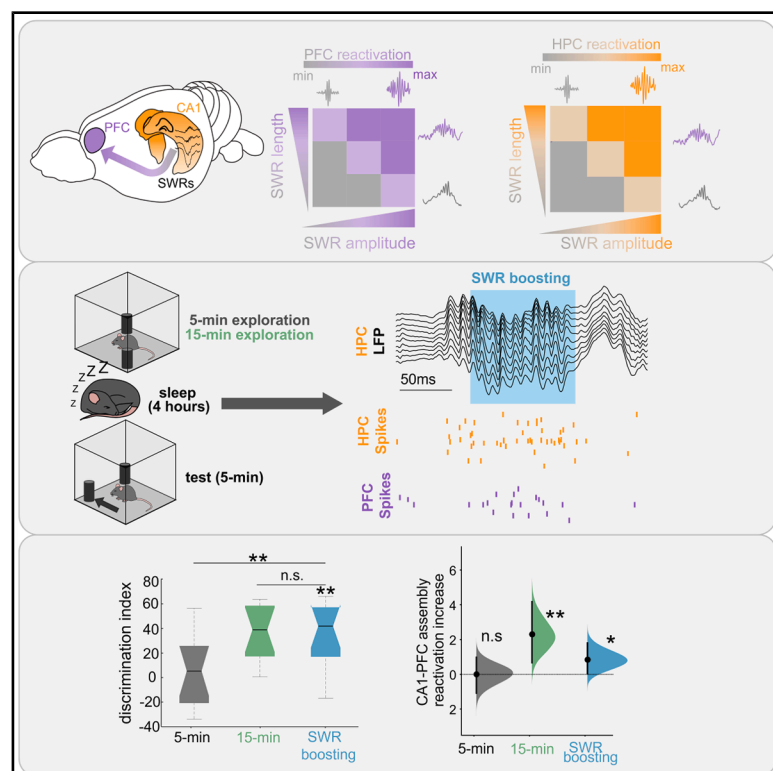


Large sharp-wave ripples promote hippocampocortical memory reactivation and consolidation during sleep

Graphical abstract



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In brief

Robinson et al. found that the reactivation of experience-related neural ensemble patterns in hippocampus and cortex during sleep is specifically associated with a subset of large SWRs. Closed-loop optogenetic boosting of SWRs during post-task sleep enhanced ensemble reactivation in the hippocampus and prefrontal cortex and improved memory performance in mice.

Highlights

- Large SWRs promote enhanced memory reactivation in hippocampus and cortex
- The fraction of large SWRs in post-task sleep correlated with memory performance
- CA1-PFC coordinated reactivation in sleep was associated with large SWRs
- Optogenetic SWR boosting in post-task sleep improved memory reactivation and recall

Robinson et al., 2026, Neuron 114, 226–236

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<https://doi.org/10.1016/j.neuron.2025.10.003>



Report

Large sharp-wave ripples promote hippocampo-cortical memory reactivation and consolidation during sleep

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<https://doi.org/10.1016/j.neuron.2025.10.003>

SUMMARY

During sleep, ensemble activity patterns encoding recent experiences are reactivated in the hippocampus and cortex. This reactivation is coordinated by hippocampal sharp-wave ripples (SWRs) and is believed to support the early stages of memory consolidation. However, only a minority of sleep SWRs are associated with memory reactivation in the hippocampus and its downstream areas. Whether that subset of SWRs has specific physiological characteristics and directly contributes to memory performance is not known. We identified a specific subset of large SWRs linked to memory reactivation in both the hippocampus and prefrontal cortex (PFC) of mice, and found that their occurrence selectively increased during sleep following new learning. Closed-loop optogenetic SWR boosting during sleep was sufficient to enhance ensemble memory reactivation in the hippocampus and PFC. This manipulation also improved subsequent memory retrieval and hippocampal-PFC coordination during waking, causally linking both phenomena to SWR-associated ensemble reactivation during sleep.

INTRODUCTION

New memories are consolidated during sleep following learning, a process that leads to the formation of stable, long-term representations.^{1,2} The hippocampus plays a key role in the initial memory encoding³ and then gradually transfers these memories to the neocortex for permanent storage.^{4–6} This process has been hypothesized to be mediated by synchronous network patterns generated in the hippocampus, known as sharp-wave ripples (SWRs).^{7–10} SWRs occur during non-rapid eye movement (NREM) sleep and waking immobility periods. They coordinate the reactivation of hippocampal neurons that encoded recent experiences and broadcast these memory representations to the neocortex and other brain areas.^{10–21} In downstream structures such as the prefrontal cortex (PFC), SWRs entrain local circuits, driving the coordinated reactivation of experience-related activity patterns.^{22–27}

Despite this wealth of evidence, an apparent paradox is that only a small subset of sleep SWRs (~10%–30%) are associated with significant memory reactivation or “replay.”^{12,15,21,27–30} This fraction could be even smaller for memory reactivation in downstream structures, such as the PFC. These observations raise the question of whether the subset of SWRs that successfully elicit memory reactivation in the neocortex, and thus presumably

contribute to memory consolidation, have unique physiological characteristics or whether their impact is determined by some additional variables that converge with SWRs. In this study, we aimed to distinguish between these two hypotheses and establish a direct link between SWR features and memory performance. Furthermore, studies in humans have shown that increased functional coordination between the hippocampus and cortex during rest following new learning correlates with successful memory recall.^{31,32} However, whether sleep SWRs mediate a persistent enhancement of hippocampo-cortical coordination and subsequent memory recall is not known.

To answer these questions, we performed neural ensemble recordings in the hippocampus and PFC of mice during a memory task and sleep, together with closed-loop optogenetic manipulation of SWRs.

RESULTS

Large SWRs are associated with stronger memory reactivation in the CA1 and PFC and modulated by learning

We implanted mice with high-density silicon probes in the dorsal hippocampus (CA1) and PFC (pre-limbic) (Figures 1A and S1). We recorded ensembles of cells in both regions ($n = 1,827$

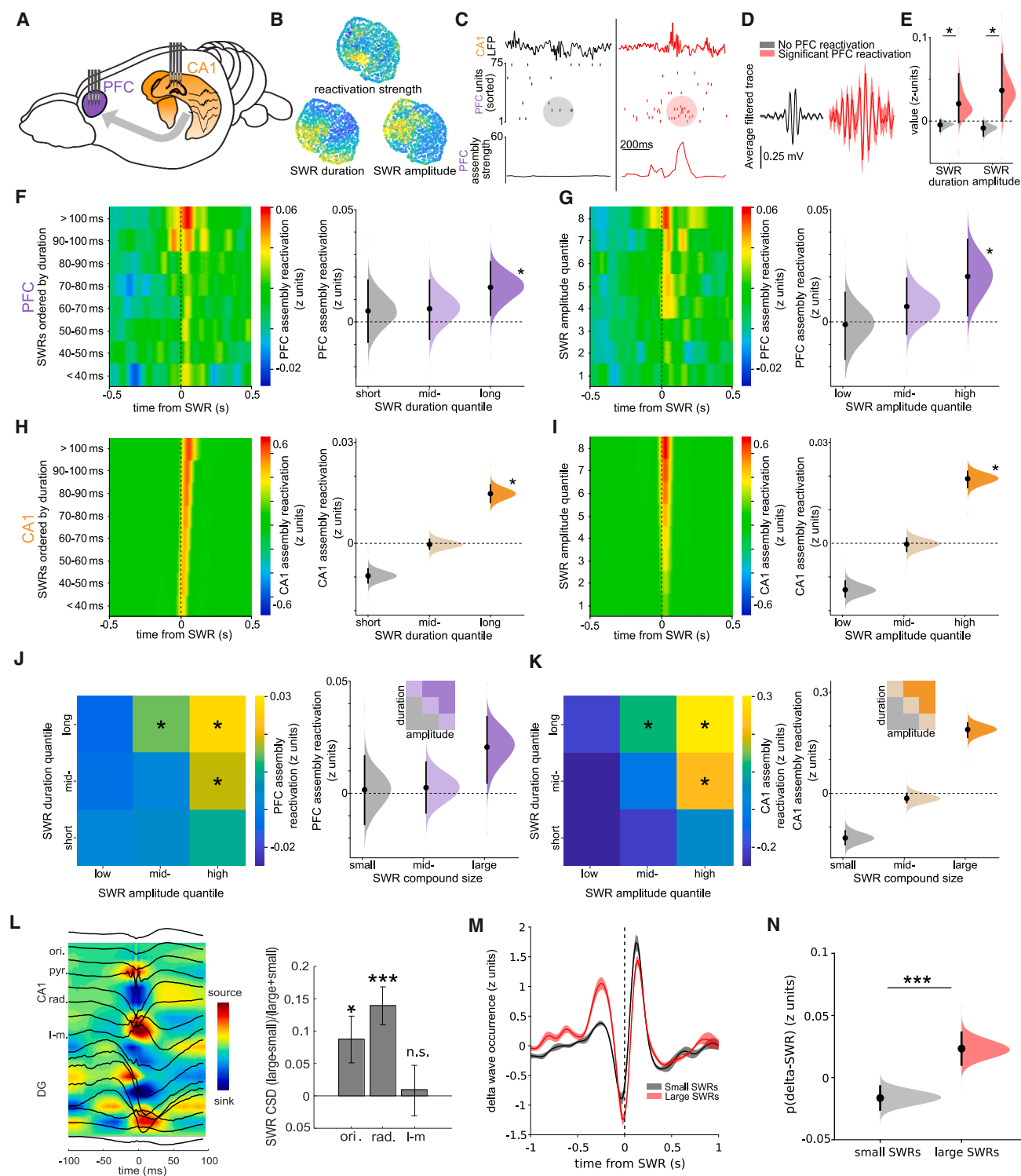


Figure 1. Enhanced memory reactivation in the hippocampus and cortex during a subset of SWRs

(A) Schematic of recording design.
(B) Visualization of SWR features using 3D UMAP embedding. Each point is a single SWR from an example session, color coded according to different features.
(C) Examples of SWR without (left) and with (right) associated PFC cell assembly reactivation. Top: CA1 local field potential (LFP). Middle: raster plots of neuronal firing, a colored circle indicates assembly activity during SWR. Bottom: assembly reactivation strength.
(D) Average filtered CA1 LFP for SWRs associated with or without significant reactivation of PFC cell assemblies.

