Assembly-Specific Disruption of Hippocampal Replay Leads to Selective Memory Deficit

Highlights

- Online decoding allows selective disruption of reactivated memory traces
- Content-related reactivation disruption in sleep led to specific recall impairment
- Disrupted spatial memory representations re-emerge in the same form after relearning
- Reactivation may facilitate the selection of correct representation after learning

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In Brief
Gridchyn et al. examine how the content of reactivated sleep activity influences memory recall. By disrupting the reactivation of specific place cell patterns, it shows recall impairment for spatial memories represented by the disrupted patterns, without impacting other, undisrupted memories.
Assembly-Specific Disruption of Hippocampal Replay Leads to Selective Memory Deficit

Igor Gridchyn, Philipp Schoenenberger, Joseph O’Neill, and Jozsef Csicsvari

SUMMARY

Memory consolidation is thought to depend on the reactivation of waking hippocampal firing patterns during sleep. Following goal learning, the reactivation of place cell firing can represent goals and predicts subsequent memory recall. However, it is unclear whether reactivation promotes the recall of the reactivated memories only or triggers wider reorganization. We trained animals to locate goals at fixed locations in two different environments. Following learning, by performing online assembly content decoding, the reactivation of only one environment was disrupted, leading to recall deficit in that environment. The place map of the disrupted environment was destabilized but re-emerged once the goal was relearned. These data demonstrate that sleep reactivation facilitates goal-memory retrieval by strengthening memories that enable the selection of context-specific hippocampal maps. However, sleep reactivation may not be needed for the stabilization of place maps considering that the map of the disrupted environment re-emerged after the retraining of goals.

INTRODUCTION

The hippocampus is implicated in all stages of learning, ranging from memory encoding and consolidation to recall (Morris, 2006), and spatial learning, including the learning of goal locations, depends on this area (Morris et al., 1982). Hippocampal principal cells tend to fire in discrete locations of the environment, and together, these place cells form a cognitive representation of the explored space (O’Keefe and Nadel, 1978). Place cells change their coding during goal learning and start firing near learned goals (Dupret et al., 2010; Hollup et al., 2001).

The waking firing patterns of place cells are reactivated in subsequent sleep and quiet immobility periods (Wilson and McNaughton, 1994). Through replaying previously encoded memory traces, reactivation has been suggested to promote memory consolidation (Buzsáki, 1989; Marr, 1971; McClelland et al., 1995). However, the exact mechanism by which reactivation facilitates the retrieval of previously acquired memories is not known. It has been suggested that repetition can strengthen labile hippocampal representations, such as those formed by place cells in a novel environment (Buzsáki, 1989; van de Ven et al., 2016). In addition to their possible role in intrahippocampal memory stabilization, reactivation may be involved in the transfer of memory traces to external brain areas and, in doing so, facilitate systems consolidation and update brain-wide memory representations (Buzsáki, 1996; McClelland et al., 1995).

Reactivation of hippocampal firing patterns often occurs during sharp-wave ripples (SWRs), which are constituted by transient 200-Hz oscillatory patterns and are triggered by the synchronized activation of hippocampal cells (Buzsáki et al., 1992; Kudrimoti et al., 1999). Several studies have detected the emergence of these 200-Hz oscillatory periods online and, through electrical or optogenetic stimulation, have disrupted SWRs and SWR-related reactivation (Ego-Stengel and Wilson, 2010; Girardeau et al., 2009; Jadhav et al., 2012; Kovács et al., 2016; Roux et al., 2017; van de Ven et al., 2016). When electrical stimulation-mediated SWR disruption was applied during sleep, it delayed the learning needed to solve complex spatial learning tasks on mazes (Ego-Stengel and Wilson, 2010; Girardeau et al., 2009). However, similar SWR blockade during active waking periods disrupted spatial working-memory performance (Jadhav et al., 2012). SWR disruption using optogenetic inhibitory archaerhodopsins (Arch and ArchT) was used to test the stabilization of hippocampal place maps that emerged in a novel environment (Kovács et al., 2016; van de Ven et al., 2016) and during spatial learning (Roux et al., 2017). In a novel environment, such disruption of SWRs before the second exposure to the novel environment did not lead to a significant reduction in the similarity of place fields (Kovács et al., 2016) but instead triggered alterations at the assembly level, suggesting that the joint firing patterns of place cells were altered (van de Ven et al., 2016). Moreover, in another study, waking SWRs that occurred during spatial goal learning were inhibited, leading to place field destabilization (Roux et al., 2017). This suggests that perhaps waking, but not sleep, SWRs are involved in the stabilization of place fields.

Collectively, these data suggest a role for SWRs in spatial learning, but it is unclear whether the disruption of SWRs alone or specifically the disruption of assembly firing patterns that encode memory traces during SWRs mediates a learning impairment. In fact, the highly synchronized network activity occurring...
during SWRs can trigger synaptic plasticity (King et al., 1999), and it may be involved in synaptic downscaling (Norimoto et al., 2018). It is, therefore, not clear whether the neuronal assembly content during SWRs has a direct role in spatial learning or whether reactivation merely reflects the built-in property of hippocampal circuits to replay neuronal assembly patterns (Dragoi and Tonegawa, 2011; Gupta et al., 2010; Stella et al., 2019). Finally, it is unclear whether the reactivation of a specific memory influences only the stabilization of that memory or whether it affects and reorganizes other, related memories as well.

To directly investigate the role of reactivated memory content, we performed content-specific disruption of reactivated firing patterns during sleep and tested whether it leads to recall impairment of those memories whose reactivation was disrupted before. At the same time, we monitored place cell activity and tested the stability of place cell representations to assess the role of reactivated firing patterns in maintaining intrahippocampal representations. Because our work used online decoding of neuronal activity combined with closed-loop optogenetic stimulation to disrupt specific memory content, we also aimed at demonstrating that higher cognitive functions, such as learning and memory recall, can be influenced by brain-machine interface (BMI) methods. Such approaches have been employed to interfere with complex brain functions online at sensory-perceptual (Cerf et al., 2010; Dadarlat et al., 2015; O’Doherty et al., 2011) or motor-action levels (Donoghue, 2008; Ifft et al., 2013; Moritz et al., 2008); yet, their application to hippocampus-dependent learning has not been tested before.

**RESULTS**

We expressed archaerhodopsin (ArchT) in the CA1 region of the hippocampus using adeno-associated virus (AAV2/1) with a CaMKII promoter (Boyden et al., 2005; Chow et al., 2010) in four rats. We implanted these animals with 128-channel, independently movable electrode arrays. The light was applied to both hippocampi using four optic fibers to inhibit the activity of CA1 neurons that expressed ArchT. Light application disrupted place cell activity both directly, through firing suppression, and indirectly, with interneuron-mediated disinhibition, influencing at least 5 mm² of the CA1 that our electrodes covered (Figures 1 and S1). Light responses were tested at the beginning of the recording session in the light-pulse session, which was followed by a rest session (pre-rest) without light application (see STAR Methods). In the light-pulse session, the mean firing rate of pyramidal cells was significantly different from pre-rest ($p < 10^{-5}$, Wilcoxon signed-rank test; Figure S2A); however, interneuron rate and the excitation/inhibition ratio were not significantly different from those in the subsequent pre-rest session (all $p > 0.19$, Wilcoxon signed-rank test; Figures S2A and S2B).

