Published in final edited form as:
Nat Neurosci. 2010 August ; 13(8): 995–1002. doi:10.1038/nn.2599.

The reorganization and reactivation of hippocampal maps predict spatial memory performance

David Dupret, **Joseph O'Neill**, **Barty Pleydell-Bouverie**, and **Jozsef Csicsvari**
MRC Anatomical Neuropharmacology Unit, Department of Pharmacology, University of Oxford, Mansfield Road, Oxford OX1 3TH, United Kingdom

Abstract

The hippocampus is a key brain circuit for spatial memory, and the spatially-selective spiking of hippocampal neuronal assemblies is thought to provide a mnemonic representation of space. Here we show that remembering newly-learnt goal locations requires the 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 for 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 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], a form of memory essential for an organism to learn and remember behaviorally-relevant places such as the location of food resources. 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 representing the information to be remembered [1,5-8]. During subsequent recall, reinstatement of memory trace activity patterns is thought to be required for successful retrieval of such information. However, initially encoded memory traces are labile and vulnerable to interference, only becoming stable through a process of consolidation [5,9,10]. Therefore, acquisition-associated activity patterns must first be stabilized during memory trace consolidation if their later reinstatement is needed to support accurate memory-related behavior [5,9,10].

Hippocampal principal cells, called place cells, fire in specific regions of the environment (i.e., 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

Correspondence: Jozsef Csicsvari and David Dupret MRC Anatomical Neuropharmacology Unit, University of Oxford, Mansfield Road Oxford OX1 3TH, United Kingdom Office: +44-1865-281130 Fax: +44-1865-271647 jozsef.csicsvari@pharm.ox.ac.ukdavid.dupret@pharm.ox.ac.ukhttp://mrcanu.pharm.ox.ac.uk/staff/jozsef.html.
AUTHORS CONTRIBUTIONS D. D. conducted the experiments. D.D., J.O. and B.P-B carried out the data analysis. D.D. and J.C. wrote the manuscript. J.C. supervised the project. All authors discussed the results and commented on the manuscript.

Note: Supplementary information accompanies the paper

Europe PMC Funders Author Manuscripts

salient places by place cells may derive from a reorganization of firing patterns as part of memory trace encoding during learning. However, the direct role of place cells in encoding memory traces has not been demonstrated. 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 demonstrated whether hippocampal representations of goal locations are acquired as a direct result of learning [17]. In addition, it remains to be determined 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 designed to 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, 150–250Hz) [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 together. 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 constitute a mechanism underlying system-level memory consolidation in which waking firing patterns are replayed 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 showing 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 stages of a spatial memory task. Moreover, to test how hippocampal network activity is altered during memory impairment we blocked NMDA-receptors (NMDARs), known to be critical for spatial memory [3,17,31-34], and assessed hippocampal network changes. We showed 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. Such goal-related reorganization was not observed, however, when goal locations were marked by visual cues. During the consolidation stage, the SWR-associated reactivation of these newly-acquired representations of goal locations predicted memory performance. Together, 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 (Supplementary Fig. 1; 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. To prevent the use of odor cues, food dust was scattered across the maze and the board was rotated relative to the start-box (see Methods). The procedure required daily memory updates of goal locations because a new set of bait-locations was introduced every day. The animals' memory performance was assessed by the number of crossings (Fig. 1b) and the time spent (Supplementary Fig. 2a,b) at goal areas (10 cm diameter circle around the

learnt bait-locations) during the probe sessions in which rewards were no longer provided. These probe sessions were performed 2h after each daily learning session (i.e., post-learning probe or “post-probe”) and, on the following day, prior to the new learning session (i.e., 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 in order to assess changes to hippocampal network activity following learning of new bait-locations.

The learning performance of animals improved rapidly on each day: the distance travelled to complete a trial showed >50% reduction following the first trial and reached an asymptotic level within a few trials, showing that rats rapidly encoded and remembered the new bait-locations for the remaining trials (Fig. 1a, Drug-free). 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 (Fig. 1b, Drug-free post-probe: P=0.0003; Supplementary Fig. 2b). These locations were remembered on the following day, as assessed during the next day’s pre-probe session (Fig. 1b, Drug-free pre-probe: P=0.010; Supplementary Fig. 2b).

To reveal 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 centre falling within a goal area (the place field center area was defined at pixels where the firing rate was larger than 80% of the peak rate; goal area was defined as a 10cm circular region around the food well; see Methods). Consistent with previous studies[14-16], we observed 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, while CA3 representations did not change (Fig. 2a-c, Drug-free; Supplementary Figs. 3 and 4a). 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 representing 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 further the similarity of place-related assembly patterns (see Methods), showed that the CA1 population similarity score between probe sessions was lower than the baseline score calculated within sessions, while these scores remained similar in CA3 (Fig. 2f). Therefore, CA1 place-related assemblies present during probe sessions reorganized following learning, while 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 representing goal locations increased gradually over trials (Fig. 2d, Drug-free CA1 with r=0.370, P<0.00001; Supplementary Figs. 3 and 4b). Moreover, CA1 assembly patterns observed during the last 10 learning trials (referred to as “end of learning”) were more similar to assembly patterns during the probe session after learning than before (Fig. 2g, Drug-free; Supplementary Fig. 5). Hence, CA1 goal representations developed gradually during learning, and those representations present at the end re-emerged in the subsequent probe session.