(legend continued on next page)

CA1 and 1,103 PFC neurons from 21 mice) while mice performed a hippocampus-dependent memory task (object displacement, see STAR Methods) and during sleep before and after the task. To analyze memory reactivation, we employed an unsupervised method to detect the synchronous coactivity of groups of neurons, or cell assemblies, during learning, as in previous work.^{19,20,33,34} We then compared whether these task-related cell assemblies in the PFC and CA1 reactivated during SWRs in subsequent sleep. We found that 35.4% of SWRs were associated with significant memory reactivation in the hippocampus and 19.7% in the PFC ($p < 0.05$, $n = 353,600$ SWRs from 21 mice). The probability of PFC reactivation was higher for SWRs associated with significant CA1 reactivation (Figure S2A).

We asked whether SWRs that elicited ensemble reactivation in downstream areas, such as the PFC, and could therefore promote memory consolidation, have specific defining features. We first represented all SWRs as points in a high-dimensional space constructed using different features and embedded them in a low-dimensional space using uniform manifold approximation and projection (UMAP) for visualization. This method revealed that SWR properties such as amplitude, duration, or frequency varied along a continuum (Figures 1B and S2). Color-coding SWRs based on the reactivation strength of concurrent PFC ensemble activity suggested a correlation between SWR amplitude and duration with PFC reactivation strength (Figure 1B). Classifying all sleep SWRs in two binary categories, those with and without significant associated memory reactivation in the PFC, confirmed that the former had significantly larger duration and amplitude (Figures 1C–1E) but did not significantly differ in other features (Figure S2).

To examine in more detail the relationship between memory reactivation strength and SWR duration and amplitude, we classified SWRs based on these features and calculated peri-SWR histograms of PFC assembly reactivation strength for each of the categories. Only the topmost duration and amplitude SWR quantiles were associated with strong PFC memory reactivation (Figures 1F and 1G). We obtained similar results when we performed the same analysis with CA1 assembly reactivation strength (Figures 1H and 1I). However, in the case of the CA1, reactivation strength increased more gradually with increasing SWR duration and amplitude in contrast to a more abrupt increase in the PFC.

SWR duration and amplitude were only moderately correlated (Figure S2), and our initial observations suggested that only a subset of SWRs with both large amplitude and duration were associated with strong memory reactivation (Figure 1B). Therefore, we classified SWR into groups of increasing joint amplitude and duration. Memory reactivation strength in both the PFC (Figure 1J) and CA1 (Figure 1K) was largest for SWRs in the top duration and amplitude quantile but weak for SWRs with large amplitude but short duration—or long duration but low amplitude.

We further examined whether small and large SWRs differ in their relationship with upstream or downstream regions. First, we analyzed the relative strength of synaptic inputs during small and large SWRs by performing current source density (CSD) analysis on hippocampal laminar recordings. Both groups of SWRs had qualitatively similar CSD profiles, suggesting that they were generated by the same synaptic inputs. However, large SWRs were associated with stronger stratum oriens and, especially, stratum radiatum current sinks compared with small SWRs (Figure 1L). In contrast, there were no significant differences in the magnitude of stratum lacunosum-moleculare sinks (Figure 1L). This result suggests that an enhanced CA3 input (that primarily targets stratum radiatum) may be the main driver of large SWR generation.

Next, we analyzed the relationship between hippocampal SWR and cortical sleep oscillations. We focused on delta waves and spindles, whose coupling with SWRs supports memory consolidation.^{36,37} Small and large SWRs alike tend to be followed by PFC delta waves, but large SWRs were significantly more likely than small SWRs to follow delta waves (Figures 1M, 1N, and S3); that is, to occur at the onset of the cortical UP state. In addition, large SWRs were also more likely to be coupled with PFC spindles than were small SWRs (Figure S5).

Finding that memory reactivation was associated with a specific subset of larger SWRs prompted us to ask whether the occurrence of these SWRs was correlated with successful memory consolidation. We tested mice in two versions of the object displacement task (Figure 2A).³⁸ In the first one, mice were allowed to explore two identical objects for three 5-min trials. For the memory recall test, conducted after a 4-h sleep session, one object was displaced to a different location. Mice displayed

(E) SWRs associated with significant PFC reactivation were longer ($p = 0.043$, $n = 69,522$ SWRs, hierarchical bootstrap test) and had higher amplitude ($p = 0.025$, $n = 284,078$) than the remaining SWRs. Black dots: mean; black lines: 95% confidence intervals; curves: the hierarchically resampled mean distributions.

(F) Left: reactivation strength of PFC assemblies around SWRs sorted by duration. Right: hierarchically bootstrapped estimates of the PFC assembly reactivation during SWRs classified by duration quantiles ($p = 0.25/0.20/0.0104$ for short, mid-length, and long ripples, respectively, hierarchical bootstrap test).

(G) Same as (F) but with SWRs sorted by amplitude ($p = 0.55 / 0.14 / 0.013$ for low-, mid-, and high-amplitude SWRs, respectively).

(H) Same as (F) but for CA1 assemblies ($p = 1/0.65/0$ for short, mid-length, and long SWRs, respectively).

(I) Same as (H) but with SWRs sorted by SWR amplitude ($p = 1/0.57/0$ for low-, mid-, and high-amplitude SWRs).

(J) Left: PFC assembly responses to SWR quantile grid split according to both duration and amplitude ($*p < 0.05$, Wilcoxon signed-rank tests). Right: PFC reactivation strength ($p = 0.42/0.34/0.0076$ for short, mid-length, and long SWRs, hierarchical bootstrap test).

(K) Same as (J) but for CA1 assemblies ($p = 1/0.96/0$).

(L) Left: averaged CSD profile of SWRs during a representative session (ori, stratum oriens; rad., str. radiatum; l-m., str. lacunosum-moleculare). Right: quantification of relative current sink magnitude per layer for large versus small SWRs ($***p < 0.05/0.001$, signed-rank test; $n = 15$ sessions from 5 mice).

(M) Mean peri-event time histogram of delta wave occurrence around small (black) and large SWRs (red). Mean $\pm$ SEM.

(N) Probability of a delta wave occurring before SWR (-400 to -100 ms) was higher for large SWRs than small SWRs ($p = 0$, hierarchical bootstrap test; $n = 18$ sessions in 10 animals).

See also Figures S1–S3.