In our experiments, rats were trained to locate food rewards on target and control cheeseboard environments (Figure 1A). The food locations were different on these cheeseboards and changed at the beginning of each recording day. Animals rapidly (within 1 to 3 trials) learned and rehearsed the reward locations in six learning and five subsequent “end-of-learning” trials on both cheeseboards. The first block contained one additional trial compared to the second block because, in the very first trial,
the animal’s behavior was different from the remaining trials. Following learning, animals were rested for 4 h during which a spike waveshape-based cell-assembly detection algorithm (Ciliberti et al., 2018; Kloosterman et al., 2014) (see also Figures 2 and S2D–S2F) determined online whether the target or the control cheeseboard environments were reactivated. Assembly detection was performed during the initial phase of high synchrony events (HSEs), and a laser pulse was triggered to disrupt the HSE firing pattern unless the algorithm could identify with high confidence that the control environment was detected (Figures 2A and 2B). Instead of SWRs, we detected HSE onset because we wanted to ensure that we detected and decoded a reactivation event as early as possible, i.e., as soon as a sufficient number of cells fired action potentials for the decoding. Moreover, this approach enabled the detection of lower-amplitude SWR, which the SWR detection procedure may have missed. These HSEs were detected when, at a given 20-ms time window, the number of the detected spikes exceeded a threshold. This threshold was initially set to 3.5 times the mean spike numbers measured in 20-ms windows during the pre-rest session, but the threshold was dynamically adjusted to achieve an approximate 1-Hz detection rate. The majority (93.7%) of the HSEs were detected in the absence of theta oscillations, during slow-wave sleep and immobile waking periods (Figure S3A). Most of the HSE detection occurred earlier than SWR times and about 50% of the detected HSEs were followed by a SWR within 50 ms (Figure S3B). The ripple-band (150–250 Hz) power following the HSE detection was significantly weaker for events in which the light was triggered compared to the cases in which light was not emitted because the control environment was decoded ($p < 10^{-5}$, Mann-Whitney U test). The duration of the HSEs that were disrupted by the light application was similar to the undisrupted ones (disrupted: $88.6 \pm 8.1$ ms, undisrupted $83.1 \pm 10.3$ ms, $p = 0.154$, Mann-Whitney U test). We next performed probe sessions (3 min each), in which animals explored without food rewards to test whether the animal remembered the learned goal locations. Subsequently, we performed food-reinforced post-learning sessions (two interleaved blocks of 5 trials each), in which animals could rehearse or relearn the previous reward configurations. This enabled us to further test the memory-recall performance of the animal in the first trial. Although the behavioral patterns of the animal were different in the learning, probe, and post-learning trials, the average firing rate of place cells in the control and target environments remained similar in these sessions (all $p > 0.154$, Mann-Whitney U test; Figure S2C).

**The Effect of Assembly-Specific Reactivation Disruption on Memory Recall**

Our procedure allowed the reactivation of the control environment only, whereas the target environment reactivation and those patterns that could not be confidently identified by the algorithm were disrupted. Therefore, in the subsequent probe and rehearsal sessions, we tested whether the animal remembered the previously learned food rewards in the control environment better than in the target environment (Figures S3A and S4). In probe sessions, animals spent significantly more time near the learned goal locations in the control than in the target environments ($p < 0.0065$, Wilcoxon signed-rank test, $n = 21$; Figure 3C), and the number of crossings at control goal zones was also significantly higher than at target zones ($p < 0.0065$, Wilcoxon signed-rank test; Figure 3C). In the first trial of the post-learning session, the normalized path length to reach the goal was significantly shorter in the control environment than in the target ($p < 0.0022$, Wilcoxon signed-rank test; Figure 3C). These effects could not be accounted for by altered recall in any animal alone because the effects remained when any of the animals were excluded from the analysis (highest $p = 0.0401$ for dwell time, highest $p = 0.0366$ for the number of crossings, highest $p = 0.0199$ for normalized path length, Wilcoxon signed-rank test). Additionally, to further test the contribution of different animals, we used a two-way ANOVA analysis with session type (i.e., control or target) and animal as factors. No difference was seen between animals (dwell time: $p > 0.43$, number of
crossings: $p > 0.38$, normalized path length: $p > 0.21$, F test), nor was there an interaction between animals and session type (dwell time: $p > 0.92$, number of crossings: $p > 0.72$, normalized path length: $p > 0.37$, F test). However, the session type was associated with differences in dwell time ($p < 0.0032$, F test), the number of crossings ($p < 0.0018$, F test), and the normalized path length ($p < 0.022$, F test). These analyses showed that animals retained the learned food locations of the control environment better than the target environment locations, indicating that our manipulation allowed the recall of goal memory of the control environment while disrupting the recall of target environment goals.

In these recordings, network-state parameters may have influenced the behavioral performance of the animal to recall the goals and the ability of our method to disrupt the recall in the target environment selectively. We examined whether the duration of non-theta periods, the delta-band (2–4 Hz) power during it, and the number of SWRs, HSEs, or specifically the disrupted HSEs influenced recall performance during control and target environments and predicted the preferential recall disruption in the target environment. However, none of these correlations were significant (all $p > 0.08$, Pearson correlation with Holm correction for multiple comparisons; Figure S3C). Previous...
work suggested that reactivation of recent experience declines over time (Ji and Wilson, 2007; Tatsuno et al., 2006; Wilson and McNaughton, 1994). Therefore, we tested whether the detection performance of the control and target assemblies declined over time (Figures S3D–S3F). In our 4-h time period during the rest session, we did not observe a decline, in agreement with a recent work that observed long reactivation of place cell patterns that were formed in a novel environment for up to 8 h (Giri et al., 2019).