The performance improvement 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 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, such that animals did not have to remember the locations to gain the reward (Supplementary Fig. 1). During this cued learning, animals ate the same number of rewards; exhibited similar stereotyped movement paths; and spent similar time at goal locations as in the absence of cues (Fig. 3a,c; Supplementary Fig. 2). However, animals did not exhibit any spatial preference in subsequent probe sessions, indicating that they did not learn the visually-guided locations (Fig. 3b, Supplementary Fig. 2b). In these control experiments, we found that CA1 goal-oriented remapping did not take place, suggesting that such reorganization occurs when a map-based strategy was used to locate hidden rewards (Fig. 3d-h, Supplementary Figs. 4 and 6).

Effect of the NMDAR blockade

These results show that, in our task, new spatial memories were encoded by CA1 place maps representing 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 observed under conditions of memory impairment. It has been shown that NMDARs are required for spatial memory [3,31-34]. Therefore, rats were injected with the NMDAR antagonist CPP (3-((R)-2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid; see Methods) after the pre-probe session [32,37] in order to interfere with their spatial memory. We found that the learning performance of CPP-treated animals improved rapidly (Fig. 1a) and was comparable to those in the drug-free condition. However, CPP-treated animals subsequently failed to remember the newly-learnt locations in both probes when there was at least 2h gap between learning and recall (Fig. 1b, all Ps>0.339; Supplementary Fig. 2b; see Supplementary Fig. 14 for shorter delays).

In association with impaired memory for goal locations, we found that NMDAR blockade prevented the stabilization of hippocampal maps representing those locations. During learning, the proportion of CA1 place cells with goal-related firing increased gradually (Fig. 2d, CPP CA1 with r=0.334, P<0.00001; Supplementary Figs. 4b and 7), as in the drug-free condition. However, under CPP the similarity between reorganized assembly patterns present 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 the drug-free case. Moreover, assembly patterns remained similar across probe sessions (Fig. 2f), as did the proportion of place cells with goal-related firing (Fig. 2c, Supplementary Figs. 4a 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 no longer remembered goal locations during subsequent probe sessions.

To test whether place cell representations are related to memory traces, we examined whether representations of goal locations predict spatial memory performance. We found that 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 representing goal locations at the end of learning (Fig. 2e, r=0.511, P=0.0014) and in the following probe session (Supplementary Fig. 8) but not at the beginning of learning nor in the probe session before (all Ps>0.131). This was the case neither under CPP nor in the Cued condition (all Ps>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.

eSWR network responses during learning

In the hippocampus, SWR events have been suggested to assist the stabilization of memory traces [7,21,22,28,35]. Although SWR activity has been traditionally described during off-line sleep/rest (sSWRs) including periods of slow-wave sleep and long waking immobility, they are also present during on-line exploratory periods (eSWRs) [35]. These eSWRs have been suggested to strengthen place cell representations and therefore they may assist in stabilizing 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/stabilization. During learning, we observed eSWRs at the bait-locations (Fig. 4a,b). The number of goal-associated eSWRs was correlated with memory performance in the drug-free condition ($r=0.524$, $P=0.0010$) suggesting that eSWRs were associated with on-line memory trace formation. However, the number of SWRs that occurred during longer (>2.4s) immobility periods (iSWRs) [35] during learning did not predict memory performance ($P>0.722$). Moreover neither eSWR nor iSWR numbers predicted memory performance under CPP (all $Ps>0.410$). Ongoing place-related activity is supplemented by increased network activity during eSWRs, strengthening the synchronized firing of cells encoding the same location and consequently promoting synaptic plasticity amongst them [35]. Since eSWRs tended to occur at reward-locations, we checked whether cells representing these places strengthened their firing synchronization during eSWRs. We compared the eSWR firing rate histograms of CA1 place cells representing goal locations to those representing 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” (Fig. 4c, 5.81±0.29Hz versus 4.22±0.36Hz, $P<0.002$). This was abolished under CPP (4.42±0.24Hz versus 3.98±0.35Hz, $P>0.319$) and was not present in the Cued condition (Supplementary Fig. 9a). Moreover, the strength of eSWR network responses measured at the end of the learning was associated with memory recall: network participation (i.e., synchrony) of CA1 place cells in eSWRs was correlated with memory performance (Fig. 4d, $r=0.418$, $P=0.011$) even when controlled for the eSWR firing rate or the proportion of place cells at goal locations ($r=0.448$, $P=0.016$; $r=0.364$, $P=0.023$ respectively, partial correlation). This was the case neither under CPP nor in the Cued version (all $Ps>0.318$).