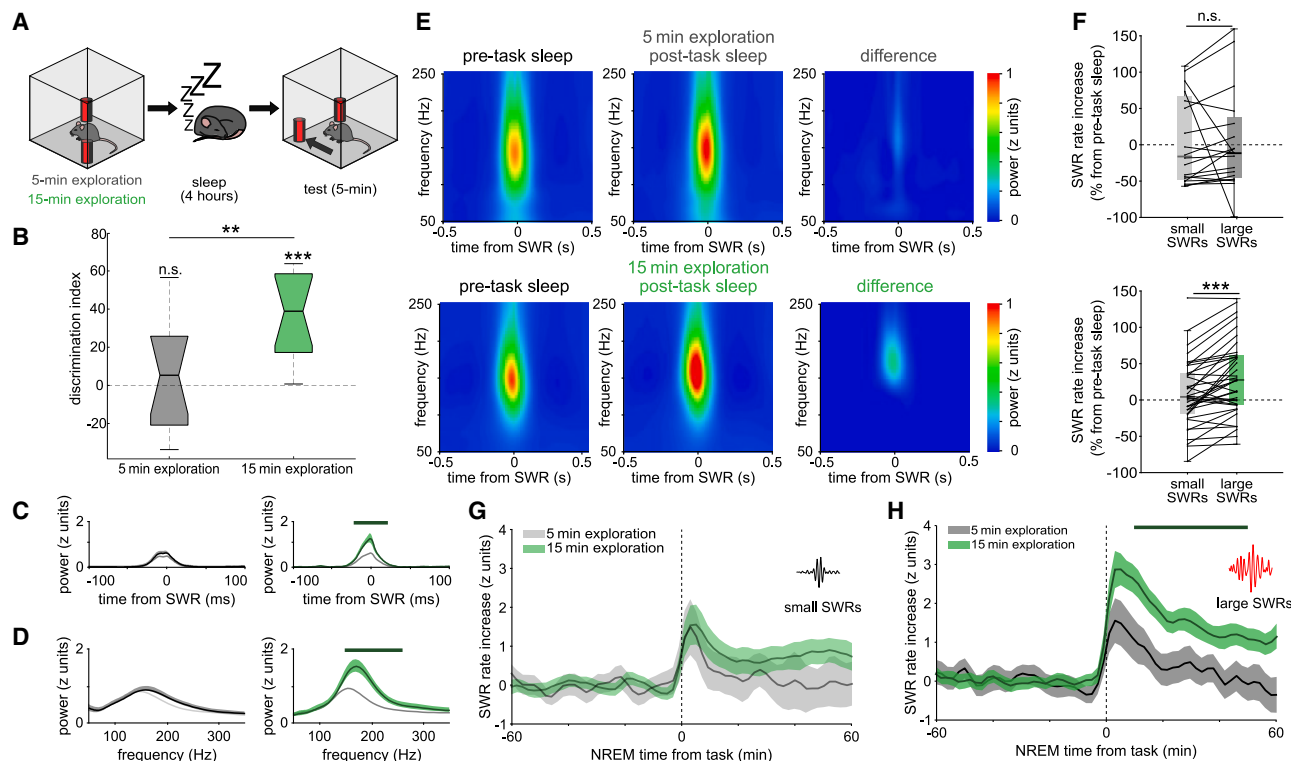


Figure 2. Increased fraction of large SWRs correlates with memory performance

(A) Animals were trained on the object displacement memory task, with either a single or three 5-min exploration sessions.

(B) Memory performance (object discrimination index) on the test session was significant for the 15-min ($n = 13$ mice, $p = 2.4 \times 10^{-4}$, Wilcoxon signed-rank test) but not the 5-min group ($n = 13$ mice, $p = 0.68$, $p = 0.011$ for 5 versus 15-min group, Wilcoxon rank-sum test).

(C) Spectral power within the ripple band (150–250 Hz) increased for SWRs following 15-min, but not 5-min, exploration ($n = 38$ and 20 sessions, respectively). A line indicates the significant interval ($p < 0.05$, Monte Carlo test).

(D) Spectral profile of SWRs was unaffected after 5-min exploration (left), while power in the ripple frequency band was enhanced following 15-min exploration ($p < 0.05$, Monte Carlo test, $n = 20/38$ sessions).

(E) Average sleep SWR wavelet spectrograms for pre-task sleep (left), post-task sleep (middle), and their difference (right), following either 5-min (top, $n = 20$) or 15-min (bottom, $n = 38$) exploration sessions.

(F) Fraction of large SWRs increased following 15-min (bottom), but not 5-min (top), object exploration sessions (5-min: $p = 0.68$, $n = 20$ and 15-min: $p = 8.9 \times 10^{-6}$, $n = 38$, Wilcoxon signed-rank test).

(G) Incidence of small SWRs in NREM (1 h of contiguous NREM sleep before and after the task) was similar between 5- and 15-min training conditions ($p > 0.05$, hierarchical bootstrap test).

(H) Incidence of large SWRs in post-task NREM sleep increased following 15-min exploration ($p < 0.05$). (C)–(G) shows the mean $\pm$ SEM. See also Figures S4–S7.

an increased exploration of the displaced object, as quantified by a discrimination index (Figures 2B and S4), establishing that they correctly remembered its original location. However, when mice were only allowed to explore the object for one 5-min trial, they did not show an exploratory bias for the displaced object during the following test (Figures 2B and S4). Mice did show an increased exploration of the displaced object after 5-min exploration when the test was conducted only 1 h later (Figure S4C). This suggests that the reduced experience during 5-min sessions was enough to support short-term memory recall but not enough for longer-term memory consolidation. Sleep structure and the amount of NREM were similar in the rest periods after both versions of the task (Figure S5).

We next compared SWR features in the sleep sessions before and after the initial object exploration. SWRs before and after the 5-min exploration sessions were similar in amplitude and dura-

tion (Figures 2C–2E). In contrast, SWRs following 15-min exploration sessions were larger on average than in the preceding sleep (Figures 2C–2E and S6). We thus divided all SWRs into “large” and “small” using a compound measure combining duration and amplitude (see STAR Methods) and calculated their relative fraction in each sleep period. Overall SWR rate (0.43 and 0.47 Hz in pre and post sleep, respectively, $p = 0.079$, Wilcoxon signed-rank test) and the relative fraction of small and large SWRs did not change following 5-min object exploration sessions (Figure 2F). Following 15-min exploration sessions, the overall SWR rate increased (0.46 and 0.52 Hz, $p = 6.2 \times 10^{-4}$), specifically due to a larger fraction of large, but not small, SWRs (Figure 2F). Furthermore, there was a significant positive correlation between the post-sleep fraction of large SWRs and task recall performance after 15-min, but not 5-min, exploration sessions (Figure S7).

We then examined the temporal dynamics of SWR occurrence. SWR rate and fraction of large SWRs after 15-min exploration increased sharply at the start of the post-learning NREM sleep and then gradually decayed but remained high for ~ 1 h of NREM (Figures 2G and 2H). Following the 5-min exploration, only a transient increase in SWR rate was observed right at the start of NREM sleep, and no specific increase in large SWR occurrence (Figures 2G and 2H). Moreover, SWR rate already increased gradually during immobility task periods during 15-min training sessions compared with 5-min training sessions (Figures S4D and S4E).

Closed-loop optogenetic SWR boosting enhanced memory reactivation and recall performance

We next wanted to establish whether there is a causal link between large SWRs and memory performance by specifically manipulating them during the post-task sleep period. For this purpose, we expressed channelrhodopsin (ChR2) in CA1 pyramidal cells using viral and transgenic approaches. Mice were implanted with silicon probes (or wire arrays) in the hippocampus and the PFC and bilateral optic fibers above the CA1 (Figure 3A). We detected SWRs in real time during post-task sleep sessions and optogenetically boosted them using a closed-loop approach (Figures 3B and S8). A 100-ms low-intensity blue-light pulse delivered upon SWR detection, prolonged the ongoing oscillation and associated spiking activity (Figure 3C). This manipulation also resulted in a boost of SWR power (Figure 3D). To test whether this manipulation could enhance memory performance, we applied it during the sleep period following 5-min object exploration sessions. SWR boosting resulted in an increase in the total fraction of large SWRs during post-task sleep similar to that naturally occurring following 15-min exploration sessions (Figure 3E). Remarkably, although after 5-min exploration sessions without optogenetic stimulation mice did not recall the displaced object, memory recall was successful in sessions with SWR boosting during sleep (Figure 3F). This result indicates that optogenetic boosting of sleep SWR was able to enhance memory performance.

To verify whether the behavioral impact of SWR boosting depends on the temporal specificity of the manipulation, we performed a control experiment. We delivered the same optogenetic stimulation but after a random delay upon SWR detection in post-task sleep following 5-min training sessions. This manipulation elicited a similar physiological response (evoked ripple-like oscillations and associated spiking in the CA1) to the SWR timed stimulation (Figure S8). In contrast, delayed stimulation did not have any significant effect on memory performance (Figure S8), suggesting that the enhancement previously observed relies on precisely timed stimulation of the CA1.

We hypothesized that the observed differences in memory performance following 5- or 15-min exploration, and the behavioral effects of optogenetic SWR boosting, could be explained by differences in post-task neural reactivation in the CA1 and PFC. To test this, we assessed sleep memory reactivation using several approaches. First, we measured the correlation of neural firing patterns in pairs of cells between the task and post-sleep SWRs. Pairwise reactivation was significant (relative to baseline sleep) in the CA1, but not in the PFC, following 5-min exploration (Figures 3G and 3H). In contrast, after both 15- and 5-min explo-

ration followed by SWR boosting, we observed significant memory reactivation in both CA1 and PFC (Figures 3G and 3H).

Next, we examined the reactivation of CA1 and PFC cell assemblies in sleep SWRs. Cell assemblies in the CA1 showed a weak reactivation following 5-min exploration, and PFC assemblies displayed no significant reactivation at all (Figures 3I–3L). In contrast, we observed robust significant assembly reactivation in both structures after 15-min exploration sessions as well as 5-min exploration with SWR boosting (Figures 3I–3L). Repeating these analyses after subsampling to match within-SWR firing rates in pre- and post-task sleep for all conditions resulted in similar enhanced assembly reactivation after 15-min training and with SWR boosting (Figure S9). Furthermore, we observed a session-wise positive correlation between the rate of optogenetically boosted SWRs and memory performance (Figure S7). Optogenetic stimulation did not alter overall sleep structure or the relative fraction of NREM sleep epochs (Figure S5). However, SWR boosting did enhance the coupling of hippocampal SWR and PFC delta waves, in a similar manner to the effects of extended training (Figure S3). Overall, these results suggest that the improvement in memory performance due to optogenetic SWR boosting was due to an enhancement of task-related neural reactivation in the CA1 and PFC.

