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The reorganization and reactivation of hippocampal maps predict spatial memory performance

David Dupret, Joseph O'Neill, Barty Pleydell-Bouverie & Jozsef Csicsvari

The hippocampus is an important brain circuit for spatial memory and the spatially selective spiking of hippocampal neuronal assemblies is thought to provide a mnemonic representation of space. We found that remembering newly learnt goal locations required NMDA receptor–dependent stabilization and enhanced reactivation of goal-related hippocampal assemblies. During spatial learning, place-related firing patterns in the CA1, but not CA3, region of the rat hippocampus were reorganized to represent new goal locations. Such reorganization did not occur when goals were marked by visual cues. The stabilization and successful retrieval of these newly acquired CA1 representations of behaviorally relevant places was NMDAR dependent and necessary for subsequent memory retention performance. Goal-related assembly patterns associated with sharp wave/ripple network oscillations, during both learning and subsequent rest periods, predicted memory performance. Together, these results suggest that the reorganization and reactivation of assembly firing patterns in the hippocampus represent the formation and expression of new spatial memory traces.

The hippocampus is important for spatial memory[1–3], which is essential for an animal to learn and remember behaviorally relevant places such as the location of food. In fact, the hippocampus is implicated in all stages of spatial memory processing, including acquisition, consolidation and recall[1,3,4]. It is thought that, during acquisition, memory traces are encoded by the collective activity of neurons that represent the information to be remembered[1,5–8]. During subsequent recall, successful retrieval of such information is thought to depend on the reinstatement of memory trace activity patterns. However, initially encoded memory traces are labile and vulnerable to interference, only becoming stable through consolidation[5,9,10]. Therefore, acquisition-associated activity patterns must first be stabilized during memory trace consolidation if they are to be reinstated to support later memory-related behavior[5,9,10].

Hippocampal principal cells, called place cells, fire in specific regions of the environment (place fields) during active waking periods. The joint activity of these place cells is thought to provide an allocentric representation of space, which forms a framework for the representation of spatial memory[1,11–13]. Consistent with this role in spatial memory, place representations of the environment are not uniform: many place cells fire preferentially at goal locations when animals perform goal-directed tasks[14–16]. Such over-representation of salient places by place cells might derive from a reorganization of firing patterns as part of memory trace encoding during learning. However, it has not been shown that place cells have a direct role in encoding memory traces. Alternative explanations are also possible: goal-related firing could arise as a result of 'non-cognitive' factors, such as the presence of reward or the use of goal-oriented stereotyped behavior. Therefore, it has yet to be determined whether hippocampal representations of goal locations are acquired as a direct result of learning[17]. In addition, it is unclear whether the reinstatement of newly formed hippocampal representations is required for successful memory recall.

Consolidation of memory traces is thought to be promoted during sleep and inactive waking periods[7,9,10,18,19] and manipulations that enhance sleep-related brain activity by reinstating the contextual cues experienced during learning improve the subsequent retention of a hippocampus-dependent task[20]. During slow-wave sleep and waking immobility, the most dominant oscillatory patterns of hippocampal network activity are the intermittent sharp wave/ripple events (SWRs, 150–250 Hz)[1,21–23]. These SWRs have been linked to spatial learning, as their partial disruption leads to behavioral impairments[24,25]. During SWRs, many hippocampal pyramidal cells fire synchronously. Moreover, these firing patterns are non-random, and resemble those observed in the previous active waking period[26–28]. This 'reactivation' of waking patterns during SWRs is believed to underlie system-level memory consolidation by replaying waking firing patterns during off-line immobility/sleep rest periods in order to stabilize memory traces[29,30]. However, it has not been demonstrated that reactivated firing patterns represent memory traces. This would require proof that reactivation of waking patterns reflects what is subsequently remembered by the animal, as expressed by behavioral performance in a memory task.

In this study we aimed to determine whether new place representations are acquired as a result of spatial learning and to test whether their reactivation and stability are associated with subsequent memory performance. To do so, we recorded hippocampal network activity during the acquisition, consolidation and recall of a spatial memory task. To test how hippocampal network activity is altered during memory impairment, we blocked NMDA receptors

MRC Anatomical Neuropharmacology Unit, Department of Pharmacology, University of Oxford, Oxford, UK. Correspondence should be addressed to J.C. (jozsef.csicsvari@pharm.ox.ac.uk) or D.D. (david.dupret@pharm.ox.ac.uk).

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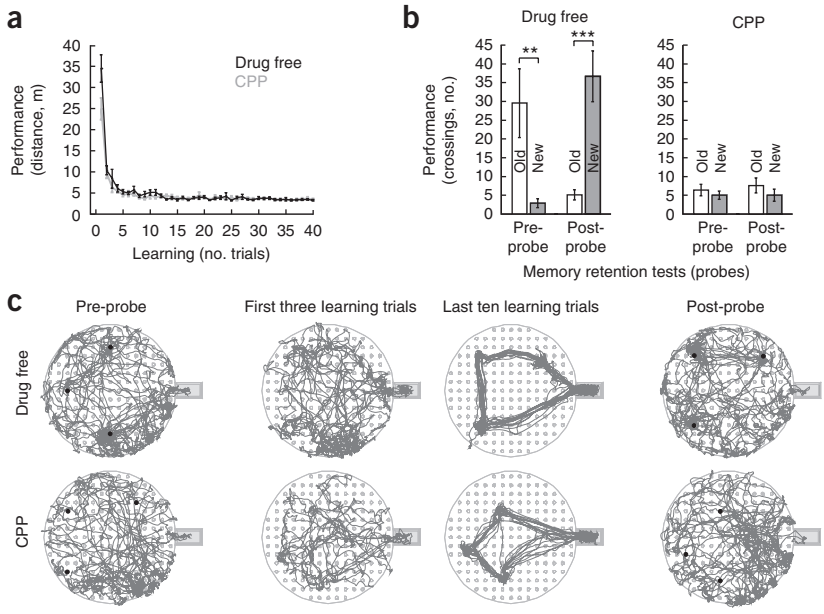

Figure 1 Daily learning of a new set of goal locations on the cheeseboard maze. Rats from the drug-free and CPP-treated conditions were trained in a matching-to-multiple-places task to locate a new set of three hidden food rewards every day on a cheeseboard maze (see **Supplementary Fig. 1** and Online Methods). (**a**) Learning performance was estimated by the distance traveled to find all rewards per trial (means ± s.e.m., all P values <0.00001, ANOVA). (**b**) Memory retention performance was estimated by the number of crossings in goal areas (means ± s.e.m., $^{**}P < 0.01$, $^{***}P < 0.001$, paired t-test; see Online Methods and **Supplementary Fig. 2**). Crossings were compared for goal locations learnt the day before (Old) and the current day (New). (**c**) Representative examples of an animal's path; for clarity, only the first 10 min of each probe session is depicted (black dots, learnt goal locations).

