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Reactivation of experience-dependent cell assembly patterns in the hippocampus

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The hippocampus is thought to be involved in episodic memory formation by reactivating traces of waking experience during sleep. Indeed, the joint firing of spatially tuned pyramidal cells encoding nearby places recur during sleep. We found that the sleep cofiring of rat CA1 pyramidal cells encoding similar places increased relative to the sleep session before exploration. This cofiring increase depended on the number of times that cells fired together with short latencies (<50 ms) during exploration, and was strongest between cells representing the most visited places. This is indicative of a Hebbian learning rule in which changes in firing associations between cells are determined by the number of waking coincident firing events. In contrast, cells encoding different locations reduced their cofiring in proportion to the number of times that they fired independently. Together these data indicate that reactivated patterns are shaped by both positive and negative changes in cofiring, which are determined by recent behavior.

The hippocampus is involved in the formation of spatial and episodic memories[1–3]. It has been suggested that one of the ways that the hippocampus participates in the formation of new memories is by encoding and temporarily storing labile memory traces of waking experience[4–6]. After encoding, these traces are thought to be reactivated, which may serve both to stabilize them and/or to facilitate their transfer to other cortical areas for long-term storage. This process of reactivation is thought to be involved in the system's consolidation of episodic memories[4,5,7].

Previous work has shown that hippocampal waking firing patterns recur in subsequent sleep. Place cells that are highly active during exploration show higher firing rates during subsequent sleep than cells that were less active[8–10]. Furthermore, the reactivated firing of individual place cells is not independent; cells with similar spatially-selective firing fields (place fields) during exploration also tend to fire together in the following sleep period[6,11]. Therefore, reactivation reflects neuronal activity patterns representing discrete places in the environment. In addition, there is evidence that the firing sequences of place cells representing movement paths are reactivated during sleep after the animal follows stereotyped movement paths (for example, on linear tracks or circular mazes)[12–14]. Reactivation is mostly seen during irregularly occurring network patterns called sharp-waves/ripple (SWR) patterns[11,14–19], which have been suggested to enable the transfer of hippocampal information to extrahippocampal areas[4,20]. SWRs are initiated in the CA3 region and are associated with the synchronized discharge of CA1 and CA3 cells and transient 200-Hz oscillatory patterns (ripples) in the CA1 region[2,21–23].

The fact that cells with similar place fields continue to fire together in the absence of sensory input during sleep suggests that such cells fire as members of a cell assembly in the CA3 and CA1 regions. The firing fields of place cells vary relative to the environmental boundaries from one environment to another[24,25], and different combinations of place cells fire together in different environments. Thus, different combinations of place cells (that is, different cell assemblies) are reactivated during sleep if they are preceded by exploration of different environments. This requires the reorganization of firing associations between cells across sleep sessions. One possibility is that such changes in firing association may be activity dependent, with the greatest changes occurring between cells that repeatedly fire together because they code for similar regions that are also frequently visited. In this scenario, reactivation would reflect both the environment in which the animal explored most recently and its recent behavior in that environment, suggesting that episodic-like memory traces are reactivated.

However, a second possibility is that exploration promotes the reactivation of the entire set of place cells (that is, the 'cognitive map') representing the most recently explored environment. In this case, firing patterns representing different regions of the environment would be reactivated with equal probability, independent of the actual behavior of the animal. Accordingly, reactivation would merely reflect the already established associations between cells needed for place cell activity[26]. In this scenario, because of the pre-existing place-map associations, nonassociative firing rate increases could lead to reactivation.

The aim of this study was to better understand the mechanism of reactivation by determining the rules governing changes in firing association between cells. We present evidence that supports the idea that reactivated firing patterns are shaped by activity-dependent, associative processes; cell pairs representing the most visited regions

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of the environment showed the strongest increases in firing association between sleep both before and after exploration. These changes in firing association could be predicted by the number of times cells fired together at short latencies. Collectively these data indicate that reactivated firing patterns are shaped by changes in firing associations that follow a Hebbian learning rule[27]; that is, changes in firing associations were predicted by the number of waking coincident firing events.