Enhanced CA1–PFC coordination during memory consolidation and recall

Recent work in humans has shown that functional connectivity between the hippocampus and cortex increases after learning and that this increase correlates with subsequent memory recall performance.^{31,32} We thus hypothesized that the coordination between CA1 and PFC during sleep would increase following 15-min, but not 5-min, exploration sessions and that a similar effect would be induced by optogenetic SWR boosting. We performed two different analyses to test this hypothesis.

First, we examined the coordinated reactivation of CA1 and PFC cell assemblies. PFC cell assemblies increased their reactivation rate in SWRs associated with significant CA1 reactivation in sleep following 15-min exploration, and after 5-min exploration followed by sleep SWR boosting, but not after 5-min exploration without stimulation (Figure S10A). Moreover, we found evidence for learning-dependent cross-regional reactivation as PFC assemblies increased their coordinated reactivation with specific CA1 assemblies throughout sleep sessions following 15-min training or 5-min exploration followed by SWR boosting (Figure S10).

To test for CA1–PFC coordination directly, we investigated the coordinated reactivation of pairs of CA1 and PFC cells in SWR before and after the task. CA1–PFC pairwise reactivation increased following 15-min, but not 5-min, exploration sessions (Figure 4A). SWR boosting after 5-min exploration also resulted in enhanced pairwise cross-regional reactivation (Figure 4A). Next, we identified cross-regional assemblies, synchronous groups of coactive cells with members from both CA1 and PFC (Figure 4B),^{25,39} and assessed their reactivation in sleep SWR before and after the task. CA1–PFC cell assemblies showed significant coordinated reactivation after 15-min exploration or 5-min exploration followed by sleep SWR boosting, but not after 5-min exploration without stimulation (Figure 4C).

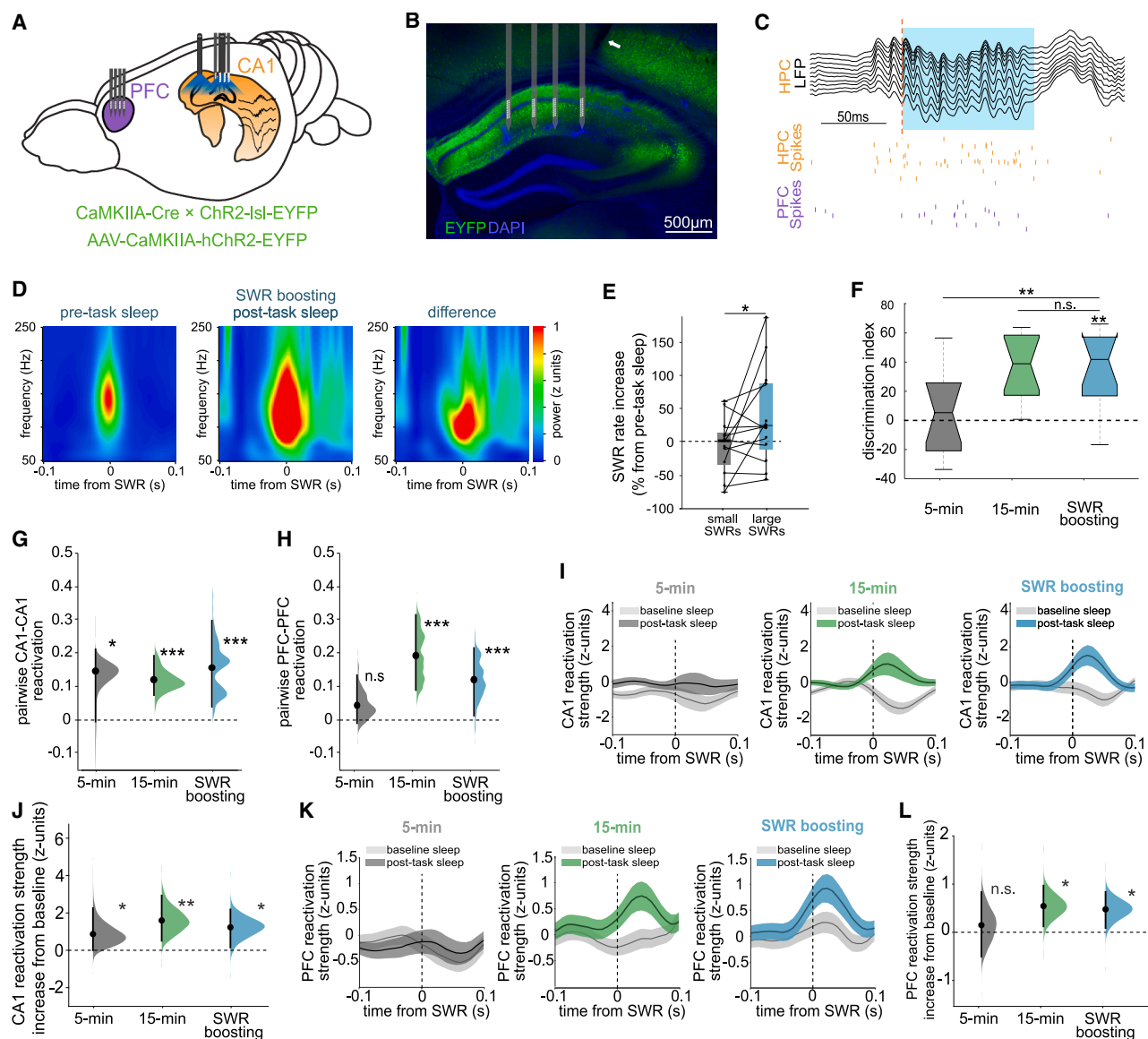


Figure 3. Optogenetic SWR boosting enhances memory reactivation and performance

(A) Schematic of CA1 and PFC recordings combined with optic fibers targeting the hippocampus bilaterally.
(B) Histological section showing the expression of channelrhodopsin-2 enhanced yellow fluorescence protein (ChR2-EYFP) in pyramidal cells; white arrow: optic fiber track.
(C) Example of optogenetic SWR boosting. Black trace is CA1 local field potential (LFP), vertical dashed bar shows SWR real-time detection, and blue rectangle optogenetic stimulation. Raster plot below shows units firing.
(D) Average sleep SWR wavelet spectrograms for pre- and post-task sleep with SWR boosting ($n = 13$ mice, $p < 0.05$, Monte Carlo test).
(E) SWR boosting resulted in an increased incidence of large SWRs in post-task sleep ($p = 0.028$, $n = 13$, Wilcoxon signed-rank test).
(F) Memory recall performance (object discrimination index) for no stimulation 5- and 15-min training sessions (same data as Figure 2B) and SWR boosting sessions ($n = 13$ mice, $p = 1.7e-3$, Wilcoxon signed-rank test; SWR boost versus 5-min $p = 8.9e-3$, Wilcoxon rank-sum test; SWR boost versus 15-min $p = 0.96$).
(G) CA1 cell pair co-firing in SWRs: 5-min exploration ($p = 0.039$, $n = 18,708$ cell pairs, hierarchical bootstrap test), 15-min exploration ($p = 0$, $n = 16,215$), and 5-min exploration followed by SWR boosting ($p = 0.001$, $n = 10,963$); black dots: mean estimates; black lines: 95% confidence intervals; curves: the resampled mean distributions.
(H) Same as (G) but for PFC: 5-min exploration ($p = 0.14$, $n = 12,739$ pairs), 15-min exploration ($p = 0$, $n = 10,729$), and SWR boosting ($p = 0.020$, $n = 11,157$).
(I) Averaged peri-SWR CA1 assembly reactivation strength following 5-min exploration, 15-min exploration, and 5-min exploration followed by SWR boosting; light gray: baseline pre-task assembly activity. Mean $\pm$ SEM.
(J) Quantification of (I) measured by hierarchical bootstrap estimate of assembly reactivation in post-task sleep relative to baseline sleep: 5-min exploration ($p = 0.032$, $n = 97$ assemblies), 15-min exploration ($p = 1.8e-3$, $n = 189$), and SWR boosting ($p = 0.016$, $n = 131$ assemblies).
(K and L) Same as (I) and (J) but for PFC assemblies: 5-min ($p = 0.35$, $n = 79$ assemblies), 15-min ($p = 0.0104$, $n = 96$), and SWR boosting ($p = 0.011$, $n = 115$).
See also Figures S3, S5, S7, and S9.