Assembly-Specific Disruption Altered the Firing of Target Environment-Encoding Cells

We then confirmed our assembly detection procedure by identifying individual place cell activity with an offline spike-clustering procedure. First, we tested whether our algorithm reliably detected assemblies of different environments. To this end, we identified place cells that selectively fired stronger in either environment during learning and tested how these cells fired during and after the detection window. First, we examined those HSEs in which the light was not turned on because they were decoded as reactivating the control environment. In these events, the firing rates of place cells that preferentially fired in the control environments during learning also showed elevated firing compared to the cells that fired more in the target environment. This effect was seen both during the assembly detection window (p < 0.014 for inhibited cells, p < 2 × 10^{-5} for disinhibited cells, Mann-Whitney U test; Figures 4A and 4B) and after, during the continuation of HSE activity (p < 0.0477 for inhibited cells, p < 0.0009 for disinhibited cells, Mann-Whitney U test; Figures 4A and 4B). Therefore, these HSEs represented the control environment during learning and tested how these cells fired during and after the detection window. We next examined those HSEs that encoded the target environment, and consequently, the light was turned on in these events immediately after the assembly detection. Cells with a firing preference for the target environment fired stronger than the control environment-encoding cells in detection windows where the target environment was decoded (p < 0.0192 for inhibited cells, p < 2 × 10^{-6} for disinhibited cells, Mann-Whitney U test; Figures 4A and 4B). However, after the light was turned on, both the control and target environment-encoding cells that were inhibited by the light reduced their rate to a similar level, whereas disinhibited cells encoding either environment showed increased firing with a similar magnitude (p > 0.92 for inhibited cells, p > 0.052 for disinhibited cells, Mann-Whitney U test; Figures 4A and 4B). Therefore, light stimulation decreased the firing of some target environment-encoding cells but also recruited some of the control environment-encoding cells through disinhibition. Interestingly, during undisrupted HSEs in which the control environment was detected, the inhibited cell group exhibited a further increase in its rate after the HSE detection, whereas the disinhibited cells did not change their rate further (p < 10^{-4}, Wilcoxon signed-rank test), suggesting differences in the firing dynamics of these cell groups during HSE.

To test the combined effect of the light application on assembly expression, we measured how strongly the control and target environment activity patterns were reactivated during the detection period and after. We compared the cofiring patterns of cells during the end-of-learning periods to those HSEs in which the control or target environments were decoded. For HSEs where the control environment was decoded and light was not emitted, control environment cofiring patterns were reactivated with similar strength during the detection window and after (p > 0.99, Z test; Figure 5A), but these HSE assembly patterns were less related to the target environment cofiring (p < 10^{-4}, Z test; Figure 5A). In contrast, for target environment-decoded HSEs, during the detection window, the target environment cofiring patterns were reactivated, but the reactivation strength weakened significantly when the light was turned on (p < 10^{-4}, Z test; Figure 5B), and weaker reactivation was seen for the control environment cofiring patterns both before and after the light onset. In addition, the correlation between the firing rates during and after the detection window was significantly higher for HSEs in which the control environment was decoded than for the target-decoded HSEs (p < 10^{-15}, Z test; Figure S5). Thus, our
Figure 5. Reactivation during Control and Target Environment-Encoding HSEs

(A and B) Reactivation of end-of-learning (control and target environment) co-firing assembly patterns were compared during and after the detection time window for HSEs decoding either the control (A) or target (B) environments. The diagram on the top illustrates the co-firing calculations and shows the expected firing of control and target environment-encoding cells during learning and in subsequent HSEs during the rest session in which either the control or target environment was decoded. The gray boxes illustrate that the co-firing of cell pairs were compared during periods of learning and rest HSEs. We expect the co-firing of control encoding cells in the control environment to be more similar than those HSEs in which the control environment was decoded, whereas for the target environment-decoding cells, a similar relationship is strong during HSEs before the light application only. In agreement with this, the reactivation of end-of-learning target patterns during target-decoded HSEs drops after detection (p < 10^{-4}, Z test), but the control patterns in control-decoded HSEs do not (n.s. p > 0.99, Z test). Data are represented as mean ± 95% CI. See also Figure S5.

procedure disrupted the reactivation of the cell-assembly activity patterns of the target, but not the control, environments.

**Hippocampal Maps of the Target Environment Were Destabilized but Re-emerged after Relearning**

Given that our HSE disruption led to environment-specific memory impairment, we next examined the stability of hippocampal place representation. We compared the place fields from the end-of-learning trials to the probe trials, as well as to the first post-learning trial. Place field similarity of the target environment map was weaker than that of the control environment for both the probe comparison (all cells: p < 0.0056, inhibited: p < 0.0142, disinhibited: p < 0.0044, Mann-Whitney U test; Figure 6B) and the first post-learning trial (all cells: p < 0.027, inhibited: p < 0.037, disinhibited: p < 0.039, Mann-Whitney U test; Figure 6B). However, in later trials (6th to 10th) of the post-learning session, the place maps of both control and target environments were similar to those of the end-of-learning session (p > 0.34 for all cells, p > 0.45 for inhibited cells, p > 0.59 for disinhibited cells, Mann-Whitney U test; Figure 6B). However, the end-of-learning representations were formed during the learning trials, and the control and target environment maps themselves in the end-of-learning session were different (Figures S6A–S6C). The trajectory of the animal in the target environment was different in the first and the late (6th to 10th) post-learning trials, whereas it was similar for the control environment. Therefore, we further tested whether differences in place field similarity remained when we simulated the firing of place cells using identical trajectories seen in the late trials of the post-learning session. The spatial firing probabilities seen in the first trial were used to simulate the firing of cells on the late trial tracks, and the place fields were recalculated from the simulated firing. Similar simulated place fields were used for the end-of-learning maps as well. The place field similarity for the simulated place fields still remained higher for the control than for the target environments (all p < 10^{-5}, binomial test; Figure S6D). Also, when late post-learning place fields were simulated in the same way, these remained similar (all p > 0.99, binomial test; Figure S6E), like they did for the original maps. This suggests that our manipulation prevented retrieval of the new place maps of the target environment that emerged during learning in the first memory trial of the post-learning trial, but not their stabilization and their consequent retrieval in later trials.

**DISCUSSION**

Here, we showed that disruption of the reactivation of specific place cell patterns leads to the recall impairment of spatial goal memories represented by these place cells, without impacting other, undisrupted memory traces. In parallel with the behavioral impairment of goal retrieval, the place map of the disrupted environment was not reinstated during memory recall. However, when the animal relearned the goal, the original map that was established during the learning, before the reactivation disruption, was reinstated.

Previous work showed that the blockade of SWRs can delay spatial learning when the animal has to both acquire the rules of a task and learn which arms contain food on a maze (Ego-Stengel and Wilson, 2010; Girardeau et al., 2009). Learning such tasks requires cortical dialog for rule acquisition and at least a partial reorganization of the hippocampal map (Durstewitz et al., 2010; Ragozzino et al., 1999; Xu et al., 2019). Therefore, in these studies, the delay in rule acquisition may have been due to the disruption of cortical dialog during SWRs, but SWR disruption may also have impacted the reorganization and stabilization of place maps through interfering with critical plasticity needed for map stabilization (Kentros et al., 1998). Moreover, electrical stimulation may have triggered spurious plasticity, causing alterations of spatial representations formed during the learning process (Dragoi et al., 2003). Our manipulation was not related to SWRs, per se, but to decoded reactivation content during HSEs to make a decision on whether the
reactivation was allowed or disrupted. It is unlikely that this manipulation caused unspecific plastic changes, considering that we primarily affected the reactivation of the target environment. Moreover, to our surprise, once the animal relearned the goals, the target environment map was similar to the original map that emerged as a result of learning before reactivation disruption. Furthermore, even the target environment maps in the probe session and the 1st post-learning trial exhibited only a weak but significant impairment in the map similarity to the end-of-learning map. This suggests that reactivation during quiet rest periods does not play a critical role in the stabilization of new cognitive maps. However, considering that reactivation disruption initially prevented the exact recall of the appropriate map, reactivation may play specific roles in stabilizing memories that

Figure 6. Place Field Stability at Different Stages of Learning

(A) Representative place fields during the end-of-learning, probe and the first post-learning trial, and the last five trials of post learning. Maximum map rates are shown on the bottom right. Top traces: behavioral traces in a representative session corresponding with the place fields of the first row below. The order of the place fields represents the order in which the trials were performed in the respective session.