RESULTS

Reactivation of waking activity patterns during subsequent sleep

First, we verified whether place-related firing patterns seen during exploration of novel and familiar environments recur in subsequent sleep. In each environment, different ensembles of place cells will fire together during exploration (**Supplementary Fig. 1** online), and are expected to be reactivated subsequently during sleep (**Fig. 1**). We compared place-field similarity (PFS) and joint-firing tendency (cofiring) of cell pairs (see Methods) during exploration with cofiring in sleep sessions before (pre) and after (post) exploration. Sleep before exploration was always preceded by exploration of a different environment to ensure the change of reactivated sleep patterns between the two sleep sessions. Reactivation of waking patterns is thought to occur mainly during periods of SWRs (**Fig. 1c**). Therefore, we compared reactivation during SWR and non-SWR periods in sleep by analyzing the cofiring of cell pairs separately in these periods. Both measures of waking firing patterns (PFS and cofiring) were significantly correlated with postsleep cofiring ($P < 10^{-8}$), but were significantly less correlated ($P < 0.0005$) with presleep cofiring (**Fig. 1d,e**). Correlations were significantly stronger with cofiring during SWR than with cofiring during non-SWR periods (familiar and novel, $P < 0.0013$; **Fig. 1d,e**). In addition, the increase in cofiring as a function of PFS was greater during SWR than non-SWR (**Fig. 2a,b**), as indicated by the slope of the regression lines (SWR > non-SWR, familiar and novel, $P < 0.001$). The number of SWRs detected in sleep sessions did not differ significantly in the pre- and postsleep sessions (novel: presleep, 367 ± 24.5 s.e.m., postsleep, 389 ± 33.1 s.e.m., $P > 0.4$; familiar: presleep, 376 ± 38.7 s.e.m., postsleep 410 ± 55.3, $P > 0.5$, paired t-test), indicating that the reduced number of SWRs during presleep do not explain the reduced correlations observed in these epochs.

We also examined whether the reactivation of waking activity is quantitatively different after exploration of novel places than after exploration of familiar ones when the configuration of firing associations amongst the active subset of cells is established for the first time. Reactivation was stronger in the novel condition ($P < 0.00001$; **Fig. 1c,d**). This effect remained whether the novel waking-sleep sessions occurred before or after familiar exploration in a given recording day (**Supplementary Fig. 2** online). The correlation between waking activity and SWR cofiring was significantly stronger in the novel condition across individual recording days in different novel environments (**Supplementary Fig. 3** online). The average speed of the animal was higher during exploration of the novel environments, but exploration speed did not correlate with reactivation strength (**Supplementary Fig. 4** online).

It has been reported previously that cofiring is stronger during SWRs after exploration in