Reactivation of waking firing patterns during rest

During off-line rest periods, sSWRs have been suggested to promote memory consolidation because waking activity patterns of place cells are reactivated during these network events [26,28,35,38]. To test whether reactivation occurs following spatial learning, we examined whether cells encoding similar places during learning fire together in subsequent sSWRs [28]. The place field similarity (PFS) of cell pairs (measured for place fields present at the end of learning) exhibited significant correlations with their joint firing tendency (cofiring) during sSWRs (Fig. 5a; Supplementary Figs. 10 and 11). For both goal-centric and start-box cells, cofiring in sSWRs after learning correlated more strongly with PFS than did cofiring before learning (Fig. 5a). However, reactivation of goal locations was even stronger than the reactivation of the start-box location (Fig. 5a, Drug-free). While CPP application did not prevent reactivation *per se*, such an enhanced reactivation of goal locations was abolished (Fig. 5a, CPP; Supplementary Figs. 10 and 11). The enhanced reactivation of goal locations was not observed in the Cued condition (Supplementary Fig. 9b). Since CPP caused a memory deficit in our task, we hypothesized that conflicting representations were reactivated under CPP. 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 the drug-free condition, 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 the recurrence of old, conflicting representations.

Finally, we tested whether reactivation of goal-related CA1 assembly patterns predicts memory performance. For each sSWR we calculated a “reactivation map” in order to determine which locations were represented by the sSWR assembly pattern (see Methods). These reactivation maps quantified how similar the sSWR assembly pattern was to waking assembly patterns representing 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. Thus, the peak of the reactivation map marked the location which the sSWR population activity represented the best. Either rate maps from the end of learning or from the following probe period were used to measure the similarity of assembly patterns (see Methods). For most sSWRs, the resulting reactivation maps highlighted one of the bait-locations (Fig. 5b). Moreover, the proportion of sSWRs representing goal locations predicted subsequent memory performance when reactivation maps were created using either end of learning (Fig. 5c, r=0.620, P=0.00005) or post-probe (r=0.362, P=0.028) representations. In contrast, this was not seen if sSWRs were taken from the rest session before learning (all Ps>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 (all Ps>0.412).

DISCUSSION

In this study we have examined 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, our evidence suggests that SWRs can facilitate memory trace strengthening during both on-line and off-line periods. Thus our report establishes 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 may map not only allocentric space but also the behavioral salience of certain discrete locations. In support of this, it has been reported that many place cells fire at goal locations during goal-oriented tasks, suggesting 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 demonstrated neither a direct link between goal-related firing patterns and spatial learning of goal locations, nor whether goal-related hippocampal maps are reinstated during memory recall. Indeed, 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. Such 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 demonstrate that newly-acquired hippocampal representations re-emerge during the recall stage and, moreover, 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. While this argument cannot be fully excluded, it is expected that the reinstated goal-oriented maps during recall facilitate efficient navigation to goal locations, thus have functional consequences for spatial memory process, even if the learning process itself had involved attention (see Supplementary Discussion).

We showed that the reorganization of place cell firing and the associated network responses observed during spatial learning of goal locations were not identical to those observed following changes of entire environments while animals are engaged in simple spatial exploration. While place cells in both CA1 and CA3 hippocampal areas reorganize following 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 in order to solve spatial problems: while 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 may 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 those that occur 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 is NMDAR-dependent [37], as was the stabilization of new spatial learning-associated maps in this study; while the establishment of new hippocampal maps is not prevented by NMDAR blockade in either case [37]. In addition, we have demonstrated that enhanced reactivation of newly-established goal representations was 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 entire environments or discrete places both involve 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 an up-regulation of synaptic plasticity and increased release of neurotransmitters such as dopamine or acetylcholine to facilitate the encoding and the consolidation of new places and events into memory.

We have demonstrated a role for SWR events in the initial strengthening of neuronal representations associated with new spatial memories. We showed that neurons encoding

newly-learnt locations exhibited increased synchronization during eSWRs. Moreover the network synchronization level during eSWRs predicted memory performance. This enhanced synchronization was not observed under NMDAR blockade or in the cued learning task. Hence increased synchronization was observed only when the animal remembered the learnt goal locations. Because neuronal assemblies encoding the current location of the animal exhibit increased synchronization during these eSWRs, this 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 encoding new reward-locations is expected to promote neuronal plasticity stabilizing these newly-formed assemblies. An increased incidence of high frequency SWR-like oscillations has been also reported during exploration of novel environments and these events have been suggested to promote the stabilization of new place maps [43].