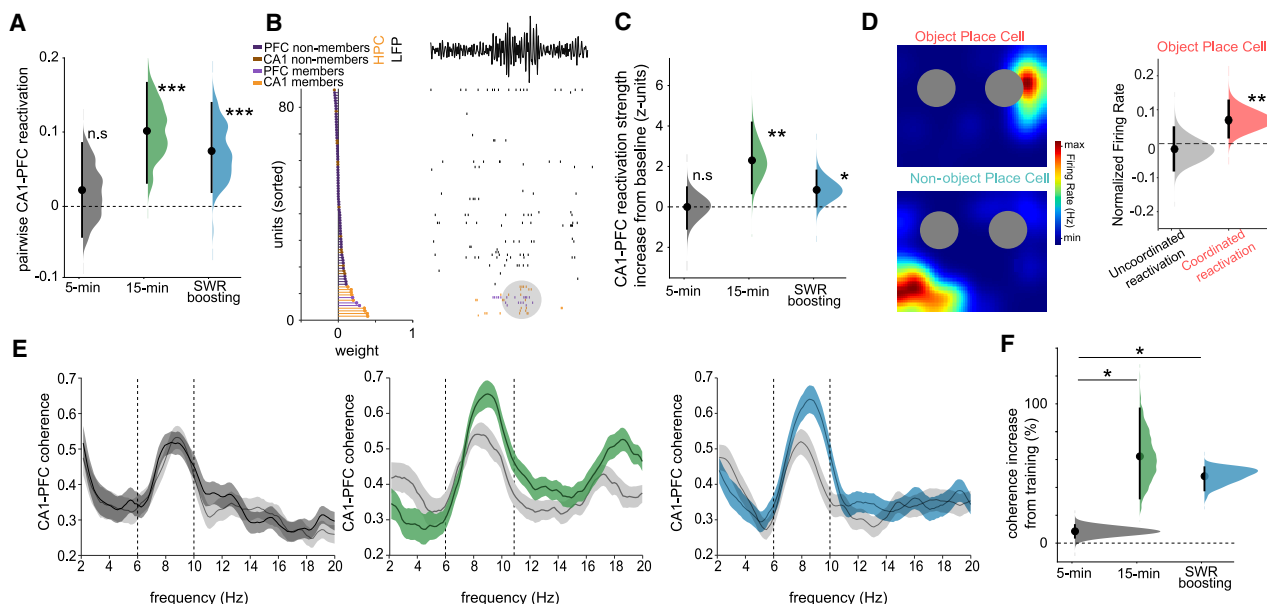


Figure 4. Coordination of CA1 and PFC activity during sleep and memory recall

(A) Hierarchically bootstrapped estimates of the CA1-PFC cell pair co-firing 5-min exploration ($p = 0.32$, $n = 10,342$ cell pairs; hierarchical bootstrap test), 15-min exploration ($p = 0$, $n = 4,573$), and 5-min exploration followed by SWR boosting ($p = 0.012$, $n = 16,334$ cell pairs).

(B) Example CA1-PFC assembly. Left: CA1 and PFC units sorted by their contribution to selected assembly (assembly members in bright color at the bottom). Right: examples of raster plots of unit firing showing assembly reactivation (shaded ovals) during a SWR (CA1 filtered local field potential [LFP] on top).

(C) Quantification of peri-SWR CA1-PFC assembly reactivation strength measured by a hierarchical bootstrap estimate of assembly reactivation in post-task sleep relative to baseline sleep: 5-min ($p = 0.032$, $n = 29$ assemblies), 15-min ($p = 1.8 \times 10^{-3}$, $n = 28$ assemblies), and 5-min with SWR boosting ($p = 0.016$, $n = 26$ assemblies).

(D) Left: rate map examples of an object place cell and a non-object place cell. objects to scale as gray circles. Right: object place cells fired more during CA1-PFC coordinated reactivation events than during uncoordinated reactivation ones in post-task sleep ($p = 0.0052$, Wilcoxon rank-sum test).

(E) CA1-PFC LFP coherence in training and test. Left: CA1-PFC coherence was similar during 5-min exploration (light gray) and the subsequent test session (black). 15-min exploration (middle) and SWR boosting following 5-min exploration (right) resulted in enhanced CA1-PFC coherence in the subsequent test session (green or blue) compared with training (light gray). Mean $\pm$ SEM. Dashed vertical lines indicate theta frequency band.

(F) Quantification of (D) measured by a hierarchical bootstrap estimate of the increase in hippocampo-prefrontal coherence during test, normalized by coherence during training (5-min versus 15-min training, $p = 0$, $n = 9/13$ sessions, respectively; 5-min versus 5-min training with SWR boosting, $p = 0$, $n = 9/5$ sessions; 15-min versus 5-min training with SWR boosting $p = 0.24$, $n = 13/5$ sessions).

See also [Figures S6](#) and [S10](#).

We also examined the representational content of coordinated cross-regional reactivation events (SWRs with significant reactivation of both CA1 and PFC assemblies). We classified all hippocampal spatially selective cells into those that encoded object locations (“object place cells”) or other locations in the maze (“non-object place cells”) (Figure 4D). Object, but not non-object, place cells selectively increased their firing rate in SWRs associated with significant coordinated CA1-PFC reactivation (Figure 4D).

Finally, we asked whether the enhanced cross-regional coordination during sleep persisted during waking memory recall. To answer this, we measured CA1-PFC theta frequency local field potential (LFP) coherence, as it has been previously shown to be associated with cross-regional communication and successful memory performance.^{40,41} We found that CA1-PFC theta coherence during the test phase was largely increased compared with the initial object exploration in both 15-min sessions and 5-min sessions followed by SWR boosting, in contrast to 5-min exploration sessions (Figures 4E and 4F). This observa-

tion suggests that extended training and artificial SWR boosting result in enhanced CA1-PFC coordination that supports successful memory recall.

DISCUSSION

Neural ensemble reactivation in the hippocampus and associated cortical areas, such as the PFC, is regarded as a cellular hallmark of memory consolidation. This process is primarily associated with a minority of SWRs (~10%–30%) during NREM sleep.^{11–13,15,42} SWRs are highly heterogeneous network events, as characterized in previous studies.^{17,43–45} However, the significance of SWR heterogeneity in memory consolidation has not been established. We found that the subset of SWRs that were associated with memory reactivation in the CA1 and PFC were those with both the largest duration and amplitude. The fraction of these large SWRs selectively increased during sleep following new learning, but only when the learning experience was sufficient for successful memory retrieval 4 h later. Previous

work reported global increases in SWR rate during sleep following learning^{19,36,38} and an association of SWR duration and amplitude with increased memory load during learning.^{17,37,46} Our results expand these observations and demonstrate that large SWRs promote ensemble reactivation in the hippocampus and downstream areas in support of memory consolidation.

Brief optogenetic activation of CA1 pyramidal cells triggered by real-time detection of sleep SWRs boosted them, mimicking the effect of extended learning on SWR features. Optogenetically boosted SWRs resulted in enhanced memory reactivation in both the CA1 and PFC, demonstrating a direct link between SWR features and the associated dynamics of neuronal population spiking activity. Remarkably, this manipulation, but not the same stimulation delivered after a random delay upon SWR detection, improved the mice's memory performance, enabling them to successfully recall a brief prior experience, normally insufficient for successful retrieval. This effect was similar to that of the extended learning experience, suggesting that sleep memory reactivation can compensate for reduced learning time or promote the consolidation of weakly encoded experiences, in line with theoretical proposals on the role of "offline" memory replay for training artificial and biological neural networks.^{47–49} During extended experience, the rate of SWRs gradually increased. We hypothesized, in agreement with previous findings,^{50,51} that awake reactivation can "tag" specific neural ensembles and prioritize their subsequent reactivation during sleep, leading to enhanced consolidation. This effect could be preferentially driven by large SWRs, as we found that they promote enhanced memory reactivation within and beyond the hippocampus. In addition, the specific sub-state within sleep when SWRs occur can also influence their role in memory processes, as suggested by a previous study.²¹

In a previous study,¹⁷ we used the same closed-loop optogenetic approach to boost SWRs during the performance of a spatial short-term memory task, resulting in an improvement in learning performance. The present study extends those results, indicating that large SWRs during sleep have an instrumental role in memory consolidation and, crucially, demonstrating that properly timed optogenetic stimulation can enhance neural ensemble reactivation in the hippocampus and in downstream structures. Multiple brain-wide phenomena, such as cortical oscillations and brain-state fluctuations, correlate with SWRs and are associated with memory consolidation.^{21,52–57} Our results suggest that the contribution of some of these processes to memory could be mediated by their modulation of SWR features that, in turn, have a direct effect in promoting neural ensemble reactivation.

A leading mechanism for the systems consolidation of new memories is the coordination of hippocampal and cortical activity promoted by SWRs.^{8,9,53,56,58,59} We found that large SWRs are more likely to follow PFC delta waves, suggesting that the onset of cortical UP states may be a privileged window for cortico-hippocampal interactions and influence memory consolidation. CA1–PFC spiking coactivity patterns increased their coordinated reactivation following an extended learning experience and correlated with successful memory recall. Furthermore, coordinated CA1–PFC reactivation events were more likely to encode object locations than other maze areas, suggesting

that they play an important role in the consolidation of behaviorally relevant memories.

We also found enhanced CA1–PFC theta coherence following extended learning experience. Importantly, optogenetically boosting SWRs in sleep increased CA1–PFC cell assembly sleep reactivation and enhanced theta coherence during subsequent awake recall. This result provides a missing link between the functional coordination of the hippocampus and cortex during sleep SWRs and persistent changes in cross-regional synchronization during waking. This finding offers a potential mechanism to explain observations in humans that found enhanced hippocampo-cortical functional connectivity following learning and its correlation with memory performance.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to, and will be fulfilled by, the lead contact, Dr. Antonio Fernandez-Ruiz (af77@cornell.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The original code that supports the findings of this study can be downloaded from GitHub (<https://github.com/ayalab1/neurocode>).
- Raw data and any additional information required to reanalyze the data reported in this paper are available from the [lead contact](#) upon request.