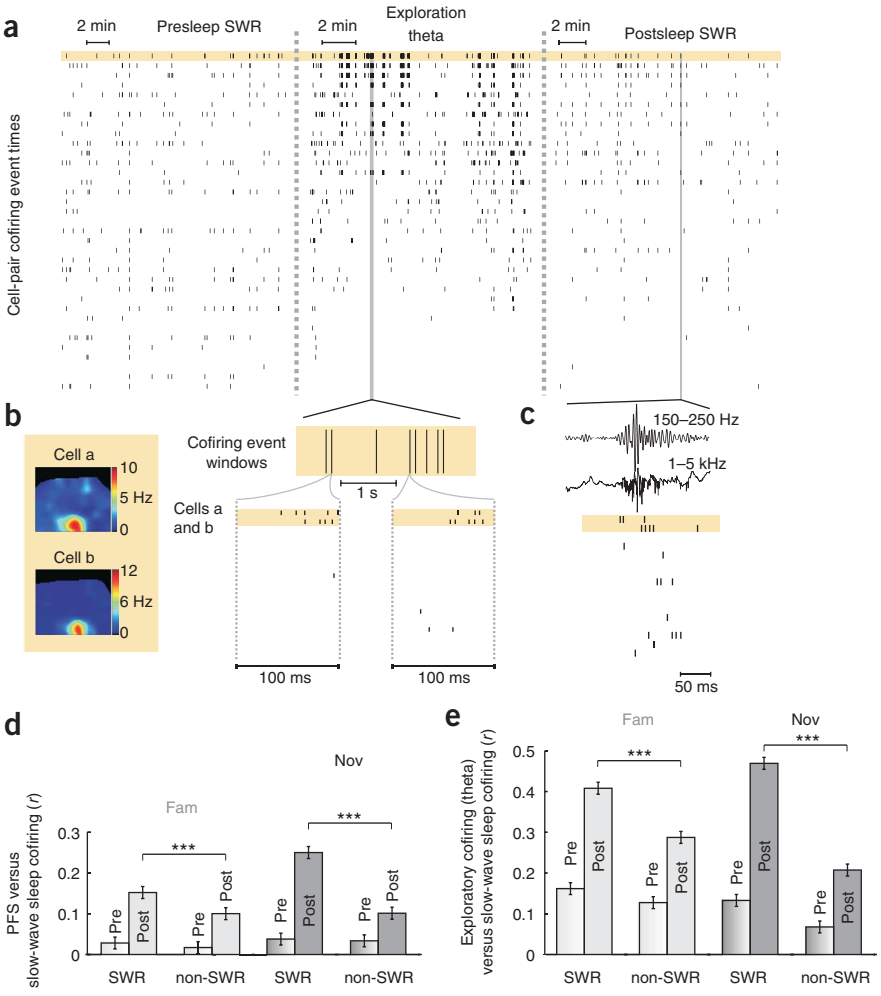

Figure 1 Place-related firing patterns are reactivated during sleep. (**a**) Raster plot marking the times of coincident firing (in 100-ms windows) of 35 CA1 pyramidal-cell pairs during exploration and SWRs. Different cell pairs, plotted in separate lines, were sorted according to the number of cofiring events during exploration of a novel environment. Note the maintained cofiring relationship of cell pairs from exploration to sleep after (post), but not before (pre), exploration. (**b**) Top raster, cofiring events for the highlighted pair in **a** are expanded for the marked period (place fields on the left). Spike raster plots of all pyramidal cells are shown below for two marked cofiring events. (**c**) Top traces, field recording during a SWR event marked in **a**. Both wide-band (1 Hz – 5 kHz) field and its band-pass filtered (150–250 Hz) trace are shown. Raster plot, spike times of pyramidal cells during the SWR. (**d**) PFS of cell pairs was more strongly correlated with sleep cofiring after exploration than before ($P < 0.00001$). Correlations between PFS and sleep cofiring were stronger during SWR than during non-SWR epochs ($P < 0.0013$) and stronger in the novel (Nov) than in the familiar (Fam) condition (Nov > Fam, $P < 0.00001$). (**e**) Exploratory cofiring showed a significantly stronger correlation with sleep cofiring after exploration than before ($P < 0.00001$). These correlations were stronger during SWR than during non-SWR epochs ($P < 0.00001$) and strongest in the novel condition (Nov > Fam, $P < 0.00001$). Error bars in **d** and **e** indicate ± s.e.m. of the correlation coefficient.