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 aged-related memory impairment exhibit impaired reactivation of firing sequences as compared to young adults [44]. Further support for a role in system-level memory consolidation comes from studies reporting coordinated reactivation across brain regions: reward-related firing patterns reactivate together in the striatum and the hippocampus [45]. Moreover, reactivated prefrontal assemblies associated with rule-learning tend to occur 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. However, reactivation itself can also be observed in animals that exhibited spatial memory deficit under NMDAR blockade. Yet, an enhanced reactivation of newly-established patterns was needed, which itself prevented interference between the reactivation of previous, already consolidated, patterns and newly-established patterns. We showed that sSWR events played 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 demonstrate how sSWRs could contribute to the consolidation of spatial memories. Further support comes from recent studies that disrupted SWR activity 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 may promote learning/consolidation by other means than reactivation. Nevertheless, they do 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 encoding 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

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 Information). 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 a sequence 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 1b). 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 the learning sessions, rats were allowed to settle down within 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 3 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 the 3-((R)-2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid [(R)-CPP, 10 $mg.kg^{-1}$, i.p., Tocris Cookson Ltd] after the pre-probe [32,37] (see Supplementary Methods). For the Cued version of the task, three identical objects (Falcon tube 50 ml, Greiner, see Supplementary Fig 1a) 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 towards 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 a total of 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 2040 CA1 pyramidal cells (drug-free=1074, 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 (0.1Hz–5kHz) 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 analogue-to-digital converter computer card (United Electronics Industries). Two 32-channel unity-gain preamplifier headstages (Axona Ltd) were used to reduce cable movement artefacts. An array of three LED clusters mounted on the preamplifier headstages was used to track the location of the animal (25 frames per s) via 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 travelled to retrieve all three rewards during each trial. Memory retention performance was assessed during the first 3 minutes 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 learnt baited locations). The pre-probe was used to evaluate the memory for the baited locations learnt the day before (i.e., 24h before learning); the post-probe was used to assess the memory for the newly learnt locations (2h 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 the 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>5cm/s) 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, 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 centre falling within <10cm 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, the cell identity on the z axis; see schematic in Figure 2f); population vectors were calculated at each x-y bin; these were then correlated between periods and averaged across all spatial bins. To avoid artefacts in the correlation measure, x-y locations visited less than 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 (cofiring) during the rest session after learning reflected the degree to which their place-fields overlapped (place field similarity, PFS) as previously 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 a total of 17,980 and 10,369 goal-centric cell pairs in the drug-free and CPP conditions respectively, and of 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 cofiring 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. Since the rat explored the maze before both rest periods, reactivation in the rest after learning might not only reflect associations from learning but also activity from the pre-probe session of the day. In order to control for associations that may reflect exposures prior to learning or baseline correlations we used a partial correlation to assess reactivation of place-related waking patterns, correlating learning-related PFS with rest cofiring after learning while controlling for rest cofiring before learning[26,49,50]. In order 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 cofiring after learning[49,50].

Reactivation maps were computed to observe whether CA1 assembly patterns present at the end of 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 (see schematic in Figure 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 reflects 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 the CPP conditions (see 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 before [35]. Briefly, eSWR firing rate histograms of CA1 pyramidal cells were calculated in 20 ms bin in reference to the eSWR peak (i.e., peak of ripple-band power) separately for their infield firing (i.e. inside the place field) and outfield firing (i.e. outside the place-field) firing [35].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank J.N.P. Rawlins and D.M. Bannerman for their critical discussions about the behavioral procedures, J.R. Huxter and K. Allen for their discussions about data analysis and manuscript, P. Somogyi, M. Capogna, C. Lever, O. Paulsen and T. Bienvenu for their constructive comments on a previous version of the manuscript and N. Campo-Urriza for her technical assistance. This work was supported by the Medical Research Council. D.D. was successively funded by fellowships from the "*Institut de France-Fondation Louis D.*" and the International Brain

Research Organization (IBRO Research Fellowship), and currently holds a Junior Research Fellowship in Neurosciences from Saint Edmund Hall College, University of Oxford.