(NMDARs), which are essential for spatial memory[3,17,31–34], and assessed hippocampal network changes. We found that, during acquisition, firing patterns of place cell assemblies were reorganized to represent newly learnt goal locations, and that these new representations reemerged during subsequent memory recall. However, we found no such goal-related reorganization when goal locations were marked by visual cues. During consolidation, the SWR-associated reactivation of these newly acquired representations of goal locations predicted memory performance. These results support the hypothesis that assembly firing patterns in the hippocampus represent the formation and expression of spatial memory traces.

RESULTS

Goal-related reorganization of hippocampal firing patterns

We developed a spatial memory task in which rats learned and subsequently recalled the locations of three hidden food rewards on a cheeseboard maze (**Fig. 1**, **Supplementary Fig. 1** and **Supplementary Methods**). The learning session included 40 trials during which animals had to retrieve all hidden rewards before returning to the start-box to collect an additional reward (**Fig. 1a**). To prevent the use of odor cues, we scattered food dust across the maze and rotated the board relative to the start-box (Online Methods). The procedure required daily memory updates of goal locations because a new set of bait locations was introduced every day. We assessed the animals' memory performance by the number of crossings (**Fig. 1b**) and the time spent (**Supplementary Fig. 2**) at goal areas (in a 10-cm-diameter circle around the learnt bait locations) during the probe sessions in which rewards were not provided. These probe sessions were performed 2 h after each daily learning session (post-learning probe or 'post-probe') and, on the following day, before the new learning session (pre-learning probe or 'pre-probe'). Pre-probe sessions also served as a control: they were compared with post-probe sessions performed on the same day to assess changes to hippocampal network activity after of the animals learned new bait locations.

The learning performance of animals improved rapidly on each day: the distance traveled to complete a trial was >50% lower after the first trial than before it, and reached an asymptotic level within a few trials, showing that rats rapidly encoded and remembered the new bait locations for the remaining trials (drug-free; **Fig. 1a**). Rats also remembered these bait locations in the subsequent post-probe session: they visited the newly learnt bait locations more frequently than those learnt the day before (drug-free post-probe, $P = 0.0003$; **Fig. 1b** and **Supplementary Fig. 2**). These locations were remembered on the following day, as assessed during the next day's pre-probe session (drug-free pre-probe, $P = 0.010$; **Fig. 1b** and **Supplementary Fig. 2**).

To investigate how spatial memories for new goal locations are represented in the hippocampus during this task, we recorded the activity of multiple place cells and oscillatory field potential patterns using multichannel extracellular techniques[13,35]. The hippocampal representation of goal locations was quantified as the proportion of cells with a place field center within a goal area (the place field center area was defined at pixels where the firing rate was >80% of the peak rate; goal area was defined as a 10-cm circular region around the food well; **Fig. 2** and Online Methods). Consistent with previous studies[14–16], we detected goal-related changes to hippocampal place maps (referred to as goal-oriented remapping). However, this was region-specific: more CA1 cells represented newly learnt locations in the probe session after learning than in the one before, whereas CA3 representations did not change (drug-free; **Fig. 2a–c** and **Supplementary Figs. 3** and **4**). The exclusion of SWR-related spiking activity did not change these results (CA1: pre-probe = 0.090 ± 0.015, post-probe = 0.203 ± 0.010, $P < 0.0001$; CA3: pre-probe = 0.099 ± 0.025, post-probe = 0.112 ± 0.029, $P > 0.698$; paired t-test). Moreover, the proportion of place cells that represented the start-box did not significantly change (CA1: pre-probe = 0.082 ± 0.020, post-probe = 0.115 ± 0.016, $P > 0.263$; CA3: pre-probe = 0.077 ± 0.037, post-probe = 0.080 ± 0.028, $P > 0.947$; paired t-test). A population vector analysis[36] used to quantify the similarity of place-related assembly patterns (see Online Methods), showed that the CA1 population similarity score between probe sessions was lower than the baseline score calculated within sessions, whereas these scores remained similar in CA3 (drug-free; **Fig. 2f**). Therefore, CA1 place-related assemblies that were present during probe sessions reorganized after learning, whereas CA3 assemblies remained stable.

We then tested whether the CA1 goal representation we observed during the post-probe session was established during learning *per se*. We detected learning-related reorganization of CA1, but not CA3, firing patterns: the proportion of CA1 place cells that represented goal locations increased gradually over trials (**Fig. 2d**, drug-free CA1 with