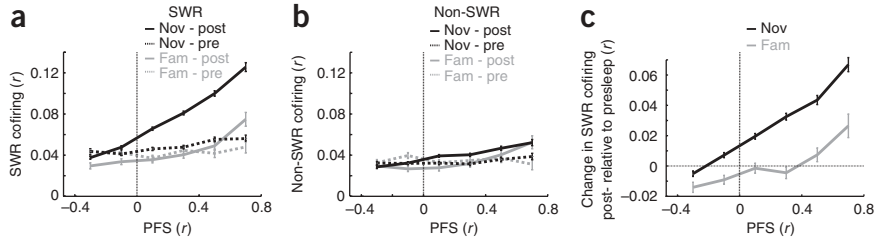

Figure 2 PFS predicts sleep cofiring (post) and cofiring change (from pre to post). (**a**,**b**) Mean cofiring during SWR (**a**) and non-SWR (**b**) events as a function of PFS. Note the wider cofiring range for SWR periods as compared with non-SWR periods. (**c**) Mean change in SWR cofiring as a function of PFS. Change in SWR cofiring was correlated with PFS (familiar, $r = 0.125$; novel, $r = 0.184$; $P < 10^{-10}$). Error bars in **b** and **c** indicate ± s.e.m.

novel places than after exploration in familiar places[16]. Indeed, in our dataset, the mean SWR cofiring correlation for all cells pairs was significantly greater during sleep after novel exploration than it was presleep (average r: presleep, 0.042; postsleep, 0.0638; t-test, $P < 0.00001$). However, this novelty-associated cofiring increase was stronger for place cells that had overlapping place fields; we observed a steeper regression slope in the novel condition when PFS was related to sleep cofiring during SWRs (novel > familiar, ANOVA, $P < 0.00001$; **Fig. 2a**).

To summarize, sleep cofiring was stronger for place cells that encoded similar places during SWRs compared with non-SWR periods, with the strongest cofiring being observed following exposure to a novel environment. Such an increase may help to orthogonalize network firing patterns representing different places and to facilitate a more accurate replay during SWR than during non-SWR periods, particularly after a single exposure to a new environment.

Activity-dependent changes in firing associations

The reorganization of firing patterns needed for reactivation during SWR patterns could be established during exploration as a result of increasing or decreasing firing associations between cells, or both. To measure changes in cell firing associations, we calculated the change in cofiring between pre- and post-SWR as a function of PFS. Both positive and negative changes were seen, and the change of firing associations was correlated (familiar, $r = 0.125$; novel, $r = 0.184$; $P < 10^{-10}$) with PFS (**Fig. 2c**).

Previous work has shown that reactivation during waking SWRs improves with experience; it is stronger in immobility SWRs that occur in later parts of exploration[11]. Moreover, such reactivation in immobility SWRs more accurately reflects waking patterns if they were preceded by behavior that involves the repetition of stereotyped movement paths[18]. This suggests that more frequently visited places show stronger reactivation. Therefore, we tested whether cofiring during SWRs was particularly enhanced for cells with overlapping fields in places where the rat spent the most time (**Fig. 3**). From our set of cell pairs with overlapping place fields ($r > 0.1$), we determined the extent of the overlap ('cofiring field'). We found that the more time that rats spent in the cofiring field, the greater the increase in cofiring from pre- to post-exploration SWR (<4 min, novel: $r = 0.186$, $P < 10^{-10}$; familiar: $r = 0.169$, $P < 7 \times 10^{-6}$; **Fig. 3c**). This correlation was significant if we excluded cofiring fields that the animal visited less frequently (<30 s; novel, $r = 0.09$; familiar, $r = 0.11$; $P < 0.005$). The time spent in cofiring fields may be related to the PFS between cells. Therefore, we repeated the analysis, using partial correlations to control for the effect of PFS. However, increased time in cofiring fields was associated with increased cofiring, even when the effect of PFS was removed (familiar, $r = 0.141$; novel, $r = 0.14$; $P < 0.0005$).