References

1. O'Keefe, J.; Nadel, L. The hippocampus as a cognitive map. Oxford University Press; Oxford,Clarendon: 1978.
2. Squire LR. Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. Psychol. Rev. 1992; 99:195–231. [PubMed: 1594723]
3. Morris RG. Elements of a neurobiological theory of hippocampal function: the role of synaptic plasticity, synaptic tagging and schemas. Eur. J. Neurosci. 2006; 23:2829–2846. [PubMed: 16819972]
4. Riedel G, et al. Reversible neural inactivation reveals hippocampal participation in several memory processes. Nat. Neurosci. 1999; 2:898–905. [PubMed: 10491611]
5. Hebb, DO. The organization of behavior. Wiley; New York: 1949.
6. Marr D. Simple memory: a theory for archicortex. Philos. Trans. R. Soc. Lond B Biol. Sci. 1971; 262:23–81. [PubMed: 4399412]
7. Buzsaki G. Two-stage model of memory trace formation: a role for "noisy" brain states. Neuroscience. 1989; 31:551–570. [PubMed: 2687720]
8. McClelland JL, McNaughton BL, O'Reilly RC. Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. Psychol. Rev. 1995; 102:419–457. [PubMed: 7624455]
9. Glickman SE. Perseverative neural processes and consolidation of the memory trace. Psychol. Bull. 1961; 58:218–233. [PubMed: 13706058]
10. McGaugh JL. Time-dependent processes in memory storage. Science. 1966; 153:1351–1358. [PubMed: 5917768]
11. O'Keefe J, Dostrovsky J. The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. Brain Res. 1971; 34:171–175. [PubMed: 5124915]
12. Ekstrom AD, et al. Cellular networks underlying human spatial navigation. Nature. 2003; 425:184–188. [PubMed: 12968182]
13. Wilson MA, McNaughton BL. Dynamics of the hippocampal ensemble code for space. Science. 1993; 261:1055–1058. [PubMed: 8351520]
14. Markus EJ, et al. Interactions between location and task affect the spatial and directional firing of hippocampal neurons. J. Neurosci. 1995; 15:7079–7094. [PubMed: 7472463]
15. Hok V, et al. Goal-related activity in hippocampal place cells. J. Neurosci. 2007; 27:472–482. [PubMed: 17234580]
16. Hollup SA, Molden S, Donnett JG, Moser MB, Moser EI. Accumulation of hippocampal place fields at the goal location in an annular watermaze task. J. Neurosci. 2001; 21:1635–1644. [PubMed: 11222654]
17. Nakazawa K, McHugh TJ, Wilson MA, Tonegawa S. NMDA receptors, place cells and hippocampal spatial memory. Nat. Rev. Neurosci. 2004; 5:361–372. [PubMed: 15100719]
18. Maquet P. The role of sleep in learning and memory. Science. 2001; 294:1048–1052. [PubMed: 11691982]
19. Marshall L, Born J. The contribution of sleep to hippocampus-dependent memory consolidation. Trends Cogn Sci. 2007; 11:442–450. [PubMed: 17905642]
20. Rasch B, Buchel C, Gais S, Born J. Odor cues during slow-wave sleep prompt declarative memory consolidation. Science. 2007; 315:1426–1429. [PubMed: 17347444]
21. Buzsaki G. Hippocampal sharp waves: their origin and significance. Brain Res. 1986; 398:242–252. [PubMed: 3026567]
22. Csicsvari J, Hirase H, Mamiya A, Buzsaki G. Ensemble patterns of hippocampal CA3-CA1 neurons during sharp wave-associated population events. Neuron. 2000; 28:585–594. [PubMed: 11144366]
23. Csicsvari J, Hirase H, Czurko A, Mamiya A, Buzsaki G. Fast network oscillations in the hippocampal CA1 region of the behaving rat. J. Neurosci. 1999; 19:RC20. [PubMed: 10436076]