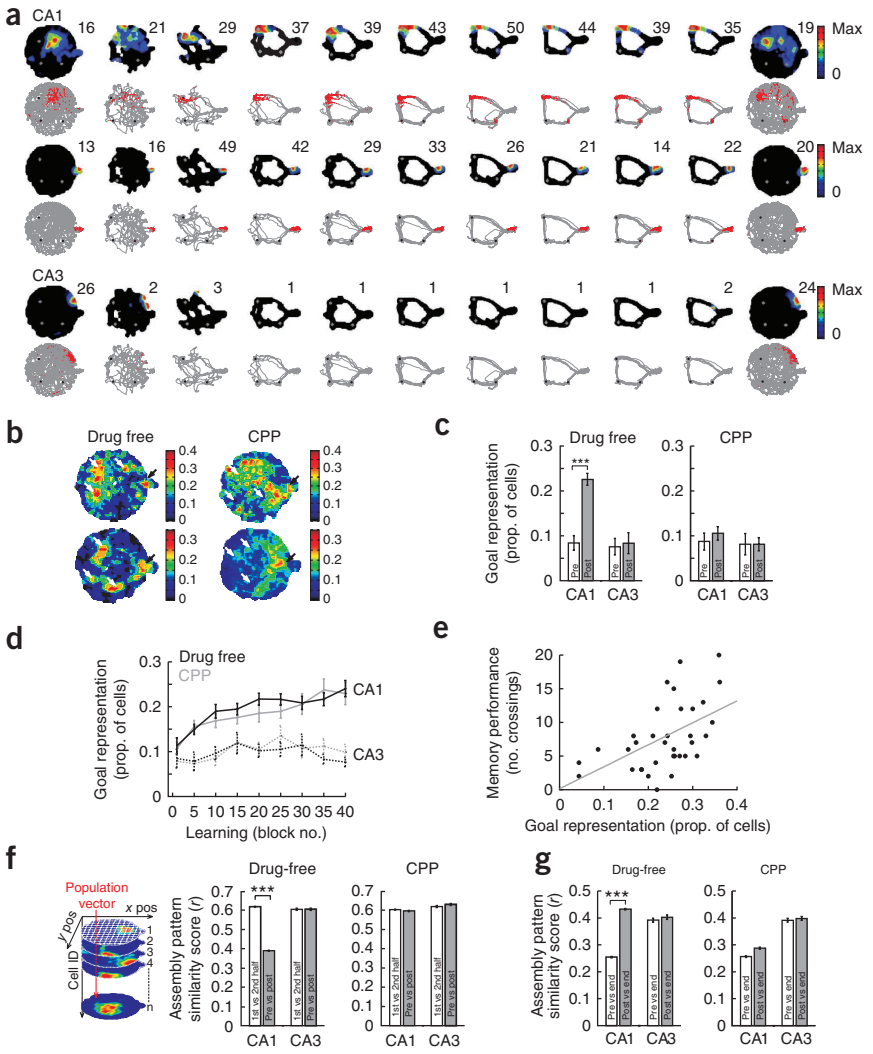

Figure 2 Goal-related reorganization of hippocampal assembly patterns. (**a**) Examples of hippocampal place cells recorded in untreated rats. Top rows, color-coded place-rate maps; bottom rows, individual spike locations superimposed on the animal's path (from left to right: pre-probe, consecutive blocks of learning trials, post-probe; see **Supplementary Figs. 3**, **6** and **7**). The upper CA1 cell reorganized its place field to a goal location (dots: goal locations) whereas the middle cell representing the start-box and bottom CA3 cell showed stable place fields across sessions. (**b**) Color-coded maps illustrating the post-probe spatial distribution of CA1 place fields in the drug-free and the CPP-treated conditions. Pixel color represents the proportion of cells with place fields that center at that *x*–*y* location (*z* scale, proportion of cells fire >80% of peak firing rate at that location). In the drug-free condition a higher proportion of cells was associated with goal locations (white arrows) and the start-box (black arrow), and bait locations were not equally represented. (**c**,**d**) Proportion of place cells representing bait locations (means ± s.e.m.; see Online Methods) during probe sessions (**c**) and across trials (**d**, CA1: solid lines, all *P* values <0.0001; CA3: dashed lines, all *P* values >0.291; ANOVA). The proportion of cells were calculated separately for each recording day and averaged. (**e**) Scatter plot showing post-probe memory performance (number of crossings) as a function of the proportion of CA1 place cells at goal locations during the end of learning (gray: regression line, $r = 0.511$, $P = 0.0014$). (**f**,**g**) Similarity score of place-related assembly patterns (means ± s.e.m.) determined using a population vectors analysis within probe (**f**, 1st versus 2nd half), between probes (**f**, pre versus post) and between each probe and end of learning (**g**, pre/post versus end). Left: schematic of the population vector analysis: rate maps were stacked into three-dimensional matrices for each waking period (the two spatial dimensions on the x and *y* axis, cell identity on the z axis); population vectors were calculated at each *x*–*y* bin; these were then correlated between periods and averaged across all bins (see Online Methods). Pre, pre-probe; post, post-probe; end, end of learning; ***$P < 0.00$, paired *t*-test.

$r = 0.370$, $P < 0.00001$; **Supplementary Figs. 3** and **4**). Moreover, CA1 assembly patterns recorded during the last ten learning trials (end of learning) were more similar to assembly patterns during the probe session after learning than during the one before (drug-free; **Fig. 2g** and **Supplementary Fig. 5**). Hence, CA1 goal representations developed gradually during learning, and those representations that were present at the end re-emerged in the subsequent probe session.

The improvement in performance during learning is reflected by the development of stereotyped paths. Therefore, the reorganization of CA1 place cells might occur as a consequence of animals altering their foraging trajectory, and not because such reorganization is required for spatial memory. Such reorganization could be explained also by the presence of a reward or by disproportionate dwell-time at reward locations. To test for these, we used a 'cued' version of the task in which food wells were visually marked by intra-maze cues, so that animals did not have to remember the locations to gain the reward (**Fig. 3** and **Supplementary Fig. 1**). During this cued learning, animals ate the same number of rewards, used similar stereotyped movement paths and spent a similar amount of time at goal locations as in the absence of cues (**Fig. 3a**,**c** and **Supplementary Fig. 2**). However, animals showed no spatial preference in subsequent probe sessions, indicating that they did not learn the visually guided locations (**Fig. 3b** and **Supplementary Fig. 2**). In these control experiments, we found that CA1 goal-oriented remapping did not take place, indicating that such reorganization occurs when a map-based strategy is used to locate hidden rewards (**Fig. 3d–h** and **Supplementary Figs. 4** and **6**).

Effect of NMDAR blockade

These results show that, in our task, new spatial memories were encoded by CA1 place maps that represented goal locations during learning, and that these goal-oriented maps were reinstated as stable representations alongside successful memory recall. Next, we tested whether the acquisition, stabilization and/or reinstatement of such goal-related firing patterns could be seen under conditions of memory impairment. Spatial memory requires NMDARs[3,31–34]. Therefore, we injected rats with the NMDAR antagonist CPP (3-((R)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid; see Online Methods) after the pre-probe session[32,37] to interfere with their spatial memory. The learning performance of CPP-treated animals improved rapidly (**Fig. 1a**) and was comparable to that of