This analysis indicates that the more cell pairs that fire together during exploration, the larger the increases in firing association from pre- to postsleep SWRs. For all possible cell pairs, we counted the total number of 100-ms time windows with joint firing during exploration. Cells with low cofiring counts (<50) showed weak, but significant, reductions in firing association (change < 0, t-test; familiar and novel, $P < 0.0001$). In contrast, pairs with higher (>50, **Fig. 4a**) cofiring counts showed increasingly positive changes; the cofiring counts were positively correlated with the change in firing association in both the familiar ($r = 0.144$, $P < 10^{-10}$) and novel ($r = 0.207$, $P < 10^{-10}$) conditions. Even after controlling for PFS, the number of cofiring events remained a strong predictor of changes in association (novel, $r = 0.186$; familiar, $r = 0.134$; $P < 0.00001$). These results are strongly indicative of activity-dependent changes in firing association, a change

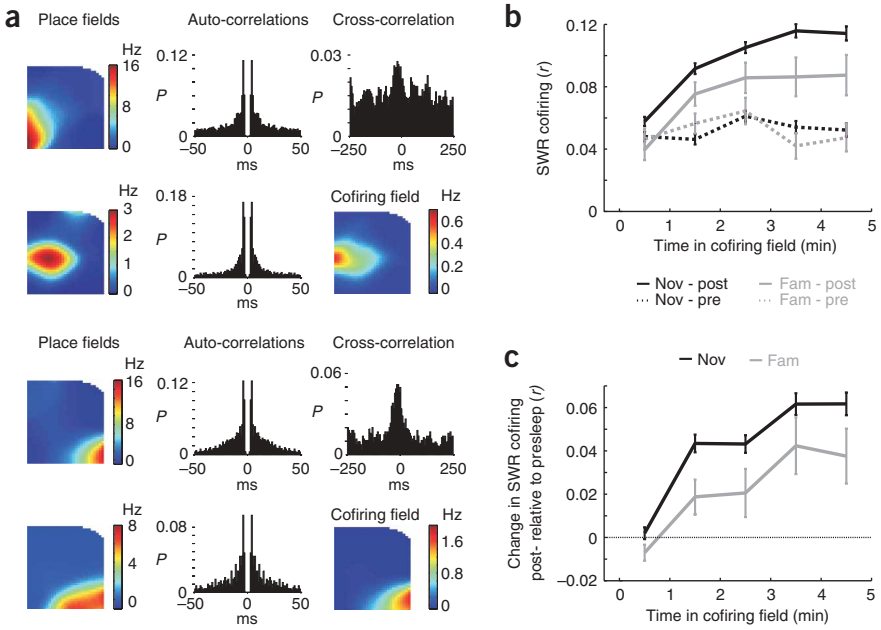

Figure 3 Cells that fire together in more frequently visited locations show stronger cofiring in SWR periods. Regions of place-field overlap were determined for cell-pairs with overlapping place fields ($r > 0.1$) by calculating their cofiring field (**Supplementary Methods** online). (**a**) Examples of cell pairs with overlapping place fields are shown with their cofiring fields, auto- and cross-correlograms. (**b**) SWR cofiring increases with the time spent in the cofiring field. (**c**) Change in SWR cofiring (from pre to post) also increases with time in cofiring field. Error bars in **b** and **c** indicate ± s.e.m.