ACKNOWLEDGMENTS

The authors thank members of the Oliva and Fernandez-Ruiz labs for providing useful feedback on the manuscript. This work was supported by NIH grants R00MH122582 and R01MH140009 (A.O.); NIH grants R01MH136355 and DP2MH136496, Sloan Fellowship, Whitehall Research Grant, Klingenstein-Simons Fellowship, Pershing Square Foundation's MIND Prize, and Pew Biomedical Scholars Award (A.F.-R.); NIH grant F32MH134673 (H.L.R.); and Eric and Wendy and Schmidt AI in Science Postdoctoral Fellowship (R.T.).

AUTHOR CONTRIBUTIONS

H.L.R., R.T., G.A.N., A.O., and A.F.-R. conceived and designed experiments and analyses and wrote the manuscript. H.L.R., G.A.N., A.G., P.P., and R.T. performed experiments. R.T. and H.L.R. conducted the analysis.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.neuron.2025.10.003>.

Received: July 4, 2025

Revised: September 15, 2025

Accepted: October 7, 2025

Published: November 7, 2025

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
AAV-CamKII α -ChR2/H13R-EYFP	Addgene	#26969-AAV5; RRID:Addgene_26969
Chemicals, peptides, and recombinant proteins		
DAPI	Thermo Fisher Scientific	Cat#D1306
DABCO	Millipore Sigma	Cat#10981
Paraformaldehyde	Thermo Fisher Scientific	Cat#A11313.36
PBS	Thermo Fisher Scientific	Cat#00302
Dura-Gel	Cambridge Neurotech	N/A
Dental Cement	UNIFAST	N/A
Metabond	C&B Metabond Quick	N/A
Experimental models: Organisms/strains		
Mouse: C57BL/6N	The Jackson Laboratory	Cat#000664; RRID:MGI:2159965
Mouse: FVB/NJ	The Jackson Laboratory	Cat#001800; RRID:IMSR_JAX:001800
Mouse: C57BL/6N x FVB/NJ F1 "Hybrids"	Sloin et al. ⁶⁰	N/A
Mouse: T29-1 Camk2a-Cre	The Jackson Laboratory	Cat#005359; RRID:IMSR_JAX:005359
Mouse: Ai32(RCL-ChR2(H134R)/EYFP	The Jackson Laboratory	Cat#024109; RRID:IMSR_JAX:024109
Software and algorithms		
MATLABR2023a	MathWorks	https://www.mathworks.com/products/matlab.html ; RRID:SCR_001622
Python 3.8.20	Python Software Foundation	https://www.python.org/ ; RRID:SCR_008394
DeepLabCut	Mathis et al. ⁶¹	https://github.com/DeepLabCut/DeepLabCut ; RRID:SCR_021391
Kilosort	Pachitariu et al. ⁶²	https://github.com/cortex-lab/KiloSort ; RRID:SCR_016422
Phy2	Cyrille Rossant, International Brain Laboratory, Cortex Lab UCL, Kenneth Harris, Matteo Carandini	https://github.com/kwikteam/phy-data
CellExplorer MATLAB Package	Petersen et al. ⁶³	https://cellexplorer.org/ ; RRID:SCR_022358
Neurocode (custom Matlab codes)	Liu et al., ²⁰ Cheng et al., ²¹ Harvey et al., ²⁵ and Karaba et al. ³⁸	https://github.com/ayalab1/neurocode
Spike2	Cambridge Electronic Design	https://ced.co.uk/products/spike2 ; RRID:SCR_000903
Other		
Silicon Probe: 128-Channel	Diagnostic Biochips	N/A
Silicon Probe: 64-Channel	Neuronexus	N/A
Micro-drive	Cambridge Neurotech	N/A
Optical Fiber 200 μ m	ThorLabs	Cat#FP200ERT
CED1401-Power1401-3A	Cambridge Electronic Design	https://ced.co.uk/products/pow3in ; RRID:SCR_016040
Intan RHD USB Interface Board	Intan Technologies	https://intantech.com/RHD_USB_interface_board.html ; RRID:SCR_019278
Intan RHD Electrophysiology Amplifier Chips	Intan Technologies	https://intantech.com/products_RHD2000.html
Basler Ace Camera	Basler	Cat#acA3100-200uc
Zeiss LSM 800	Cornell University Biotechnology Resource Center	RRID:SCR_015963

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

All experiments complied with guidelines established by the National Institutes of Health and approved by the Cornell University Institutional Animal Care and Use Committee (IACUC). All mice were housed in vivariums on a 12-hour light/dark cycle with a maximum of 5 animals per cage with *ad libitum* access to water and food. After surgery, mice were individually housed. Adult C57BL/6J (The Jackson Laboratory #000664, ~25–35g, ~3–6 months) or adult ‘hybrid’ offspring (~35–45g, ~3–6 months) were used for experiments not involving optogenetic manipulation. Hybrid mice were the first-generation offspring from a cross between C57BL/6J and FVB/NJ (The Jackson Laboratory, #001800).⁶⁰ For optogenetic manipulation, transgenic animals or viral approaches (see Viral Injections) were used to express Channelrhodopsin (ChR2/H134R) in pyramidal neurons. For the transgenic approach, offspring of Ai32 (The Jackson Laboratory, #024109) and CamKII α homozygous animals (The Jackson Laboratory, #005359) were used to express ChR2/H134R in pyramidal neurons.

METHOD DETAILS

Surgical Procedures

For silicon probes and viral injections, mice were anesthetized with isoflurane, and coordinates were taken following stereotaxic techniques following previously reported methods.^{19,21,38} Briefly, fur was removed using Nair and skin was washed three times with alcohol and iodine. A skin incision was then made and craniotomies were performed. For hippocampal virus injections, craniotomies were made at AP -2.3 mm, ML $\pm$ 1.6 mm, DV -1.1 mm from Bregma, and AP -2.7 mm, ML $\pm$ 2.5 mm, DV -1.25 mm. For CA1 silicon probe implantation, craniotomies were made at AP -2.5 mm, ML -2.0 mm. For the PFC, probes were inserted in a craniotomy made at AP +1.85 mm, ML $\pm$ 0.35 mm, DV 1.5 mm. Silicon probes (128-channels probes from Diagnostic Biochips or 64-channel probes from Neuronexus) were attached to micro-drives (Cambridge Neurotech or custom-printed drives). For optogenetics, 200 μ m optical fibers (ThorLabs, part# FP200ERT) were glued to a probe. A stainless-steel screw (JI Morris, 000-120 x 1/16) attached to a thin wire (Phoenix Wire Inc., cat# 36744MHW) was used to connect the ground screw over the cerebellum. Next, custom 3D printed baseplates with copper mesh attached were glued to the skull using metabond (C&B Metabond Quick Adhesive Cement System). Probes were inserted above the target region, and the base of microdrives was secured to the skull using dental cement (UNIFAST Trad Acrylic Resin). Craniotomies were covered with Dura-gel (Cambridge NeuroTech Dura-Gel). Ground wires were connected to probes’ ground wires and finally to the shielding copper mesh. Animals were allowed to recover for a week before recording started, and they were given carprofen subcutaneously for the first 3 days. After recovery, probes were slowly moved between 50 μ m to 200 μ m steps per day until the desired location was reached, where LFP physiological landmarks and characteristic LFP patterns were used to identify hippocampal and cortical layers.^{38,64}

Viral Injections

Stock AAVs were acquired from Addgene. AAV-CamKII α -CamKII α luciferase from Addgene.¹³ gc/ml, Addgene #26969-AAV5) was used. Specifically, 400nl of AAV-CamKII α -ChR2/H134R-EYFP full virus titer was injected into the hippocampus bilaterally (see Surgical Procedures). Injections were delivered through a pulled pipette using Nanoject III (Drummond Scientific Company, 3-000-207). Animals were allowed two weeks for recovery and for viral expression to take effect before probe implantation (see Surgical Procedures). The pAAV-CamKII α -hChR2(H134R)-EYFP was a gift from Karl Deisseroth (Addgene plasmid # 26969; <http://n2t.net/addgene:26969>; RRID: Addgene_26969).⁶⁵

Recording System and Data Preprocessing

Recordings were conducted with Intan RHD USB interface board or Intan Recording System. The sampling rate was 20,000 Hz, and amplification and digitization were done in the head stage by Intan RHD Electrophysiology Amplifier Chips. Acquisition was done with RHX Data Acquisition Software. LFPs were down-sampled to 1250 Hz before analysis. For video recording, an overhead mounted Basler Ace camera (Basler, acA3100-200uc) was used to record video simultaneously with neural signals. The camera’s frame rate (40 Hz) was recorded as digital inputs to Intan recording systems. Mice were tracked with custom DeepLabCut⁶¹ models trained on recorded videos and tracking was synchronized with individual frame timestamps.