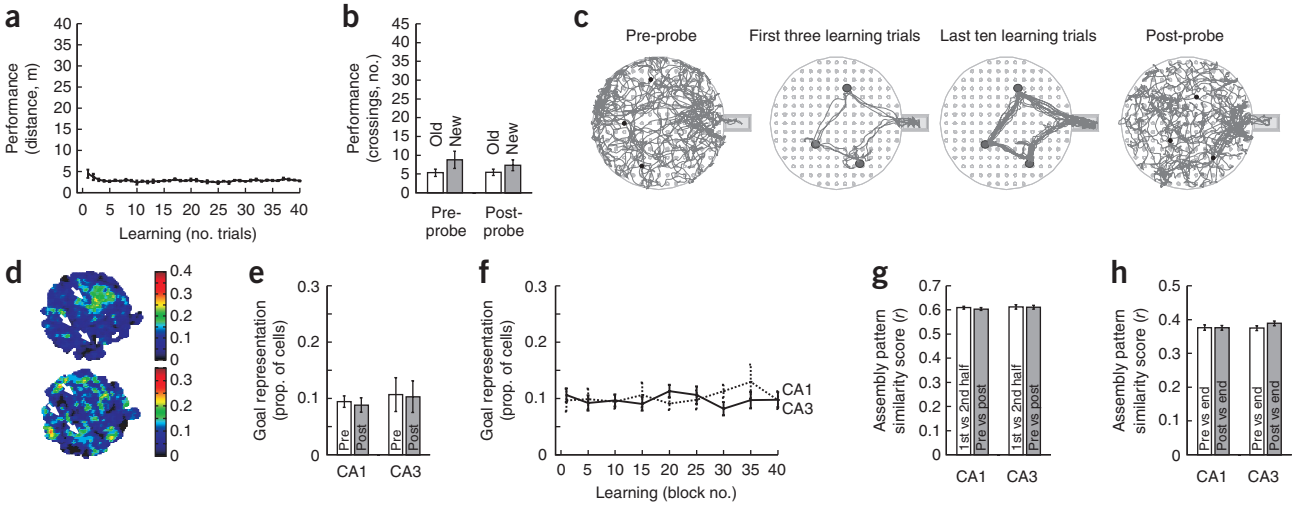

Figure 3 Locating rewards during the Cued version of the cheeseboard maze task. (**a**) Learning performance was estimated by the distance traveled to find all three rewards per trial (means ± s.e.m., $P > 0.209$, ANOVA). (**b**) Memory performance was estimated by the number of crossings in goal areas during probe sessions (means ± s.e.m.; see Online Methods and **Supplementary Fig. 2**). Crossings were compared for locations learnt the day before (Old) and the current day (New; all P values >0.185, paired t-test). (**c**) Representative examples of an animal's path (small black dots, goal locations; gray circles, intra-maze cues); for the probes, only the first 10 min is depicted for clarity. Note that animals followed similarly efficient movement paths during the cued learning as in the absence of intra-maze cues (**Fig. 1c**). (**d**) Color-coded maps illustrating the post-probe spatial distribution of CA1 place fields. Pixel color represents the proportion of cells with place fields that center at that x–y location (z scale, proportion of cells fire >80% of peak firing rate at that location). White arrows, learnt bait locations. (**e**,**f**) Proportion of place cells representing bait locations (means ± s.e.m.; see Online Methods) during probe sessions (**e**, pre-probe compared to post-probe: all P values >0.692, paired t-test) and across trials (**f**, CA1: solid line, CA3: dashed line, all P values >0.785, ANOVA). The proportion of cells was calculated separately for each recording day and averaged. Note that the proportion did not change during the cued learning. (**g**,**h**) Assembly patterns similarity score (means ± s.e.m.) determined using a population vector analysis (see Online Methods) within probe (**g**, 1st versus 2nd half), between probes (**g**, pre versus post; 1st versus 2nd half compared to pre versus post: all P values >0.401, paired t-test) and between each probe and end of learning (**h**, pre/post versus end; pre versus end compared to post versus end: all P values >0.122, paired t-test). Hippocampal assemblies remained similar in all three periods. pre, pre-probe; post, post-probe; end, end of learning.

untreated rats. However, CPP-treated animals subsequently failed to remember the newly learnt locations in both probes when there was at least a 2-h gap between learning and recall (all P values > 0.339; **Fig. 1b** and **Supplementary Fig. 2**, see **Supplementary Fig. 7** for shorter delays).

In association with impaired memory for goal locations, we found that NMDAR blockade prevented the stabilization of hippocampal maps that represented those locations. During learning, the proportion of CA1 place cells with goal-related firing increased gradually (CPP CA1 with $r = 0.334$, $P < 0.00001$; **Fig. 2d** and **Supplementary Figs. 4** and **7**), as in the drug-free condition. However, in CPP-treated rats the similarity between reorganized assembly patterns at the end of learning and those in the following probe session (**Fig. 2g**, CA1: $P < 0.0001$, paired t-test) was reduced in comparison to untreated rats. Moreover, assembly patterns remained similar across probe sessions (**Fig. 2f**), as did the proportion of place cells with goal-related firing (**Fig. 2c** and **Supplementary Figs. 4** and **5**). Therefore, goal-related CA1 place cell representations developed under NMDAR blockade during learning, in conjunction with animals' learning of goal locations. However, these newly acquired representations did not stabilize, in line with the fact that animals did not remember goal locations during subsequent probe sessions.

To test whether place cell representations are related to memory traces, we investigated whether representations of goal locations predict spatial memory performance. We found that the presence of goal-oriented hippocampal maps during learning predicted the animal's future memory performance, as did the maintenance of these maps in the probe session: memory performance, as estimated by the number of crossings in goal areas, was correlated with the proportion of CA1 place cells that represented goal locations at the end of learning ($r = 0.511$, $P = 0.0014$; **Fig. 2e**) and in the following probe session (**Supplementary Fig. 8**) but not at the beginning of learning nor in the probe session before (all P values > 0.131). This was not the case in either the CPP-treated or the Cued condition (all P values > 0.137). These results show that NMDAR-dependent mechanisms are important for the stabilization of hippocampal goal-related firing patterns and for the recall of associated memories.

Exploratory SWR network responses during learning

In the hippocampus, SWR events have been suggested to help to stabilize memory traces[7,21,22,28,35]. Although SWR activity has been traditionally described during off-line sleep or rest (sSWRs) including periods of slow-wave sleep and long waking immobility, they are also found during on-line exploratory periods (eSWRs)[35]. These eSWRs have been suggested to strengthen place cell representations and therefore they might help to stabilize newly formed goal representations[35]. We tested whether eSWR network responses predict memory performance, and whether they are sensitive to NMDAR blockade during the on-line stage of memory trace formation and stabilization. During learning, eSWRs occurred at the bait locations (**Fig. 4a**,**b**). The number of goal-associated eSWRs was correlated with memory performance in the drug-free condition ($r = 0.524$, $P = 0.0010$), indicating that eSWRs were associated with on-line formation of memory traces. However, the number of SWRs that occurred during longer (>2.4 s) immobility periods (iSWRs)[35] during learning did not predict memory performance ($P > 0.722$). Moreover, neither eSWR nor iSWR numbers predicted memory performance in rats treated with CPP (all P values >0.410).