(B) Place field similarity comparing the end-of-learning sessions with the probe and the first and the last five trials of post learning. Place field similarity was measured for all place cells (all), but inhibited and disinhibited cells were also analyzed separately. Control cells versus target cells, **p < 0.0056, *p < 0.04, ***p < 0.0033, n.s. p > 0.14, Mann-Whitney U tests. Data are represented as mean ± SEM.

See also Figure S6.
associate a map with the appropriate learning context. Recognizing the appropriate learning context and ultimately selecting the right hippocampal map may require extrahippocampal processing and inputs (Place et al., 2016). Therefore, sleep reactivation may stabilize the association of hippocampal maps to learning context representations outside the hippocampus. Considering that our manipulation detected HSEs and not SWRs, not all the detected HSEs may have been involved in extrahippocampal interactions. Also, we cannot exclude an alternative explanation of these data in which our manipulation did destabilize the target environment map to a certain degree, but, upon relearning, the similar behavioral conditions led to the formation of a map reminiscent to that which emerged in the first learning. Furthermore, SWR-associated reactivation may have additional roles in downscaling unstable synapses (Norimoto et al., 2018). In this regard, our manipulations may have interfered with plastic processes, which may have included the downscaling of CA3-CA1 synapses that were uncorrelated during target environment map expression. This interference with plasticity may have been a cause for the transient disruption of target environment map stability. Alternatively, such plasticity impairment may have contributed to the memory recall impairment without influencing map stability.

In our experiments, we also examined the role of specific reactivated patterns in relation to spatial learning. We were able to selectively influence the recall of recently learned memories through controlling the content of reactivation during quiet immobility and sleep periods following learning. Therefore, we causally demonstrated that hippocampal reactivation content preferentially facilitates the recall of the reactivated memories without impacting the recall of other memories that were also reactivated in the same rest period. These findings demonstrate that, at least at the level of the hippocampus, reactivation does not trigger wider circuit reorganization that may occur during generalization or when associations are made between related memories. However, we cannot exclude the possibility that, in more complex tasks, consolidation in the hippocampus may also involve wider reorganization and non-specific, reactivation content-independent changes. Moreover, our work examined memory recall that occurred on the same day after shorter 4-h time periods. Wider memory reorganization and generalization may take several days or weeks. Although the animal experienced the same two cheeseboards over several days with different goal configurations, the maps representing these boards remained different and less similar than the maps that were present in the beginning and the end of learning in the same environment. The absence in the convergence of map similarity indicates that the hippocampus maintained different representations of the two environments because of the consistently different spatial context.

Finally, our method demonstrated that BMI approaches could successfully influence the stabilization and the subsequent recall of recently learned hippocampus-dependent memories. However, closed-loop stimulation triggered by neuronal population activity decoding may have wider applications in studying learning and memory recall beyond weakening or disrupting the consolidation of specific traces. Early detection and disruption of neuronal patterns during recall stages may be able to selectively disrupt memory recall without causing lasting memory impairment and forgetting. Controlling the activity of cFos-expressing engram cells at the recall stage alters memory recall (Liu et al., 2012; Ramirez et al., 2013; Tanaka et al., 2019). Furthermore, waking SWRs are needed for certain spatial memory decisions (Jadhav et al., 2012; Pfeiffer and Foster, 2013), and the prolongation of these SWRs improves memory performance (Fernández-Ruiz et al., 2019). Therefore, it may be sufficient to perform content-specific disruption during SWRs that occur in decisions requiring memory recall. Finally, in certain conditions, the recall of specific memories could be potentiated by closed-loop stimulation of non-specific neuro-modulatory pathways that regulate memory recall. For example, the stimulation of the ventral tegmental area (VTA) may show a memory enhancement effect, as demonstrated by the finding that the spatial valence of an environment can be altered during sleep through VTA stimulation triggered by the activity of a single place cell (de Lavilledon et al., 2015). Taking these considerations into account, closed-loop stimulation approaches using online assembly decoding during sleep or recall stages can have wide-ranging potential to disrupt or facilitate the consolidation and recall of specific memories.

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.neuron.2020.01.021.
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AUTHOR CONTRIBUTIONS

J.C., P.S., and I.G. designed the study, I.G. conducted the experiments and analyzed the data. J.C., J.O., and I.G. wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

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STAR METHODS

KEY RESOURCES TABLE

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LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and datasets should be directed to and will be fulfilled by the Lead Contact, Jozsef Csicsvari (jozsef.csicsvari@ist.ac.at). This study did not generate new unique reagents.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Four male rats (Long Evans, aged 4-6 months, 300–400 g) were used in this study. The animals were housed in a separate room and were taken to the recording room each day prior to the experiments. They shared a cage with littermates before the surgery. All procedures involving experimental animals were carried out in accordance with Austrian (Austrian Federal Law for experiments with live animals) animal law under a project license approved by the Austrian Federal Science Ministry.

METHOD DETAILS

Surgery

Animals were injected with a recombinant AAV to express Archaeorhodopsin-GFP in the dorsal CA1 area (AAV2/1.CaMKII::ArchT.GFP.WPRE.SV40, obtained from the Penn Vector Core facility, 6.41*10^{15} genome copies per ml). Before injection, the virus solution was diluted 1:24 with a physiological NaCl solution. 300 nL of the virus was injected at each of the four sites into dorsal CA1 bilaterally: anterior-posterior (AP), mediolateral (ML) and dorsoventral (DV) 

- 3.2 AP, ± 2.0 ML, 2.15 DV;
- 3.87 AP, ± 2.67 ML, 2.06 DV;
- 4.53 AP, ± 3.33 ML, 2.06 DV;
- 5.2 AP, ± 4.0 ML, 2.19 DV.