Europe PMC Funders Author Manuscripts

24. Girardeau G, Benchenane K, Wiener SI, Buzsaki G, Zugaro MB. Selective suppression of hippocampal ripples impairs spatial memory. Nat. Neurosci. 2009; 12:1222–1223. [PubMed: 19749750]
25. Ego-Stengel V, Wilson MA. Disruption of ripple-associated hippocampal activity during rest impairs spatial learning in the rat. Hippocampus. 2010; 20:1–10. [PubMed: 19816984]
26. Kudrimoti HS, Barnes CA, McNaughton BL. Reactivation of hippocampal cell assemblies: effects of behavioral state, experience, and EEG dynamics. J. Neurosci. 1999; 19:4090–4101. [PubMed: 10234037]
27. Wilson MA, McNaughton BL. Reactivation of hippocampal ensemble memories during sleep. Science. 1994; 265:676–679. [PubMed: 8036517]
28. O'Neill J, Senior TJ, Allen K, Huxter JR, Csicsvari J. Reactivation of experience-dependent cell assembly patterns in the hippocampus. Nat. Neurosci. 2008; 11:209–215. [PubMed: 18193040]
29. Sutherland GR, McNaughton B. Memory trace reactivation in hippocampal and neocortical neuronal ensembles. Curr. Opin. Neurobiol. 2000; 10:180–186. [PubMed: 10753801]
30. Rasch B, Born J. Maintaining memories by reactivation. Curr. Opin. Neurobiol. 2007; 17:698–703. [PubMed: 18222688]
31. Morris RG, Anderson E, Lynch GS, Baudry M. Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. Nature. 1986; 319:774–776. [PubMed: 2869411]
32. McDonald RJ, et al. NMDA-receptor blockade by CPP impairs post-training consolidation of a rapidly acquired spatial representation in rat hippocampus. Eur. J. Neurosci. 2005; 22:1201–1213. [PubMed: 16176363]
33. Steele RJ, Morris RG. Delay-dependent impairment of a matching-to-place task with chronic and intrahippocampal infusion of the NMDA-antagonist D-AP5. Hippocampus. 1999; 9:118–136. [PubMed: 10226773]
34. Shimizu E, Tang YP, Rampon C, Tsien JZ. NMDA receptor-dependent synaptic reinforcement as a crucial process for memory consolidation. Science. 2000; 290:1170–1174. [PubMed: 11073458]
35. O'Neill J, Senior T, Csicsvari J. Place-selective firing of CA1 pyramidal cells during sharp wave/ripple network patterns in exploratory behavior. Neuron. 2006; 49:143–155. [PubMed: 16387646]
36. Leutgeb S, et al. Independent codes for spatial and episodic memory in hippocampal neuronal ensembles. Science. 2005; 309:619–623. [PubMed: 16040709]
37. Kentros C, et al. Abolition of long-term stability of new hippocampal place cell maps by NMDA receptor blockade. Science. 1998; 280:2121–2126. [PubMed: 9641919]
38. Nakashiba T, Buhl DL, McHugh TJ, Tonegawa S. Hippocampal CA3 output is crucial for ripple-associated reactivation and consolidation of memory. Neuron. 2009; 62:781–787. [PubMed: 19555647]
39. Leutgeb S, Leutgeb JK, Treves A, Moser MB, Moser EI. Distinct ensemble codes in hippocampal areas CA3 and CA1. Science. 2004; 305:1295–1298. [PubMed: 15272123]
40. Muller RU, Kubie JL. The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells. J. Neurosci. 1987; 7:1951–1968. [PubMed: 3612226]
41. King C, Henze DA, Leinekugel X, Buzsaki G. Hebbian modification of a hippocampal population pattern in the rat. J. Physiol. 1999; 521(Pt 1):159–167. [PubMed: 10562342]
42. Losonczy A, Makara JK, Magee JC. Compartmentalized dendritic plasticity and input feature storage in neurons. Nature. 2008; 452:436–441. [PubMed: 18368112]
43. Cheng S, Frank LM. New experiences enhance coordinated neural activity in the hippocampus. Neuron. 2008; 57:303–313. [PubMed: 18215626]
44. Gerrard JL, Burke SN, McNaughton BL, Barnes CA. Sequence reactivation in the hippocampus is impaired in aged rats. J. Neurosci. 2008; 28:7883–7890. [PubMed: 18667620]
45. Lansink CS, Goltstein PM, Lankelma JV, McNaughton BL, Pennartz CM. Hippocampus leads ventral striatum in replay of place-reward information. PLoS Biol. 2009; 7:e1000173. [PubMed: 19688032]

46. Peyrache A, Khamassi M, Benchenane K, Wiener SI, Battaglia FP. Replay of rule-learning related neural patterns in the prefrontal cortex during sleep. Nat. Neurosci. 2009; 12:919–926. [PubMed: 19483687]

47. Muller RU, Kubie JL. The firing of hippocampal place cells predicts the future position of freely moving rats. J. Neurosci. 1989; 9:4101–4110. [PubMed: 2592993]

48. Skaggs WE, McNaughton BL, Wilson MA, Barnes CA. Theta phase precession in hippocampal neuronal populations and the compression of temporal sequences. Hippocampus. 1996; 6:149–172. [PubMed: 8797016]

49. Pennartz CM, et al. The ventral striatum in off-line processing: ensemble reactivation during sleep and modulation by hippocampal ripples. J. Neurosci. 2004; 24:6446–6456. [PubMed: 15269254]

50. Tatsuno M, Lipa P, McNaughton BL. Methodological considerations on the use of template matching to study long-lasting memory trace replay. J. Neurosci. 2006; 26:10727–10742. [PubMed: 17050712]