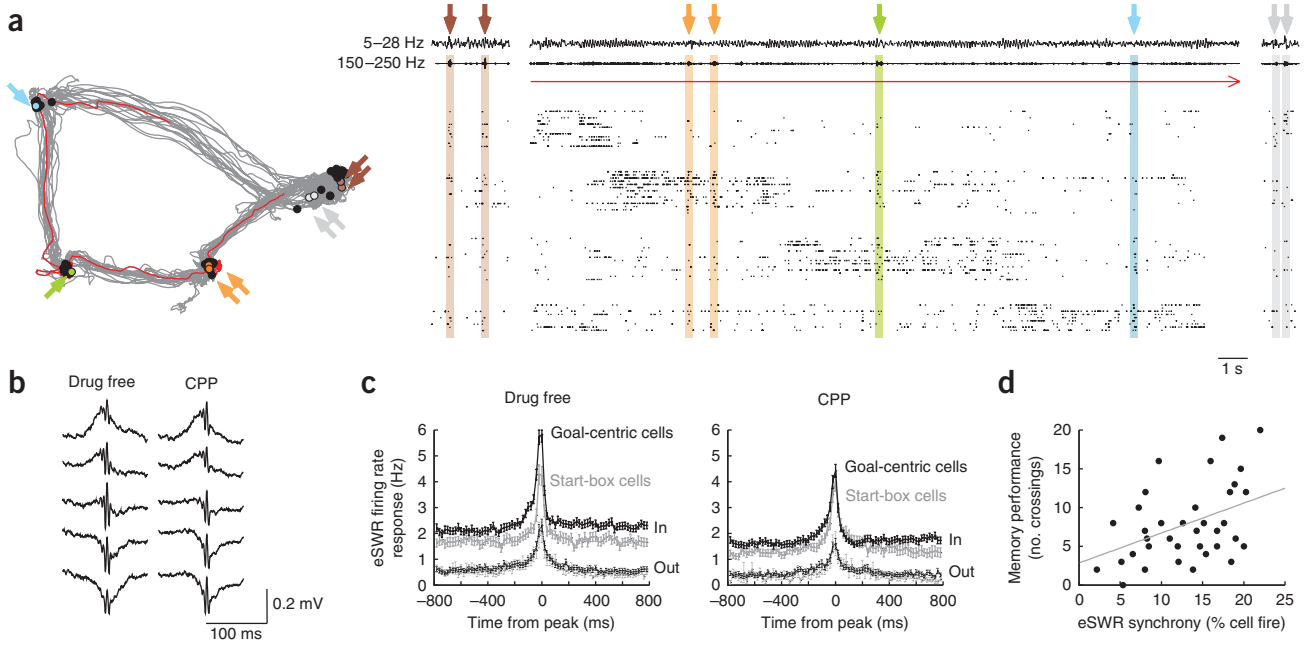

Figure 4 eSWR-associated activity of CA1 place cells. (**a**) Left, representative examples of a rat's path (gray lines) from the end of learning with eSWR locations superimposed (filled dots). Right, example of network response during a single trial (red track). Top traces, band-pass filtered (theta 5–28Hz and SWR 150–250Hz bands) local field potential. Raster plot, spike timing of simultaneously recorded CA1 pyramidal cells (one cell per row). The vertical tics indicate the action potential times of these cells. Note the spatial tuning of cells around bait locations and their eSWR firing response (arrows). (**b**) Traces of averaged eSWRs from the same rat in drug-free and CPP-treated conditions. (**c**) eSWR firing rate histograms (means ± s.e.m., see Online Methods) of CA1 'goal-centric' and 'start-box' cells inside (In) and outside (Out) their place fields in drug-free and CPP-treated conditions. (**d**) Scatter plot showing post-probe memory performance (number of crossings) as a function of 'eSWR synchrony' (percentage of CA1 pyramidal cells that fire in eSWR) at the end of learning (gray: regression line, $r = 0.418$, $P = 0.011$).

Ongoing place-related activity is supplemented by increased network activity during eSWRs, strengthening the synchronized firing of cells that encode the same location and consequently promoting synaptic plasticity amongst them[35]. As eSWRs tended to occur at reward locations, we checked whether cells that represented these places strengthened their firing synchronization during eSWRs. We compared the eSWR firing rate histograms of CA1 place cells that represented goal locations with those of cells that represented the start-box (referred to as 'goal-centric' and 'start-box' cells, respectively). In the drug-free condition, the infield firing rate of goal-centric cells during eSWRs was higher than that of start-box cells (5.81 ± 0.29 Hz versus 4.22 ± 0.36 Hz, $P < 0.002$; **Fig. 4c**). This difference was abolished under CPP treatment (4.42 ± 0.24 Hz versus 3.98 ± 0.35 Hz, $P > 0.319$) and was not found in the Cued condition (**Supplementary Fig. 9**). Moreover, the strength of eSWR network responses at the end of learning was associated with memory recall: network participation (that is, synchrony) of CA1 place cells in eSWRs was correlated with memory performance ($r = 0.418$, $P = 0.011$; **Fig. 4d**) even when controlled for the eSWR firing rate or the proportion of place cells at goal locations ($r = 0.448$, $P = 0.016$ and $r = 0.364$, $P = 0.023$, respectively; partial correlation). This was not the case under CPP treatment or in the Cued version (all P values >0.318).

Reactivation of waking firing patterns during rest

It has been proposed that sSWRs promote memory consolidation during off-line resting periods because waking activity patterns of place cells are reactivated during sSWRs[26–28,35,38]. To test whether reactivation occurs after spatial learning, we investigated whether cells that encode similar places during learning fire together in subsequent sSWRs[28]. The place field similarity (PFS) of cell pairs (measured for place fields at the end of learning) correlated significantly with their joint firing tendency (co-firing) during sSWRs (**Fig. 5a** and **Supplementary Figs. 10** and **11**). For both goal-centric and start-box cells, co-firing in sSWRs after learning correlated more strongly with PFS than did co-firing before learning (**Fig. 5a**). However, reactivation of goal locations was even stronger than the reactivation of the start-box location (drug-free; **Fig. 5a**). Although CPP application did not prevent reactivation *per se*, it abolished the enhanced reactivation of goal locations (CPP; **Fig. 5a** and **Supplementary Figs. 10** and **11**). The reactivation of goal locations was not enhanced in the Cued condition (**Supplementary Fig. 9**).

As CPP caused a memory deficit in our task, we hypothesized that conflicting representations were reactivated under CPP treatment. Because place representations reorganized during learning, we compared the reactivation of goal-related firing patterns that were present at the beginning and the end of the learning session. Under NMDAR blockade, but not in untreated rats, firing patterns from the beginning of learning were still reactivated strongly in the subsequent rest period (**Supplementary Fig. 12**). Thus, NMDAR blockade prevented the boosted reactivation of new goal-related patterns, allowing old, conflicting representations to be reactivated.

Finally, we tested whether reactivation of goal-related CA1 assembly patterns predicts memory performance. For each sSWR we calculated a 'reactivation map' to determine which locations were represented by the sSWR assembly pattern (see Online Methods). These reactivation maps quantified how similar the sSWR assembly pattern was to waking assembly patterns that represented different locations. Each pixel on these reactivation maps reflects the similarity of the activity pattern in the sSWR to the assembly activity pattern derived from the combined place-rate maps seen at that location during exploration.