Two weeks after virus injection, animals were implanted with 28 independently movable electrode groups under deep anesthesia using isoflurane (0.5%–3%), oxygen (1–2 l/min), and an initial dose of buprenorphine (0.1 mg/kg). Our electrodes were arranged in two bundles of 12 tetrodes and 2 eight-wire octrodes targeting CA1 bilaterally. The tetrodes and octrodes were constructed from four and eight 12 μm tungsten wires correspondingly (H-Formvar insulation with Butyral bond coat, California Fine Wire, Grover Beach CA), twisted and then heated in order to bind them into a single bundle. The tips were then gold plated to reduce their electrode impedance to 200–400 kΩ. During surgery, two craniotomies were centered above CA1 and the electrodes were implanted into coordinates DV: 1.4, between ± 2.2 and ± 4.6 ML and –4.9 and –3.7 AP in 1.4 mm depth from the brain surface. The fibers were located between the tetrodes in the first and last rows of the tetrode bundles in positions with coordinates –3.7 AP, ± 3.0 ML and –4.5 AP, ± 3.8 ML. Two screws positioned above the cerebellum served as ground and reference electrodes. Six additional stainless-steel anchor screws were used in order to permanently attach the microdrive assembly to the skull. The paraffin wax-coated electrodes and the microdrive apparatus were then daubed with dental acrylic to encase the electrode-microdrive assembly and anchor it to the screws in the skull. Following a recovery period of 7 days, the tetrodes were lowered in 50–150 μm steps each day into the CA1 region over a further period of 7–14 days.

Data Acquisition

The extracellular electric signals from tetrodes were pre-amplified using a headstage (4 x 32 channels, Axona Ltd, St. Albans, Hertfordshire, UK). The amplified local field potential and multiple-unit activity were continuously digitized at 24 kHz using a 128-channel data acquisition system (Axona Ltd, St. Albans, Hertfordshire, UK). Four 32-channel unity-gain preamplifier panels were
used to reduce cable-movement artifacts. Wide-band (0.4 Hz to 9 kHz) recordings were taken, and the amplified local field potential and multiunit activity were continuously digitized at 24 kHz. Two bundles of light-emitting diodes mounted either side of the headstage were used to track the location and head direction of the animal during all recording sessions, sampled at 50 Hz. Green/yellow laser light for Archaerhodopsin activation was provided by two 561-nm diode-pumped solid-state (DPSS) laser systems equipped with an acousto-optic modulator (Omicron). The light was coupled into an optic fiber connected to a fiber-optic rotary joint (Doric Lenses), which also split the light into four 200 mm per 0.48 NA patch cords, which transmitted the light to the microdrive. Laser intensity was set to reach 25mW total power at every tip of the implanted fiber stub. The light pulses were triggered with TTL pulses sent through the parallel port of the computer, running the acquisition and real-time decoding software.

**Apparatus and training procedures**

The cheeseboard mazes were similar to what has been described previously (Dupret et al., 2010). They consisted of a PVC circular board (120 cm in diameter, 2 cm in thickness) with a total of 177 food wells (2.5 cm in diameter, 1.5 cm in depth) drilled into the surface of the maze in evenly spaced parallel rows and columns (8 cm between the centers of each well). Two PVC start-boxes, one gray and one black, (27 cm long, 19 cm wide and 59 cm high) were equipped with a door (35 cm high and placed along the edge of the board. The tops of the boxes were open to allow tracking of the animal inside. In addition, a 20 cm wood plank was added between the start box entrance and cheeseboard so that animals would traverse slightly longer trajectories during learning trials, develop higher speed before reaching the goal and cover a larger proportion of the environment, thus generating place cell population activity that is easier to decode. The two environments were visually separated by a black curtain and distinguished by multiple white cue cards attached to the curtains.

Following the surgery recovery period (7 days), animals were exposed to both cheeseboard environments. Animals were placed on food restriction (> 85% of initial weight with a 10 g gain each week), once they had passed the recovery period and exceeded their pre-surgery weight. Animals were then retrieved to retrieve food pellets (MLab rodent tablet 20mg, TestDiet). Once animals were habituated to the cheeseboards and started consuming the pellets, they were trained to leave the start box, retrieve hidden pellets on the cheeseboard and return to the start box. A group of visible food pellets was spread out on the surface of the cheeseboard while the rat was inside the start box. Then, the door was opened to allow the animal to retrieve the rewards. Once all the rewards had been collected, the door was reopened, and the rat was gently guided back to the start box. That procedure was repeated until the rat began to leave the start box and return to it after collecting all the rewards (~3-4 days). The same baited locations were used from one day to the other during this initial training. In addition, animals spent two h in the sleep box every day. The sleep box represented a metal cylindrical bin 50 cm in diameter and 50 cm high with a towel at the bottom.

To prevent the use of an odor-guided search strategy during these experiments, food pellet dust was scattered across the maze before each experiment, the board was periodically wiped (using a towel used to handle the rat daily) and the board was randomly rotated at an angle multiple of 90° (due to the symmetry of the board) relative to the start-box between learning trials and between rest and probe sessions. This initial phase of the experiment ended when animals were familiar with the whole procedure. During the training phase, the animal was trained equally in both cheeseboard environments. No probes were performed during the training phase to prevent habituation to the absence of food, but more learning trials were performed after the sleep. The behavior was considered satisfactory when the animal was able to steadily perform 40 trials in every environment per day and memorize a goal location in every environment within the first 3 trials. When the behavior reached a satisfactory level and enough tetrodes (at least 14) were positioned in the CA1 layer, the recordings started.

**Behavioral paradigm: double cheeseboard spatial memory test**

Each recording experiment consisted of a sequence of five separate sessions: a light-pulse session (20 min), an immobility/sleep rest session (“pre-rest”) (45 min), a learning session (11 trials in each environment), an immobility/sleep rest session with closed-loop assembly-related optogenetic disruption (4 h), a probe test (“probe,” 3 min in each environment) and repeated learning session (“post-learning,” 10 trials in each environment). During the light-pulse session, the animal was rested on the sleep box while regular laser pulses (100 ms) were applied at 1 Hz for 20 min. The order of presentation of the environments as control and target roles of environments were chosen pseudo-randomly every day before the recording began. During the two probe tests (3 min in each environment) animals were allowed to explore the cheeseboard environment without food rewards. During the learning session, animals were given two blocks of successive trials in each environment to find the hidden reward placed in a randomly selected food well. During the learning session, the first block consisted of 6 learning trials (end-of-learning), and the second block consisted of 5. During the post-learning sessions, all blocks consisted of 5 learning trials. Before the assembly-specific inhibition started in the rest session after learning, for 5 min all synchrony events were disrupted while the training of the model required for decoding the environments was completed.

In total, optogenetic disruption was performed for 21 recording days in four animals (6, 4, 5 and 6 sessions per animal).