Europe PMC Funders Author Manuscripts

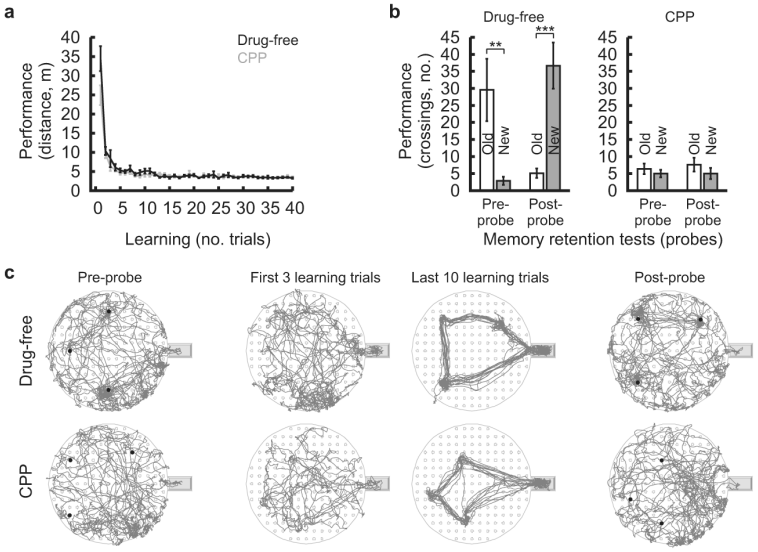

Figure 1. Daily learning of a new set of goal locations on the cheeseboard maze

Rats from the drug-free and the CPP conditions were trained in a matching-to-multiple-places task to locate a new set of 3 hidden food-rewards every day on a cheeseboard maze (Supplementary Fig. 1 and Methods). Learning performance was estimated by the distance travelled to find all rewards per trial (**a**, means±s.e.m, all Ps<0.00001, ANOVA). Memory retention performance was estimated by the number of crossings in goal areas (**b**, means ±s.e.m, **: P<0.01, ***: P<0.001, paired t-test; see Methods and also Supplementary Fig. 2b). Crossings were compared for goal locations learnt the day before ("Old") and the current day ("New"). Representative examples of animal's path (**c**); for clarity, only the first 10 min of each probe session are depicted (black dots: learnt goal locations).

Europe PMC Funders Author Manuscripts

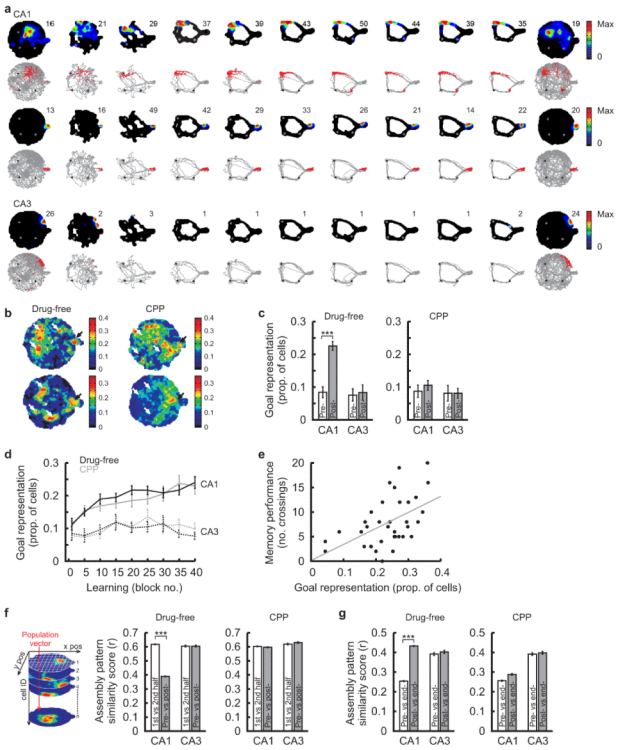

Figure 2. Goal-related reorganization of hippocampal assembly patterns

(a) Examples of hippocampal place cells recorded in the drug-free condition. Color-coded place rate maps (top rows) and individual spike locations superimposed on the animal's path (bottom rows) are shown; see Supplementary Figs. 3,6,7 for further examples. Note that the upper CA1 cell reorganized its place field to a goal location (dots: goal locations) while the middle cell representing the start-box and bottom CA3 cell exhibited 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 conditions. Pixel color represents the proportion of cells with place fields center at that x-y location (z scale=proportion of cells fire >80% of peak firing rate at that location). Note in the drug-free condition the higher proportion of cells associated with goal locations (white arrows) and the start-box (black arrow), and that bait-locations were not equally represented.

(c-d) Proportion of place cells representing bait-locations (means±s.e.m; see Methods) during probe sessions (**c**) and across trials (**d**, CA1: solid lines, all Ps<0.0001; CA3: dashed lines, all Ps>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 (grey: 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 Methods).

Europe PMC Funders Author Manuscripts

pre-: pre-probe, post-: post-probe, end-: end of learning, ***: P<0.00, paired t-test.

Europe PMC Funders Author Manuscripts

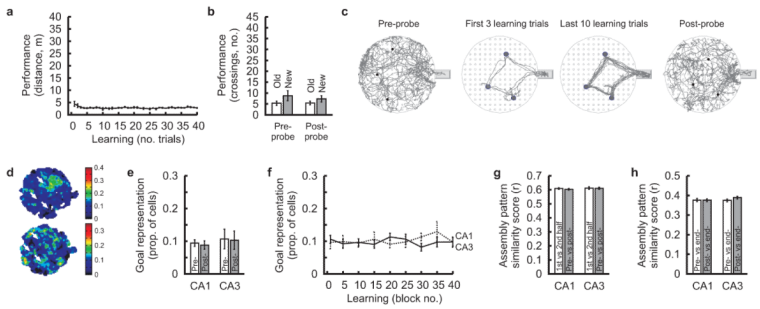

Figure 3. Locating rewards during the Cued version of the cheeseboard maze task

(a-c) Learning performance was estimated by the distance travelled to find all 3 rewards per trial (**a**, means±s.e.m, P>0.209, ANOVA). Memory performance was estimated by the number of crossings in goal areas during probe sessions (**b**, means±s.e.m; see Methods and also Supplementary Fig. 2b). Crossings were compared for locations learnt the day before ("Old") and the current day ("New"; all Ps>0.185, paired t-test). Representative examples of animal's path (**c**; small black dots: goal locations; grey-filled circles: intra-maze cues); for the probes, only the first 10 min are depicted for clarity. Note that animals followed similarly efficient movement paths during the cued learning as they had in the absence of intra-maze cues (see Figure 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 center at that x-y location (z scale=proportion of cells fire >80% of peak firing rate at that location). The white arrows indicate the learnt bait-locations.