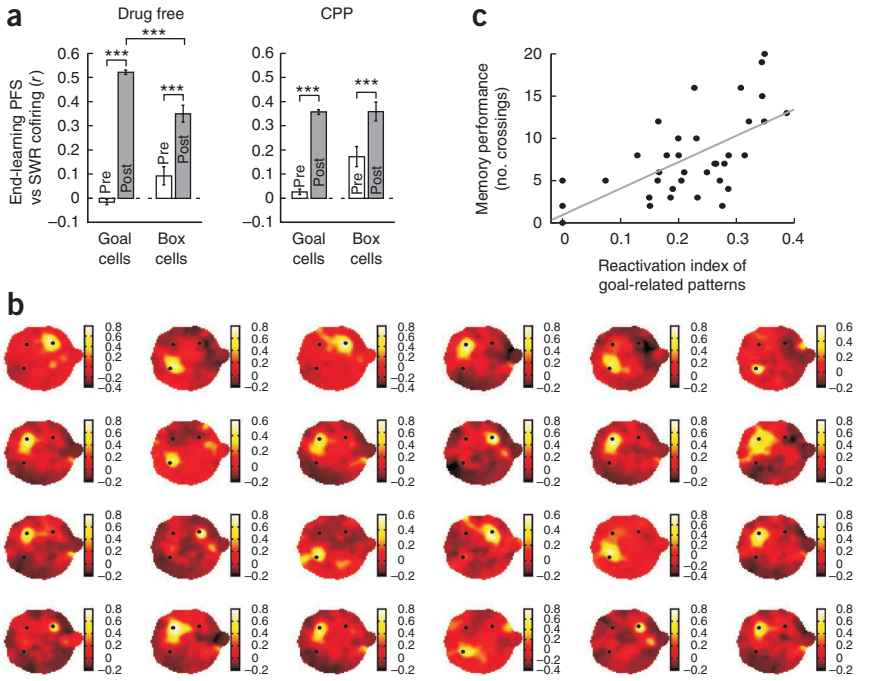

Figure 5 Reactivation of CA1 place-related assembly patterns. (**a**) Correlation between place field similarity (PFS) and sSWR co-firing calculated for 'goal-centric' and 'start-box' cell pairs (means ± s.e.m., $***P < 0.001$, paired *t*-test). The PFS was calculated using place fields established at the end of learning and sSWR co-firing was calculated in rest periods before (pre) and after (post) learning. Correlation coefficients represent the partial correlations of the PFS with the co-firing of one rest session, each controlled by the co-firing of the other rest session (see Online Methods). (**b**) Representative examples of individual sSWR reactivation maps (black dots: learnt goal locations). For each map, the pixel color represents the correlation coefficient between assembly firing patterns that occurred during a single sSWR and those representing that *x*–*y* location on the maze during the waking period (see Online Methods). Correlation coefficients are highest at one of the bait locations (*z* scale: correlation coefficient). (**c**) Scatter plot showing post-probe memory performance (number of crossings at a given goal location) as a function of the proportion of sSWRs in which assembly patterns represented the same goal location (gray: regression line, $r = 0.620$, $P = 0.00005$).

Thus, the peak of the reactivation map marked the location that was best represented by the sSWR population activity. We used rate maps either from the end of learning or from the following probe period to measure the similarity of assembly patterns (see Online Methods). For most sSWRs, the resulting reactivation maps highlighted one of the bait locations (**Fig. 5b**). Moreover, the proportion of sSWRs that represented goal locations predicted subsequent memory performance when reactivation maps were created using either end of learning ($r = 0.620$, $P = 0.00005$; **Fig. 5c**) or post-probe ($r = 0.362$, $P = 0.028$) representations. In contrast, this effect was not seen if sSWRs were taken from the rest session before learning (all values >0.193). This correlation was significant even when we controlled for the proportion of place cells at goal locations (end of learning: $r = 0.502$, $P = 0.0007$; post-probe: $r = 0.317$, $P = 0.038$; partial correlation). These relationships were not found under CPP treatment (all *P* values >0.412).

DISCUSSION

In this study we have investigated how hippocampal neuronal assemblies represent memory traces during the acquisition, consolidation and recall stages of a spatial memory task. We have shown that hippocampal neurons encoded newly learnt goal locations through the reorganization of assembly firing patterns in the CA1 region but not in CA3. Hence, CA1 hippocampal neurons represented mnemonic traces associated with the spatial memory task. We further showed that the stabilization of new CA1 assemblies that encode goal locations, and the successful retrieval of goal-associated spatial memories, both required NMDAR-dependent mechanisms. Finally, we provide evidence that SWRs can facilitate the strengthening of memory traces during both on-line and off-line periods. Our findings establish a predictive link between hippocampal network activities during memory trace formation and future memory performance.

Given that the hippocampus is necessary for spatial memory, the discovery of place selectivity in hippocampal principal cells has provided a framework within which changes to ensemble firing patterns might underlie the encoding of spatial memory traces[1]. This suggests that hippocampal firing patterns might map not only allocentric space but also the behavioral salience of certain locations. In support of this theory, it has been reported that many place cells fire at goal locations during goal-oriented tasks, indicating that salient locations are over-represented in the hippocampal code[15,16]. Similarly, there are indications that place cells reorganize to goal locations as a result of task demands, such as when the animal switches from random foraging to goal-directed behavior in a familiar environment[14]. Nonetheless, previous work has neither demonstrated a direct link between goal-related firing patterns and spatial learning of goal locations, nor established whether goal-related hippocampal maps are reinstated during memory recall. Persistent caveats include the suggestion that such goal-related firing could reflect the absolute presence of a reward, disproportionate dwell-time at those locations, or task-associated stereotypy of movement. Here we were able to exclude these caveats: reorganization of firing patterns occurred in the spatial, but not cued, version of the task even though the animal received rewards at the same locations and followed similar movement patterns in each. Moreover, hippocampal firing patterns related to the start-box were unchanged by learning, though this location was rewarded as well. The stability of the start-box-related firing patterns could be explained by the fact that the place-reward association for the start-box remained constant from day to day while it changed daily for the hidden rewards.

Our findings further show that newly acquired hippocampal representations re-emerge during the recall stage, and we provide evidence that such re-emergence is necessary for successful memory retrieval. Therefore, our results support the hypothesis that hippocampal assembly firing patterns represent the formation and expression of spatial memory traces. However, there is an alternative hypothesis in which task-dependent spatial attention drives goal-oriented remapping. Although this argument cannot be fully excluded, we expect that the reinstated goal-oriented maps during recall facilitate efficient navigation to goal locations, and therefore have functional consequences for spatial memory processes, even if the learning process itself involves attention (see **Supplementary Discussion**).

We showed that the reorganization of place cell firing and the associated network responses recorded during spatial learning of goal locations were not identical to those recorded after changes of entire environments while animals are engaged in simple spatial exploration. Although place cells in both CA1 and CA3 reorganize after exposure to a different environment[13,39,40], we found that reorganization of firing patterns associated with spatial learning did not take place in all hippocampal fields: it was present in the CA1 region but not in CA3. This suggests specialization within the hippocampus to solve spatial problems: whereas CA1 place representations are flexible, adapting to task requirements, CA3 representations are stable, providing invariant representations of the whole environment independent of task demand. Such stability of CA3 maps might be needed to maintain a reliable reference frame representing the familiar environment wherein new goal locations have to be located.