**Real-time decoding**

During the closed-loop optogenetic disruption sessions, initial processing included spike detection and feature extraction (see Spike Sorting section below). Next, high synchrony events (HSE) were detected using multiunit activity (MUA) in the time windows of fixed length (20 ms). Based on the pre-rest recording, the mean number of spikes in all tetrodes in the 20 ms time windows was calculated.
HSE detection was triggered when the number of spikes in a time window exceeded a threshold of 3.5 times the baseline mean established in the pre-rest session. Moments in time, when the synchrony level first reached the baseline level (i.e., mean spike number) before and after the peak were defined as the beginning and the end of the HSE correspondingly. The HSE detection threshold was then adaptively recalculated using the Newton-Raphson method every minute based on the actual number of detected HSEs so that the effective HSE detection rate would be near 1 Hz. The beginning of the HSE was then adjusted to the time of the first spike in the HSE window. After HSE detection, real-time decoding (see below) was used to determine, which of the two environments was encoded. If the HSE encoded a control environment with high fidelity, the event was allowed to proceed (i.e., the event was not disrupted optogenetically). Otherwise, if no confident reactivation of the control environment was detected, the light pulse of 100 ms was triggered to disrupt the reactivation. In both cases, detection refractory period of 150 ms was applied to avoid multiple detections of the same HSE as well as spiking “rebound” after the light pulse ceases.

The real-time decoding was based on the method presented in (Kloosterman et al., 2014). The method utilizes Bayesian decoding under the assumptions of the spatio-temporal Poisson process and uses the Kernel Density Estimation (KDE) procedure for non-parametric estimation of probability distributions. According to Bayes formula, spikes of place cells and the position of an animal are related in the following way:

\[
P(x_t | a_{1:m}) = \frac{P(a_{1:m} | x_t)P(x_t)}{P(a_{1:m})}
\]  

(1)

where \(x_t\) is the position of the animal at time \(t\) and \(a_{1:m}\) is a population vector of spikes, emitted in a time window at time \(t\). According to the assumption of the spatio-temporal Poisson process:

\[
P(a_{1:m} | x_t) = (4\pi t)^m \prod_{i=1}^{m} \lambda(a_i, x) [e^{-4\pi t/\lambda}] 
\]  

(2)

where \(\lambda(a_i, x)\) and \(\lambda(x)\) are generalized rate function and marginal rate functions of the spatio-temporal Poisson process correspondingly. They can be calculated from average spiking rate \(\mu\), distribution of spike locations \(p(x)\), distribution of animal positions \(p(x)\) and joint probability distribution of spike waveshape features and spikes \(p(a, x)\):

\[
\lambda(x) = \mu \frac{p(x)}{\pi(x)}
\]  

(3)

\[
\lambda(a, x) = \mu \frac{p(a, x)}{\pi(x)}
\]  

(4)

The distributions, in turn, can be estimated from the observations of the position of an animal and spikes emitted at those positions using the KDE.

KDE is a non-parametric way to estimate a probability density function of a random variable:

\[
f(x) = \frac{1}{n} \sum_{i=1}^{n} K_h(x - x(i))
\]  

(5)

where \(x(1), ..., x(n)\) is the data sample, \(h\) is the kernel width and \(K\) is the kernel density estimator, which in the original method, as well as in our adaptation of it, is a Gaussian kernel:

\[
K_h(x) = \exp \left( -\frac{1}{2h^2} x^2 \right)
\]  

(6)

Using this method, the distributions above are calculated as follows:

\[
p(a, x) = \frac{1}{N} \sum_{n=1}^{N} K_h(a - \hat{a}_n)
\]  

(7)

\[
p(x) = \frac{1}{N} \sum_{n=1}^{N} K_h(x - \bar{x}_n)
\]  

(8)

\[
\pi(x) = \frac{1}{R} \sum_{r=1}^{R} K_h(x - \bar{x}_r)
\]  

(9)
where \( N \) is the total number of observed spikes, \( R \) is the total number of observed position samples, \( H_x \) and \( H_a \) are kernel width parameters, \( \{\hat{a}_i\}_{i=1}^N \) are observed spike waveshape features and \( \{\hat{x}_{i\in R,x}\} \) are the observed animal positions.

Probability distributions \( p(x) \) and \( \pi(x) \) as well as marginal rates \( \lambda(x) \) were pre-computed immediately before the sleep session. Then, during the real-time decoding, for every spike in the detected HSE, the probabilities \( p(a,x) \) and the generalized rates \( \lambda(a,x) \) were computed. Finally, conditional probabilities \( P(a=m|x) \) and conditional likelihoods \( P(x_t|a=m) \) were calculated. The position with the highest likelihood was considered to be encoded in the HSE. A 1D models based on linearized trajectories were used to make real-time decoding as efficient as possible. We linearized trajectories by projecting 2D trajectory points to a straight line that was the best fit (in the sense of least-squares) to the actual trajectories.

The decoding confidence was estimated by calculating the difference between two maximum likelihoods in the two environments:

\[
C(a_1,m) = \max_{x \in E_1} P(x_t|a_1,m) - \max_{x \in E_2} P(x_t|a_1,m)
\]

where \( E_1 \) is the set of positions from the first environment, and \( E_2 \) is the set of positions in the second environment.

We aimed at disrupting all the events except for the top most confident ones (10%--25%), ones encoding the control environment. The initial confidence threshold was set based on the distribution of confidences in the pre-rest HSEs and was adaptively adjusted every three minutes based on the actual percentage of disrupted events to enforce the overall detection rate follows the preset confidence levels. In the post hoc analysis, the same procedure was used to identify those HSEs that confidently encoded target environments but, in this case, taking those events that most confidently encoded the target environment.

Decoding performance
Two measures evaluated the performance of the decoding model: median value of a decoding error, which is the distance between a decoded position and the real position of an animal, and binary decoding accuracy, which shows how often the method correctly decoded one of the two environments (Figures S2D--S2F). The effective reaction time of the closed-loop system was defined as the time interval between an occurrence of the first spike with which the synchrony threshold was exceeded and occurrence of significant deflection in the LFP caused by the light pulse. According to this measure, the reaction time was 1.04 ms ± 0.09 ms (mean ± SD, \( n = 21 \) sessions). This time includes data acquisition, spike detection, feature extraction, and environment decoding.

QUANTIFICATION AND STATISTICAL ANALYSIS

Local Field Potential (LFP) analysis
SWR detection was performed as previously described (Csicsvari et al., 1999). Local field potentials were band-pass filtered (150–250 Hz), and a reference signal (from a channel that did not contain ripple oscillations) was subtracted to eliminate common-mode noise (such as muscle artifacts). The power (root mean square) of the filtered signal was calculated for each electrode and summed across electrodes designated as being in the CA1 pyramidal cell layer. The SWR detection threshold (6 SD above baseline) was always set in the pre-rest session, and the same threshold used throughout.

To calculate the power of theta and delta oscillations, the raw signal from an electrode designated as being in the CA1 area was band-pass filtered (2–4 Hz for delta, 6–10 Hz for theta) and the root mean square of the filtered signal was calculated in the 60 s intervals.