(e-f) Proportion of place cells representing bait-locations (means±s.e.m; see Methods) during probe sessions (**e**, pre- compared to post-: all Ps>0.692, paired t-test) and across trials (**f**, CA1: solid line, CA3: dashed line, all Ps>0.785, ANOVA). The proportion of cells were 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 Methods) within probe (**g**, 1st versus 2nd half), between probes (**g**, pre- versus post-; 1st versus 2nd half compared to pre- versus post-: all Ps>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 Ps>0.122, paired t-test). Note that hippocampal assemblies remained similar in all three periods.

pre-: pre-probe, post-: post-probe, end-: end of learning.

Europe PMC Funders Author Manuscripts

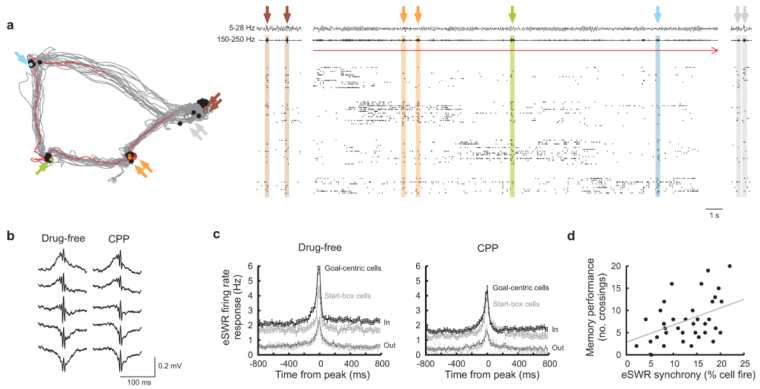

Figure 4. eSWR-associated activity of CA1 place cells

(a) Left: representative examples of the rat's path (grey 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 conditions.

(c) eSWR firing rate histograms (means±s.e.m, see Methods) of CA1 "goal-centric" and "start-box" cells inside ("In") and outside ("Out") their place fields in drug-free and CPP 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 (in grey: regression line, r=0.418, P=0.011).

Europe PMC Funders Author Manuscripts

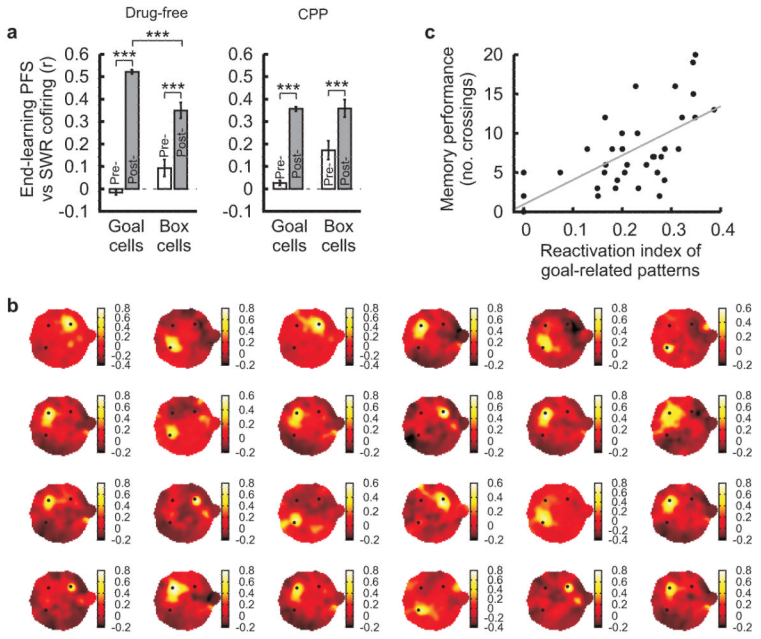

Figure 5. Reactivation of CA1 place-related assembly patterns

(a) Correlation between place field similarity (“PFS”) and sSWR cofiring 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 while sSWR cofiring was calculated in rest periods before (“pre-”) and after (“post-”) learning. Correlation coefficients represent the partial correlations of the PFS with the cofiring of one rest session, each controlled by the cofiring of the other rest session (see 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 Methods). Note that 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 (in grey: regression line, r=0.620, P=0.00005).

Europe PMC Funders Author Manuscripts