We further showed that the reorganization of CA1 firing patterns to new goal locations was gradual during spatial learning, spanning many trials on a single day of training, unlike the reorganization that occurs in newly encountered environments[13]. However, the enhanced reactivation of newly learnt locations reported here is similar to the enhanced reactivation of new representations formed in novel environments[28]. Moreover, the stabilization of new place maps representing novel environments depends on NMDARs[37], as does the stabilization of new spatial learning-associated maps described here, whereas the establishment of new hippocampal maps is not prevented by NMDAR blockade in either case[37]. In addition, we have shown that enhanced reactivation of newly established goal representations is NMDAR-dependent. These findings highlight the important role of NMDAR-dependent hippocampal plasticity during learning and are consistent with other behavioral studies showing that formation of spatial memories involves NMDARs[3,17,31–34]. They suggest that spatial learning of either entire environments or discrete places involves NMDAR-dependent plasticity to update the hippocampal representation of space according to task demands and/or environmental changes. NMDAR-dependent mechanisms also promote reactivation of the newly established representation to strengthen it, preventing interference with pre-existing representations. Such a mechanism could also involve upregulation of synaptic plasticity and increased release of neurotransmitters such as dopamine or acetylcholine to facilitate the encoding and consolidation of new places and events into memory.

We have shown that SWR events are involved in the initial strengthening of neuronal representations associated with new spatial memories. Neurons that encoded newly learnt locations showed increased synchronization during eSWRs. Moreover, the degree of network synchronization during eSWRs predicted memory performance. This enhanced synchronization was not seen under NMDAR blockade or in the cued learning task. Hence, we found increased synchronization only when the animal remembered the learnt goal locations. Because neuronal assemblies that encode the current location of the animal show increased synchronization during these eSWRs, such synchronization has been suggested to strengthen these representations by facilitating neuronal plasticity[35]. Indeed, SWRs have been hypothesized to promote neuronal plasticity amongst active cells, and this process might be related to dendritic spiking[41,42]. Therefore, the enhanced synchronization of neuronal assemblies that encode new reward locations is expected to promote neuronal plasticity that stabilizes these newly formed assemblies. An increased incidence of high-frequency SWR-like oscillations has also been reported during exploration of novel environments and these events have been proposed to promote the stabilization of new place maps[43].

The reactivation of behaviorally governed neuronal patterns during rest periods has been suggested to be involved in memory consolidation[29,30]. However, experimental data linking reactivated neuronal activity patterns to memory consolidation have been largely lacking. As an indication for this, animals with age-related memory impairment show impaired reactivation of firing sequences when compared to young adults[44]. Further support for a role of reactivation in system-level memory consolidation comes from studies that report coordinated reactivation across brain regions: reward-related firing patterns reactivate together in the striatum and the hippocampus[45]. Moreover, prefrontal assemblies associated with rule-learning tend to be reactivated during hippocampal SWRs[46].

Here, we have been able to show in a spatial memory task that reactivation of neuronal patterns representing newly learnt places predicts subsequent memory performance. Reactivation also occurs in animals treated with an NMDAR blocker that caused spatial memory deficit. However, prediction of memory performance required enhanced reactivation of newly established patterns, which prevented interference between the reactivation of previous, already consolidated, patterns and newly established patterns. We showed that sSWR events had a special role in the reactivation of goal-related firing patterns: hippocampal population activity in most sSWRs represented one of the goal locations, and the number of times a given goal location was reactivated predicted how well that location was subsequently remembered, as expressed by the animal's memory performance. These findings show how sSWRs could contribute to the consolidation of spatial memories.

Further support for this idea comes from recent studies in which SWR activity was disrupted after spatial learning in a radial maze: electrical stimulation was applied after SWR had been detected, suppressing the full expression of SWRs and leading to mild learning impairment[24,25]. These studies did not examine network spiking activity, and therefore could not exclude the possibility that SWRs promote learning or consolidation by means other than reactivation. Nevertheless, they support our evidence for a functional link between the reactivation of learning-related assembly patterns and subsequent spatial memory performance.

In summary, our data suggest that SWRs have a dual role in the stabilization of spatial memories. During on-line periods they facilitate neuronal plasticity amongst spatially active cells that encode goal locations. During off-line rest periods, SWRs could further strengthen learning-related assembly patterns within the hippocampus and could trigger system-level consolidation by synchronizing neuronal activity in several brain regions.

METHODS

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

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

D.D. conducted the experiments. D.D., J.O. and B.P.-B. analyzed data. D.D. and J.C. wrote the manuscript. J.C. supervised the project. All of the authors discussed the results and commented on the manuscript.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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ONLINE METHODS

Subjects and electrode implantation. All procedures were carried out in accordance with the Animals (Scientific Procedures) Act, 1986 (UK) and associated procedures under an approved project license. We implanted seven adult male Long-Evans rats with 16 independently movable wire-tetrodes that were positioned above the right dorsal hippocampus (see **Supplementary Methods**). Following a one-week postoperative recovery period, rats were reduced to and maintained at 85% of their age-matched preoperative weight. Water was available *ad libitum*. During this period, tetrodes were lowered to the CA1 and CA3 regions of the dorsal hippocampus.

Training. Each daily experiment consisted of five recording sessions in the following order: a probe test (pre-probe), an immobility/sleep rest session (pre-rest), a learning session, an immobility/sleep rest session (post-rest) and a probe test (post-probe; see **Supplementary Fig. 1**). Hippocampal neuronal assembly activity was continuously monitored during these sessions. The two probe sessions (~25 min) were never rewarded. After both the pre-probe and learning sessions, rats were allowed to settle down in the start box for the rest sessions (~25 min). During the learning session, rats were given successive trials (~40 trials) to locate a new set of three hidden rewards placed in randomly selected food-wells every day. As these baited locations changed from day to day but stayed fixed within a given day, this 'matching-to-multiple-places' procedure required frequent updating of memory for goal locations in an otherwise unchanging environment. The same paradigm was used for the NMDAR blockade experiments during which rats were injected with 3-((R)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid ((R)-CPP, 10 mg kg^{-1}, intraperitoneal, Tocris Cookson) after the pre-probe[32,37] (**Supplementary Methods**). For the Cued version of the task, three identical objects (Falcon tube 50 ml, Greiner, see **Supplementary Fig. 1**) were placed near the baited food-wells during the learning trials and rats were trained to retrieve the hidden rewards from those visually marked locations. In this circumstance, the task was solved by using a guidance strategy that consisted of moving toward intra-maze cues identified to be closely associated with the goals. These intra-maze cues were removed for the two probe tests. Three rats were tested in all three experimental conditions. 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 the towel used to handle the rat daily) and the board was rotated relative to the start-box between learning trials and between rest and probe sessions. In all, 12, 8 and 9 sequences of probe-rest-learning-rest-probe recorded in the familiar recording room were analyzed for the drug-free, CPP and Cued conditions, respectively, for a total of 2,040 CA1 pyramidal cells (drug-free, 1,074; CPP, 612; cued, 354) and 690 CA3 pyramidal cells (drug-free, 257; CPP, 221; cued, 212) included in the analysis (see **Supplementary Methods**).