Spike sorting
For the post hoc analysis of unit activity previously recorded data continuously sampled at 24 kHz was processed offline. The spike detection in the local field potential and sorting was performed similarly as previously described (Csicsvari et al., 1999). Action potentials were extracted by first computing power in the 800–9000 Hz range within a sliding window (12.8 ms). Action potentials with a power of > 6 SD from the baseline mean were selected and spike features were then extracted by using principal components analyses. The detected action potentials were then segregated into putative multiple single units by using manual clustering software. Only units with clear refractory periods in their autocorrelation and well-defined cluster boundaries were used for further analysis. We further confirmed the quality of cluster separation by calculating the Mahalanobis distance (Harris et al., 2001) between each pair of clusters. Periods of waking spatial exploration, immobility, and sleep were clustered together and the stability of the isolated clusters was examined by visual inspection of the extracted features of the clusters over time. Pyramidal cells and interneurons in the CA1 region were discriminated by their autocorrelations, firing rate (average 0.1–5 Hz) and waveforms, as previously described (Csicsvari et al., 1999). In this way, we were able to identify the activity of 2578 CA1 pyramidal units and 67 interneurons.

Unit classification according to light response and environmental firing preference
By light responses: to classify the light response of individual units, their firing was assessed during the light-pulse session through the application of brief (100 ms, at 1 Hz) laser pulses. Neurons in the ‘inhibited’ group exhibited a firing rate reduction of at least 25% during illumination, whereas ‘disinhibited’ neurons showed an increase in firing rate above 50% relative to their baseline rates. According to these criteria, the activity of \( n = 455 \) pyramidal cells and \( n = 59 \) interneurons was suppressed by light, and \( n = 845 \) pyramidal cells were disinhibited during illumination.
By environmental firing preference: units were classified as preferentially firing in the control environment (‘control-environment encoding’) if their mean firing rate was higher in the control environment than in the target environment and vice versa. In addition, units that satisfied coherence and sparsity criteria (see Spatial firing rate maps section) only in one of the environments were classified as encoding the corresponding environment.

Histology and reconstruction of recording positions
The rats were euthanized with an overdose of pentobarbital and were transcardially perfused with 0.9% saline (wt/vol) followed by 4% formaldehyde (wt/vol). The brains were extracted and stored in 4% formaldehyde. At least 24 h later, the brains were quickly frozen, cut in sagittal sections (100 μm) using a microtome and mounted. ArchT-GFP expression in dorsal CA1 was verified in each animal by checking fluorescence of the green fluorescent protein (GFP) tag.

Behavioral performance
The number of crossings of the goal area was defined as the number of entrances into a 10 cm diameter circle centered on the learned bait locations. Dwell time was defined as the proportion of probe time spent in the goal area. Normalized path length to reach the goal in the post-learning session was defined as a ratio of the length of the actual trajectory taken by the animal to reach the goal and the shortest possible path, i.e., a straight line between the first tracked location of the animal after leaving the start box and the goal location. Learning curves were calculated based on the distance traveled to retrieve the reward for each trial. Since the baseline time and distance changed daily, we subtracted the shortest possible trajectory from the learning curves and then averaged them point-wise to compute an overall mean learning curve.

Additionally, for each of the aforementioned behavioral performance measures, we calculated a discrimination score of preferential disruption of recall in the target environment in the following form:

$$RC = \frac{PM_C - PM_T}{PM_C + PM_T}$$

where PM_C and PM_T are the behavioral performance measure in the control and target environments correspondingly. The values of the normalized path length were subjected to a log transformation, as the transformed values were more normally distributed according to the Shapiro test. Values of discrimination score for the normalized path length were negated to have a positive value indicating better performance in the control environment that in the target and vice versa.

Z-scoring of rates
In some analyses, to achieve equal contribution of every cell’s rate change, the firing rates were z-scored. The z-score was calculated for each cell separately, taking their overall firing during the rest session, so the z-score indicates how many SD above the baseline mean a cell fired during HSEs.

Occupancy maps
Position estimates were based on tracking the middle positions between LEDs on the headstage.

The x–y plane of the cheeseboard was divided into bins of 6x6 cm² and occupancy-maps were calculated during exploratory epochs (speed > 5 cm/s) measuring the amount of time spent in each spatial bin by the animal, based on the tracking data. The number of periods (τ = 20 ms) spent in each bin were counted using a Gaussian kernel $K(x)$ with a bandwidth of $\sigma = 10$ cm and centered at the center of each spatial bin:

$$occ(x_0) = \sum_x \tau K \left( \frac{x_0 - x}{\sigma} \right)$$

where iteration is over all observed positions $x$.

Poisson simulations of place fields
To assure that the change observed in the rate maps was not due to behavioral differences, we used Poisson simulation of place cell firing along the trajectories, recorded at the end of post-learning sessions (last five trials) using firing rate maps of end-of-learning and first and last five trials of the post-learning. First, the expected number of spikes in 20 ms window (i.e., for every sampled position) was calculated based on the assumed position and rate map of the corresponding session and environment. Then the simulated number of spikes was randomly generated based on the assumption of the Poisson distribution of numbers of spikes. This procedure was repeated 200 times for each cell.

Spatial firing rate maps
The x–y plane of the cheeseboard was divided into bins of 6x6 cm and rate-maps were calculated during exploratory epochs (speed > 5 cm/s) by dividing the number of spikes recorded in each bin by the occupancy and then smoothing with a Gaussian filter with a standard deviation (SD) of 10 cm. Bins with less than 250 ms occupancy time were not considered. The number of spikes in each bin
was counted using a Gaussian kernel with a bandwidth of 10 cm. The peak rate was defined as the rate in the bin with the highest rate in the firing rate map. Only neurons satisfying place cell criteria (maximum sparsity of 0.3, minimum coherence of 0.5 (Skaggs et al., 1996)) in learning and/or post-learning sessions were used for place field similarity analyses.

**Cofiring correlations**
We used cofiring correlations as a measure of reactivation. Cofiring of pairs of cells was calculated as the correlation of vectors of their spike numbers in a given session (or a time interval group defined by the time of HSEs encoded the control and target environments) in windows of 100 ms. The co-firing correlation was calculated as the correlation of vectors of pairwise co-firings.

**Statistical analyses**
For analysis of place field similarity only cells with rates falling in the 0.5 Hz-5 Hz range were used. In bar plots, mean ± SEM is shown. Statistical analyses were done in Python using the scipy package for scientific computing (https://www.scipy.org). All tests reported were done two-sided. As the data was typically not normally distributed, we used non-parametric tests. For paired comparisons Wilcoxon signed-rank test was used, while Mann-Whitney U test was used for unpaired data. P values and statistical tests for all experiments were reported in the appropriate figure legends and/or text. No blinding was done for data analysis.