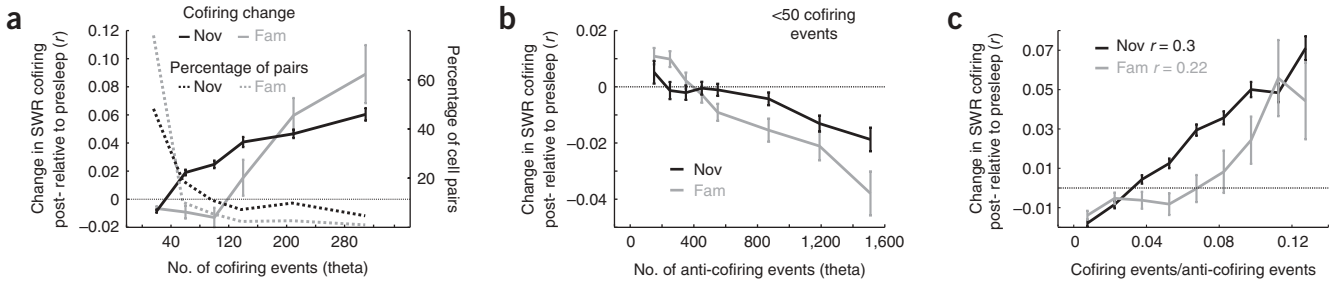

Figure 4 Ensemble firing patterns during sleep reflect the number of times that cells fire together during exploration. (**a**) Solid lines mark the mean change in SWR cofiring (from pre to post) as a function of the number of windows in which the cells fired together during exploration (cofiring events). Dotted lines mark the proportion of pairs that fired together for different cofiring event counts. The change in SWR cofiring was correlated with the number of cofiring events (familiar, $r = 0.144$; novel, $r = 0.207$; $P < 10^{-10}$). Note that fewer cofiring events were required for increases in cofiring from pre to post sleep in the novel condition. (**b**) Cell pairs with few cofiring events (<50) showed a reduction in firing association that was correlated with the number of times that each cell in the pair fired separately (anti-cofiring event) during exploration (familiar: $r = -0.117$, $P < 10^{-10}$; novel: $r = -0.065$, $P < 7 \times 10^{-10}$). (**c**) The ratio of cofiring events to anti-cofiring events predicts the change in cofiring from pre to post sleep (familiar, $r = 0.22$; novel, $r = 0.3$; $P < 0.00001$). Error bars indicate ± s.e.m.

which cannot be completely explained by static features of the place map (that is, the similarity between pairs of place fields).

Cofiring increases are stronger than expected by firing-rate change

It has been suggested before that if reactivation simply reflects pre-existing 'cognitive map' cell assemblies, the firing rate changes of cells alone should explain the strength of reactivation[26]. To investigate the potential interaction between rate and reactivation, we first tested the relationship between changes in cell-pair firing rate and cofiring. We calculated the firing rate and cofiring for each pair during SWRs in each sleep session. From the pre- to the postexploration sleep session, the change in firing rate correlated with the change in cofiring during both familiar and novel exploration (novel, $R = 0.19$; familiar, $R = 0.08$; $P < 0.00001$). Thus, a relationship between changes in rate and cofiring exists in our data. Nevertheless, if associative changes do occur, they should remain significant after the effect of firing rate has been statistically removed. Therefore, we tested whether the number of cofiring counts still predicted the changes in firing association between pre- and postsleep after controlling for changes in rate across the sleep sessions. The correlation between the number of cofiring events during exploration and the change in sleep-SWR cofiring remained significant (novel, $r = 0.17$; familiar, $r = 0.12$; $P < 0.00001$). This analysis supports the conclusion that the observed reactivation is driven by associative changes and not solely by changes in rate during sleep. Next, we tested whether the number of cofiring events predicted changes in association, independent of both firing rates and the configuration of place maps. We again carried out the partial correlation analysis described above, while controlling for rate changes and PFS. Even under these conditions, the number of cofiring events significantly predicted the change in SWR cofiring (novel, $r = 0.15$; familiar, $r = 0.11$; $P < 0.00001$). Thus, the number of times that cells fired together during exploration was a robust predictor of the change in cofiring, independent of any influence relating to changes in firing rate or to the configuration of place maps in the environment.