Data acquisition. Wide-band (0.1 Hz–5 kHz) recordings of local field potentials and multiple-unit activity were amplified 1,000-fold using a 64-channel amplifier (Sensorium) and continuously digitized at 20 kHz using a 64-channel analog-to-digital converter computer card (United Electronics Industries). Two 32-channel unity-gain preamplifier headstages (Axona) were used to reduce cable movement artifacts. An array of three LED clusters mounted on the preamplifier headstages was used to track the location of the animal (25 frames per s) through an overhead video camera (Sony). The animal tracking was synchronized with the electrophysiological recording. The animal's location was constantly monitored throughout the daily experiment. The data were analyzed off-line using custom-made software, including all unit isolation and field analysis, and occasionally the STAT 5.4 UNIX software package (Perlman G., 1980) and the R software environment (http://www.r-project.org/).

Behavior. Behavioral performance was calculated off-line using the animal's position records in the tracking data. Learning performance was assessed by calculating the distance traveled to retrieve all three rewards during each trial. Memory retention performance was assessed during the first 3 min of each probe by both scoring the number of crossings (**Figs. 1** and **3**) and the time spent (**Supplementary Fig. 2**) in the goal areas (10 cm in diameter centered on the learned bait locations). The pre-probe was used to evaluate memory for the baited locations learnt the day before (24 h before learning); the post-probe was used to assess memory for the newly learnt locations (2 h after learning). Differences between groups were analyzed using a Student's *t*-test or an ANOVA which was followed by a *post hoc* comparison using Tukey's HSD *post hoc* as appropriate. Memory performance (number of crossings) from the probe session following learning was used for correlation analysis.

Spatial firing rate maps. The *x*–*y* plane of the cheeseboard was divided into bins of 5 × 5 cm^2 and hippocampal place rate-maps were calculated during exploratory epochs (speed >5 cm s^{-1}) by a kernel-based method in which both the firing rate and occupancy maps were smoothed with a Gaussian kernel function[28,35]. Hippocampal place cells were screened for their spatial tuning using a coherence value of at least 0.6 and a sparsity value of no more than 0.3. Coherence reflects the similarity of the firing rate in adjacent bins, and is the *z*-transform of the correlation between the rate in a bin and the average rate of its eight nearest neighbors[47]. Sparsity corresponds with the proportion of the environment in which a cell fires, corrected for dwell time[48], and is defined as $(\Sigma PiRi)^2/\Sigma PiRi^2$, where Pi is the probability of the rat occupying bin i and Ri is the firing rate in bin i. For each place cell, the place field center was defined as the *x*–*y* locations where the cell fired >80% of its peak firing rate. The hippocampal representation of goal locations was then quantified as the proportion of cells with a place field center falling within a <10-cm circular region from the center of a baited well. The similarity score of place-related assembly patterns was estimated using a population vector analysis[36]: rate maps were stacked into three-dimensional matrices for each waking period (the two spatial dimensions on the *x* and *y* axis and the cell identity on the *z* axis; see schematic in **Fig. 2f**); population vectors were calculated at each *x*–*y* bin; these were then correlated between periods and averaged across all spatial bins. To avoid artifacts in the correlation measure, *xy* locations visited for <100 ms in either waking period were not considered for analysis. Analyses using the end of learning were performed using the last 10 learning trials. Analyses over the course of learning trials were performed by calculating firing rate maps using a successive window that encompassed 5 trials altogether.

Reactivation. Reactivation of place-related waking patterns was assessed by testing whether the tendency of pairs of cells to fire together (co-firing) during the rest session after learning reflected the degree to which their place fields overlapped (place field similarity, PFS) as described[28]. The cell pairs were taken from hippocampal place cells with the peak firing rate pixel of their place map within 30 cm from either one of the food-wells or the start-box, and referred to as 'goal-centric cells' or 'start-box cells', respectively. This analysis included 17,980 and 10,369 goal-centric cell pairs in the drug-free and CPP conditions, respectively, and 2,979 and 1,734 start-box cell pairs in the drug-free and CPP conditions, respectively. The PFS was established by calculating the correlation coefficient of their place rate-maps during learning. To measure the co-firing during rest, we first established for each cell its instantaneous firing rate counts (IFRC) in 100-ms windows centered on the peak of sSWRs and then calculated the correlation coefficient between the IFRCs for each cell pair. As the rat explored the maze before both rest periods, reactivation during the rest after learning might not only reflect associations from learning but also activity from the pre-probe session. To control for associations that might reflect exposures before learning or baseline correlations we used a partial correlation to assess reactivation of place-related waking patterns, correlating learning-related PFS with rest co-firing after learning while controlling for rest co-firing before learning[26,49,50]. To verify the degree to which baseline correlations existed in the rest session before learning, the correlation was reversed, and the correlation was controlled by the co-firing after learning[49,50].

Reactivation maps were computed to determine whether CA1 assembly patterns present at the end of the learning period or reinstated during the post-probe were present during the rest periods. We compared sSWR firing patterns from the rest periods to waking population activity from each spatial bin in the maze. Rest population firing vectors were established for each sSWR separately by calculating the IFRCs for all cells. Waking population activity was calculated as described above using the population vector analysis (**Fig. 2f**). Then we calculated the correlation coefficient between each sSWR-population vector and the waking population vectors from each *x*–*y* location in the maze. Peaks in these correlation maps are expected to correspond to regions in space where the waking population activity most strongly resembles the sSWR-population activity and thus presumably reflect the rat's internal representation of its position at the time scale of a single ripple event. To test whether the relative strength of the reactivation of

goal-related assembly patterns predicted subsequent memory performance, we used a partial correlation to calculate the similarity between each sSWR-population vector and the waking population vectors from either the end of learning or the post-probe, controlled for the corresponding waking population vectors from the pre-probe. Thus, for each sSWR event, each spatial bin was represented by a partial coefficient reflecting the similarity between the rest assembly patterns at that moment and the learning-related assembly patterns at that location. The reactivation index of goal-related assembly patterns corresponded to the proportion of sSWRs representing a goal location as indicated by the highest positive correlation coefficients on the map. The frequency of sSWRs did not differ significantly between the rest periods before and after learning and between the drug-free and CPP-treated conditions (**Supplementary Fig. 13**).

eSWR responses. The eSWR network response of spatially active pyramidal cells and pyramidal cells that fire outside their place field was calculated as described[35]. Briefly, eSWR firing rate histograms of CA1 pyramidal cells were calculated in 20-ms bins in reference to the eSWR peak (peak of ripple-band power) separately for their infield firing (inside the place field) and outfield firing outside the place field)[35].

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