Behavioral Tasks

Before surgery, animals were exposed to an open field environment to decrease neophobia after implantation. After surgery and recovery, animals were handled daily and habituated to the experimenter, room, open field environment, and recording cables for at least a week before the start of behavioral experiments. Recordings of brain signals were done during the object displacement memory task, as previously described.³⁸ Specifically, ~2 hours of pre-sleep was recorded before a 5-minute open field exploration. After open-field habituation, mice were trained to explore two identical objects for 5-minute periods. Depending on the experiment, animals were allowed a single 5-minute exploration of objects or three 5-minute exposures to objects. After object training, animals were allowed to sleep either 1 or 4 hours post-sleep, depending on experimental conditions. After post-sleep, animals were tested in a

5-minute single trial where one of the identical objects was moved to a new location. A discrimination index was used to assess memory performance.⁶⁶ Specifically, the discrimination index is calculated as:

$$\text{Discrimination Index} = (\text{timeObject A} - \text{timeObject B}) / (\text{timeObject A} + \text{timeObject B}) \times 100$$

time Object A is the time during the test exploring the unmoved object, and time Object B is the time during the test exploring the moved object. The discrimination index results in a score between 1 and -1, where 1 corresponds to time spent studying only the moved object, -1 corresponds to exploring the unmoved object. Only sessions where animals explored for at least 5 seconds were used for behavioral experiments.³⁸

Optogenetic manipulations and real-time SWR Detection

During the first 2-hours of post-task sleep, real-time SWR detection was conducted as done previously.^{17,19} Three LFP channels were used for online SWR detection. An LFP channel with the strongest ripple amplitude was selected for the ripple channel, filtering between 100 and 300 Hz. Another channel with the largest sharp-wave amplitude was selected to detect sharp-wave, filtering between 10 and 40 Hz. A third noise channel was selected by choosing a channel in the neocortex where no SWR activity was present. The noise channel was filtered between 100 and 300 Hz. The root-mean-square (RMS) of the three signals was then computed by a custom-made diode bridge rectifier (1N4001 diodes, 560 kOhm resistor, 68 nF capacitor). The filtered and rectified LFP were sent to a programmable real-time processor (Cambridge Electronic Design, CED) at 20,000 Hz sampling rate. Custom scripts in SpikeDetect2 (CED) were used to manually set the detection thresholds for ripple, sharp-wave, and noise for each animal individually. SWRs were defined as events crossing the ripple and sharp-wave thresholds but not crossing the noise channel threshold.

Once a SWR was detected, a small amplitude, 100 ms, light-pulse was delivered through bilateral optic fibers. On average, the delay between SWR detection and optogenetic pulse delivery was 22.9 ± 0.2 ms ($n = 10,727$ events). The light intensity was manually adjusted for each animal on the day of the experiment while the animal was in its homecage, where light power was increased until a ripple was produced. A trapezoid pulse with a 20 ms initial ramp and 20 ms ramp after the pulse was used.^{21,67} As a control, in different sessions, SWR were detected during post-task sleep and the same light-pulse was delivered after a random delay between 500 and 1,000ms (i.e. “Delayed stimulation” see [Figures S8D and S8E](#)^{17,19}).

Tissue Processing and Immunohistochemistry

After experiments, mice were deeply anesthetized under high-concentration (4-5%) isoflurane anesthesia. Mice were then perfused transcardially with 0.9% phosphate buffer saline (PBS) solution, after which 4% paraformaldehyde (PFA) solution was perfused. Brains were kept for 24 hours in PFA before sectioning them into 70 μ m thick slices.^{68,69} Slices were then stained with DAPI (Thermo Fisher, #D21490) and incubated for 30 minutes before washing. After washing, slices were mounted on glass slides with a Polyvinyl alcohol mounting medium with DABCO antifading (Millipore Sigma, #10981). Slides were imaged with a confocal microscope (Zeiss LSM 800 - acquired through Cornell University Biotechnology Resource Center, with NYSTEM (CO29155) and NIH (S10OD018516)) to image DAPI and virally expressed EYFP (see [Virus Injections](#)).

QUANTIFICATION AND STATISTICAL ANALYSES

Spike Sorting and Single Unit Classification

Spike sorting was performed semi-automatically using Kilosort⁷⁰ (<https://github.com/cortex-lab/KiloSort>). After sorting, manual curation was done in Phy (<https://github.com/kwikteam/phy-data>) using custom-designed plugins to acquire single well-isolated units (<https://github.com/petersenpeter/phy1-plugins>). Clusters were manually inspected for waveforms, auto-correlograms, and isolation distance metrics. Multi-units, noise clusters, or poorly clustered units were discarded, whereas well-isolated units were classified into putative cell types using the CellExplorer Matlab package (<https://cellexplorer.org/>).⁶³ Three cell types were assigned: pyramidal, narrow-waveform interneurons, and wide-waveform interneurons. Two key metrics separated different putative cell types: the burst index and trough-to-peak latency.⁷¹ The burst index was calculated as the average number of spikes in the 3-5 ms bins of a cell's autocorrelogram divided by the average number of spikes in the 200 to 300 ms bins. The average waveform from the recording site with maximum amplitude was taken to determine the trough-to-peak latency. Only well-isolated neurons with at least 100 spikes were kept for further analysis.

Sleep and Brain States Classification

Sleep and brain state scoring was done as previously described.^{25,38,72,73} State scoring used broadband LFP, narrow band theta frequency, and estimated electromyogram (EMG) to differentiate brain states. From broadband LFP, spectrograms were calculated using a fast Fourier transform with 10 s epochs and a sliding window of 1 s. Principal Component Analysis was then computed from the Z-transform of the spectrogram from 1 to 100 Hz. The first component reflected the power in the low frequency range (<20 Hz) with opposite weighted power at higher frequencies (>32 Hz) and distinguished non-rapid eye movement (NREM) from non-NREM periods.⁷³ For theta epochs, a channel with high theta power was selected, and theta dominance was quantified as the ratio of power in the 5-10 Hz and 2-16 Hz frequency bands.⁷² For EMG, the zero-lag correlation of filtered signal between 300-600 Hz (Butterworth

filter with filter padding between 275–625 Hz) between recording sites was used to estimate EMG activity. Pairwise correlations between channels of non-adjacent probe shank were calculated, and the mean of all correlations in time bins of 0.5 s was used as an EMG score.⁶⁷ Soft sticky thresholds were used on broadband LFP, narrow band theta, and EMG were used to determine states. High LFP in the first principal component of broadband spectrogram and high EMG were considered NREM, high narrow band theta activity and low EMG were considered rapid eye movement (REM), and remaining data (i.e., high EMG) were taken as waking state.

To prevent variations in the total amount of recorded NREM sleep from affecting our results given that SWR rate and reactivation are known to change throughout NREM sleep, we defined “baseline sleep” as the first cumulative hour of NREM sleep in the rest session recorded before training, and “post-task sleep” as the first cumulative hour of NREM sleep in the rest session recorded after training.

SWR analyses

For offline SWR detection, the wide-band signal was band-pass filtered (difference-of-Gaussians; zero-lag, linear phase FIR), and instantaneous power was calculated by clipping at 4 SD, rectified, and low-pass filtered. The low-pass filter cut-off was at a frequency corresponding to p cycles of the mean bandpass (for 80–250 Hz band-pass, the low-pass was 55 Hz). One channel around the CA1 pyramidal layer was chosen for the ripple detection, and one channel from CA1 str. radiatum was chosen for sharp wave detection. Candidate events were identified as local minima in the sharp-wave band-filtered signal, and the corresponding ripple power was recorded. K-means clustering was used to define clustering of SWR from non-SWR events, and manual curation was used to better define the boundary and remove outliers. The events were then expanded until the (non-clipped) ripple power fell below 1 SD and the sharp-wave band-filtered signal fell below 0.5 SD above the local (± 5 s) median; short events (< 15 ms) and longer events (> 400 ms) were discarded.

LFP signals were extracted around detected ripple events. For each event, a wavelet spectrogram was computed on the detrended LFP segment within ± 500 ms of the ripple peak. The mean wavelet spectrogram for SWRs in baseline sleep and SWRs in post-task sleep, and each was corrected by subtracting power at -200 ms. Subsequently, the minimum and maximum power within the 150 Hz frequency band during the ± 100 ms window around the event were used to normalize the spectrogram as $w = (w - \min) / (\max - \min)$.

To compare SWR spectral power in the ripple band (e.g. Figure 1E), the power values in the ripple band (150–250 Hz) within ± 50 ms around the ripple peak from the baseline sleep spectrogram and the post-task sleep spectrogram for each session were normalized together by subtracting the combined mean of both and dividing by their combined standard deviation. A Monte-Carlo test was then used to compare the normalized baseline sleep power to the normalized post-task sleep power for each task condition.

To compare SWR spectral profiles (e.g. Figure 1F), the mean spectra within ± 100 ms around the ripple peak from baseline sleep and post-task sleep for each session were normalized together by subtracting the combined mean of both conditions and dividing by their combined standard deviation. A Monte-Carlo test was then used to compare the normalized baseline sleep spectra to the normalized post-task sleep spectra for each task condition.