**DATA AND CODE AVAILABILITY**
Data used in this study will be made available upon request by contacting the Lead Contact, Jozsef Csicsvari (jozsef.csicsvari@ist.ac.at). The real-time decoding software is available online under https://github.com/igridchyn/lfp_online.
Supplemental Information

Assembly-Specific Disruption of Hippocampal Replay Leads to Selective Memory Deficit

Igor Gridchyn, Philipp Schoenenberger, Joseph O'Neill, and Jozsef Csicsvari
Figure S1. Optogenetic disruption of CA1 network activity, Related to Figure 1

(A) ArchT-GFP expression in the dorsal CA1 area of a virus-injected rat. A sagittal section is shown. Scale bar denotes 200 µm.
(B) Proportion of identified pyramidal cells exhibiting inhibitory or disinhibitory responses and the remaining ‘unaffected’ cells.
(C) Pyramidal light response distribution calculated as of the ratio of rates in 100 ms windows before and during the light application. The vertical dashed line indicates no change in rate during light application.
Figure S2. Firing rates across sessions and environments and performance of the online decoding algorithm, Related to Figure 1

(A) Average firing rates of interneurons and pyramidal cells in light pulses and the pre-rest session. Light-pulse vs. pre-rest: pyramidal cells ****P<10^{-5}, interneurons: n.s. P>0.19, Wilcoxon signed-rank test.

(B) Excitation/inhibition rate in the light pulses and pre-rest session was not significantly different (P>0.49, Wilcoxon signed-rank test).

(C) Average firing rates of pyramidal cells in the control and target environments during end-of-learning and in probe and post-learning sessions. Rates in control environment vs. rates in target environment: all P>0.15, the Mann-Whitney U test.

(D) The performance of the online decoding algorithm detecting the location of the animal during the learning trials in a representative session. The shown probability distribution demonstrates the relationship between actual animal position and the decoded position in the two cheeseboards. Linearized positions from that start box to the goal were used for decoding.

(E) Median decoding error at different recording sessions. Different colors mark different animals. Error bars: 95% CI. Performance of the decoding was evaluated on the last five trials of the learning session using cross-validation: the model was built using 100 ms windows with odd numbers, while evaluation was performed using the 100 ms windows with even numbers.

(F) Classification accuracy measuring the probability of the algorithm choosing the correct environment at a decoding time window during the waking session. Data are represented as mean ± SEM unless stated otherwise.
Figure S3. Properties of detection and decoding of HSEs and correlation of rest-state parameters with behavior, Related to Figures 2 and 3

(A) Proportion of HSE that was detected during non-theta stages of the rest session.
(B) Cross-correlation of HSE detection times with SWR detection times (mean ± SEM).
(C) Correlation of physiological characteristics of the rest session (non-theta periods, the delta band power during it, number of the HSEs or the disrupted HSEs and the number of SWR) with behavioral score in control and target environment and the discrimination score of preferential
disruption of recall in the target environment. Each cell of the table contains coefficient of 
correlation (reflected in the background color of the cell), confidence interval (for significance 
levels corresponding to the multiple comparisons correction) and p-value (corrected for multiple 
comparisons with Holm correction). 
(D) Number of HSE in 15 minute intervals of the rest session confidently encoding the control or 
target environment (mean ± SEM). 
(E) Maximum probability of the spatial bin decoded from the HSE detection window averaged 
over 3 min intervals. 
(F) Firing rates of control and target environment-encoding cells in the rest session averaged 
over 15 minute intervals (mean ± SEM).
Figure S4. Impaired recall in the target cheeseboard environment following the assembly-specific disruption, Related to Figure 3

(A-C). Behavioral traces from three representative behavioral sessions. Blue dot represents the goal location in the control environment; red dot represents a goal location in the target environment.
**Figure S5. Correlation of firing rates during and after the HSE detection**, Related to Figures 4 and 5

Correlation of firing rates (mean ± 95% CI) during and after the HSE detection time window for cells with firing preference for the control and target environments. Control vs. target-decoded HSE correlations: ****P<10⁻⁴, Z-test. The diagram on the top illustrates the time windows before after the HSE detection and a corresponding SWR. Raster plots on the bottom show the expected firing of control and target environment-encoding cells in the respective conditions.
A 1st trial vs. end-of-learning

Place field similarity, r

- **Control**
- **Target**

<table>
<thead>
<tr>
<th>All</th>
<th>Inhibited</th>
<th>Disinhibited</th>
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B 6th trial vs. end-of-learning

Place field similarity, r

- **Control**
- **Target**

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C Comparison of control and target maps trials 8-9 vs. trials 10-11

Place field similarity, r

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D End-of-learning vs. 1st trial of post-learning, simulated place fields

Place field similarity, r

- **Control**
- **Target**

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E End-of-learning vs. last 5 trials of post-learning, simulated place fields

Place field similarity, r

- **Control**
- **Target**

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Figure S6. Place cells remap their place fields after the beginning of learning but remain stable in the end-of-learning trials, Related to Figure 6

(A) Place field similarity comparing the first learning trial with the end-of-learning. Place field similarity was measured for all place cells (all) but inhibited and disinhibited cells were also analyzed separately. Control cells vs. target cells, n.s. P>0.2, Mann-Whitney U tests.

(B) Place field similarity comparing the sixth learning trial with the remaining learning trials during the end-of-learning. Place field similarity was measured for all place cells (all) but inhibited and disinhibited cells were also analyzed separately. Control cells vs. target cells, n.s.: all P>0.14, Mann-Whitney U tests.

(C) Comparison of environment maps in the learning session, trials 8-9 vs. trials 10-11 for the same (i.e. control environment in trials 8-9 vs. control environment in trials 10-11 and target environment in trials 8-9 vs. target environment in trials 10-11) and different (i.e. control environment in trials 8-9 vs. target environment in trials 10-11 and target environment in trials 8-9 vs. control environment in trials 10-11) environments. For comparing maps of the different environments, the maps were rotated to align positions of the start boxes and cheeseboards. ****P < 10^-6, Mann-Whitney U test.

(D) Place field similarity comparing the end-of-learning with the simulated place fields of the post-probe for the first trial of post-learning. The spatial firing probabilities seen in the first trial was used to simulate the firing of cells on the late (i.e. last five) trial tracks and the fields were recalculated from the simulated firing. Place field similarity was measured for all place cells (all) but inhibited and disinhibited cells were also analyzed separately. 200 place fields were simulated for every session and environment and comparison of all possible pairs was performed. Significance was defined with binomial test from proportion of pairwise comparisons, showing significant difference with the Mann-Whitney U test. Control cells vs. target cells, ****P<10^-5, binomial test.

(E) Place field similarity comparing the end-of-learning with the simulated place fields of the post-probe for the last five trials of post-learning. The spatial firing probabilities seen in the last five trials was used to simulate the firing of cells on the late (i.e. last five) trial tracks and the fields were recalculated from the simulated firing. Place field similarity was measured for all place cells (all) but inhibited and disinhibited cells were also analyzed separately. 200 place fields were simulated for every session and environment and comparison of all possible pairs was performed. Significance was defined with binomial test from proportion of pairwise comparisons, showing significant difference with the Mann-Whitney U test. Control cells vs. target cells, n.s. P>0.99, binomial test.

Data are represented as mean ± SEM.