SWR start and end are defined as part of the offline SWR detection described above. SWR peak was defined as the moment of maximal ripple amplitude power within the event. SWR asymmetry was defined as how far the ripple peak deviated from the center of the event, computed as the absolute deviation of the peak from the midpoint between ripple start and end: $|(peak - start) / (end - start) - 0.5|$, with values near 0 indicating a symmetrical event. SWR frequency was defined as the frequency at which the wavelet spectrogram reached its maximum within ± 100 ms of the ripple peak, and SWR amplitude was the corresponding power at that frequency. To visualize the SWR features, we included SWR duration, SWR amplitude, SWR frequency, SWR asymmetry, and reactivation strength of PFC cell assemblies (see below), and reduced the dimensions using an unsupervised Uniform Manifold Approximation and Projection (UMAP)^{21,74} and plotted the first two dimensions, color-coding by feature.

To select SWRs with respect to their size in terms of both duration and amplitude, we designed a compound measure combining these features. For each session, SWR amplitude and SWR duration were z-scored using the mean and standard deviation of the SWR amplitude and SWR duration values, respectively, measured from baseline sleep SWRs. SWR size was defined as the minimum of these two values, and events with an SWR size exceeding the mean size of baseline sleep SWRs were classified as “large SWRs,” and other SWRs were classified as “small SWRs”.

Cortical rhythm detection

Delta waves and spindles were detected as described previously.^{53,58} Briefly, the prefrontal LFP was band-pass filtered (0–6 Hz) and z-scored, yielding $D(t)$. Upward-downward-upward zero-crossings of the derivative of the z-scored signal were defined as the start, peak, and the trough of a candidate delta wave, respectively. The delta wave index was computed as $D(t_{peak}) - D(t_{trough})$, and events with delta wave index exceeding a threshold (the threshold was user defined but in all cases was between 4 and 6) were retained as delta waves. Delta waves briefer than 150 ms or longer than 500 ms were discarded.

For sleep spindles, the prefrontal LFP was band-pass filtered (9–17 Hz) and z-scored. The squared magnitude of its Hilbert transform was smoothed using a 100-ms Gaussian window, yielding $S(t)$. Spindles corresponded to epochs where $S(t)$ remained above 2.5 for more than 0.5 s but less than 3 s, and peaked at > 5 .

LFP coherence analyses

Coherence was measured between LFP signals in the PFC (pre-limbic cortex) and CA1 pyramidal layer. LFP epochs recorded during movement periods in the task were concatenated. Epochs that contained artifacts were rejected. Spectra were estimated by Fourier transform of the LFP. Coherence was calculated as the magnitude of the summed cross-spectral density between two LFP time series, normalized by respective power spectra.

Current Source Density analysis

To address the contribution of different synaptic inputs to the LFPs recorded in the CA1 subregion, we employed current source density (CSD) analysis^{21,34,75} (Figure 1L). First, event-triggered LFP signals were isolated for small and large SWR separately. For each of them, CSD analyses were applied to the profile of the CA1 LFPs. The different layers (i.e., stratum oriens – ori., radiatum – rad. and lacunosum-moleculare – l-m.) associated with the main inputs into CA1 (i.e., CA2, CA3 and entorhinal inputs respectively) were identified by the voltage reversal of LFP signals and depth profile of currents sources and sinks (from CSD analysis), assuming current sources as positive CSD values and current sinks as negative CSD values as described previously.^{21,34,75} The strength of each input was estimated as the power of the CSD in their corresponding layers. Specifically, the CSD value for each input (ori., rad. or l-m.) was calculated as the absolute magnitude of the corresponding sinks in each layer. Then, a *ratio* was calculated as the CSD magnitude during SWRs as:

$$CSD\ index = \frac{(|CSD\ SWR_{small}| - |CSD\ SWR_{large}|)}{(|CSD\ SWR_{small}| + |CSD\ SWR_{large}|)}.$$

Cell assembly analyses

Cell assemblies were detected using an unsupervised statistical framework based on a hybrid principal component analysis (PCA) followed by independent component analysis (ICA) as reported previously.^{20,33,58} In brief, for each region (the hippocampus and the prefrontal cortex), spike trains of each neuron were binned in 50-ms intervals for the whole session, the pairwise firing correlation coefficients were computed for all pairs. Next, we calculated the number of assemblies based on those principal components whose eigenvalues exceeded the threshold for random firing correlations (using the Marcenko-Pastur law), ensuring that each of the detected patterns explained more variance of the spike train correlation matrix than other patterns that would result from independently firing neurons. Independent component analysis (fast-ICA algorithm) was then used to determine for each assembly (component) the vector of weights with which each neuron's firing contributes to that assembly. The strength of each assembly *i*'s activation for a given time bin *k* was computed as follows:

$$S_{ik} = Z_k^T P Z_k$$

where *Z* is the population activity matrix of the z-scored firing rate of each unit, and *P* is the outer product of the component *i*'s weights, in which the diagonal has been set to zero so that isolated spikes from individual units do not contribute to *S*.

The goal of the cell assembly analysis was to study the patterns associated with the learning experience (i.e. object exploration). However, because the task design required the animals to be habituated to the environment in advance, we expected a number of cell assemblies to have formed during this habituation period, reflecting task-related variables (such as the environment layout, distal cues, etc.) that are orthogonal to the learning experience. We therefore discarded cell assemblies with significant reactivation in baseline sleep before object exploration from subsequent analyses. Unless otherwise specified, throughout the text, the term 'assembly reactivation' refers to the reactivation in SWRs of cell assemblies identified using this PCA-ICA based approach.

To detect cross-structural cell assemblies, we followed a procedure described previously.^{25,39} Briefly, we combined the recordings in the hippocampus and the prefrontal cortex and performed assembly detection as described above. For a given assembly, the weight vector was normalized to have unit Euclidean norm, and units whose weights exceeded $1/\sqrt{N}$, where *N* is the number of recorded units, were considered assembly members. Cell assemblies with members in both the hippocampus and the prefrontal cortex were retained as cross-structural cell assemblies.

Assembly responses to SWRs were quantified as the mean reactivation strength within 0–100 ms of SWR onset. To allow pooling across assemblies, responses were z-scored within each assembly. For analyses requiring classification of individual SWR responses (Figures 1, S2, and S7), responses exceeding $z = 1.96$ ($P < 0.05$) were considered significant; SWRs without PFC significant reactivation were considered as SWRs in which none of the detected PFC cell assemblies reached a significant response.

For the downsampled control analyses in Figure S9, we repeated the above analyses after downsampling spiking activity to make sure that our results are not driven by any overall firing rate changes from baseline sleep to post-task sleep. For the down-sampling procedure, the mean multiunit response to SWRs (0–100 ms) was computed for baseline sleep SWRs and post-task sleep SWRs. The smaller of the two rates was retained as the response rate-to-be-matched, and we discarded random spikes within the 0–100 ms SWR window of the interval with the larger response rate until the two response rates were matched. In other words, if the response rate increased from baseline sleep to post-task sleep, then spikes were discarded from post-task sleep SWRs.

Pairwise reactivation

Pairwise reactivation analyses were performed as described previously.^{20,76} Briefly, we binned the activity of all units during SWRs in baseline sleep, and used this spike count matrix to compute a pairwise cross-correlation matrix C_{baseline} . Similarly, we binned the activity of all units in 100 ms during movement intervals of training of the object task, and used this spike count matrix to compute a pairwise cross-correlation matrix C_{behavior} . Finally, we binned the activity of all units during SWRs in post-task sleep, and used this spike count matrix to compute a pairwise cross-correlation matrix C_{post} . We used linear regression between C_{baseline} and C_{behavior} and between C_{baseline} and C_{post} to remove the portion of the variance that can be explained by preexisting patterns. The reactivation of pairwise behavioral patterns in post-task sleep was quantified as the correlation between the resultant residuals R_{behavior} and R_{post} . This method was used in Figures 3G, 3H, and 4A.

Hierarchical bootstrap

Hierarchical bootstrap⁷⁷ was performed to analyze data with hierarchical structure. Briefly, bootstrap datasets were created by resampling with replacement following levels of hierarchical order in the order of animals followed by sessions followed by the within-session observations. Each resampled bootstrap dataset was used to calculate an estimate of the mean, in a total of 5000 resampling times.

For the pairwise reactivation analysis, hierarchical bootstrap was used to estimate a distribution of correlation values between R_{behavior} and R_{post} . To this end, R_{behavior} and R_{post} were resampled together according to the hierarchical structure, and the correlation between them was computed for each bootstrap sample.

In all cases (whether estimating a mean or a correlation), the P-value reflects the direct comparison of the distribution of estimates to zero, or comparisons of different distributions to each other, after controlling for the other nesting variables.

Statistical Analysis

Data analysis was performed using custom routines in Python and MATLAB (MathWorks). No specific analysis was used to estimate minimal population sample or group size, but the number of animals, sessions and recorded cells were larger or similar to those employed in previous related work.^{21,28,29,38} Unless otherwise noted, non-parametric Wilcoxon rank-sum or Wilcoxon signed-rank test was used for unpaired and paired data comparisons respectively, and ANOVA and Kruskal-Wallis was used for multiple comparisons. All statistical tests were two-tailed with $p < 0.05$ as the cutoff for statistical significance, which is indicated by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$). Error bars show SEMs, and boxplots show median (central mark), 75th (box), and 90th (whiskers) percentile, unless indicated otherwise. Due to experimental constraints of optogenetic experiments, experimenters were not blind to these manipulations.