Reduction of cofiring is also activity dependent

We found specific reductions in firing association between cells with nonoverlapping exploratory firing patterns (**Fig. 2c**). To test whether the reduction in firing association between the cells was activity dependent, we counted the number of times that either cell fired on its own (anti-cofiring events). Of the cell pairs with few (<50) cofiring events, the change in cofiring was negatively correlated to the number of anti-cofiring events (novel, $r = -0.065$, $P < 7 \times 10^{-10}$; familiar, $r = -0.117$, $P < 10^{-10}$; **Fig. 4b**). In both the novel and familiar conditions, cells that fired together <50 times, but had >500 anti-cofiring events, had significantly higher individual firing rates during SWR sleep than those with <500 anti-cofiring events (ANOVA, $P < 0.0001$). In this way, cells that encode different places and show high individual SWR rates will show divergent firing patterns. Finally, the ratio of cofiring counts to anti-cofiring counts predicted the change in firing association from pre- to postsleep (**Fig. 4c**). Cells with a greater tendency to fire independently showed negative changes. Thus, both the negative and positive changes in firing association occur as a result of activity-dependent processes.

The change in cofiring is also related to the joint-firing interval

Various time windows have been suggested as being optimal for neuronal plasticity, including gamma and theta oscillation wavelengths[28–32]. Therefore, we sought to determine the optimal time interval for exploratory cell-pair firing that may lead to changes in firing associations. We measured the mean of the minimum spike

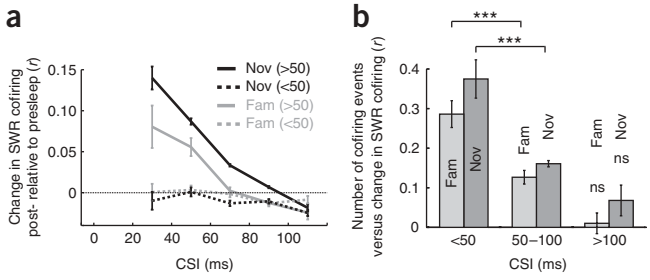

Figure 5 Average cofiring change as a function of the mean CSI. (**a**) The minimum interval between spike pairs in a ± 200-ms spike-centered window (CSI). Only pairs with >50 cofiring events were included. All CSIs were between 15 and 125 ms. In cell pairs with many cofiring events (>50), the change in SWR cofiring negatively correlated with CSI (familiar, $r = -0.181$; novel, $r = -0.24$; $P < 10^{-10}$). Error bars indicate ± s.e.m. (**b**) The correlation between the number of exploratory cofiring events and the change in SWR cofiring was strongest for pairs that fired together at short latencies (<50 ms versus 50–100 ms, $P < 0.00001$ for both the familiar and novel conditions). Error bars indicate ± s.e.m. of the correlation coefficient.

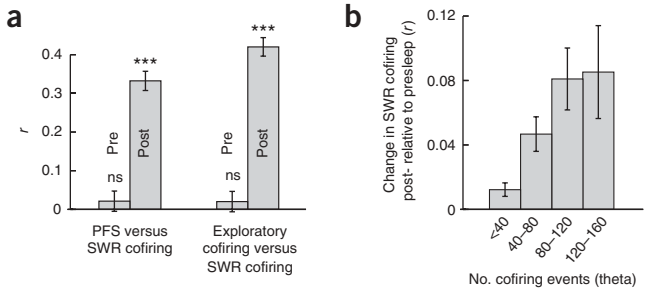

Figure 6 Reactivation of CA3-CA1 cell ensembles after exploration in novel environments. (**a**) We recorded 1,440 CA3-CA1 pyramidal-cell pairs during exploration in novel environments ($n = 10$ sessions). Both the PFS and cofiring during exploratory theta epochs of CA3-CA1 cell pairs predicted SWR cofiring in post sleep ($P < 0.00001$). No relationship was seen between waking patterns and cofiring in presleep sessions ($P > 0.1$). Error bars indicate ± s.e.m. of the correlation coefficient. (**b**) The change in cofiring from pre to post sleep was correlated with the number of CA3-CA1 cell pair cofiring events ($r = 0.11$, $P < 0.005$). Error bars indicate ± s.e.m.

interval between cell pairs. Across the population, cell pairs with a <50-ms mean cofiring spike intervals showed a significantly stronger increase in association than those with >100-ms intervals (familiar and novel conditions, ANOVA, $P < 0.0001$). Overall, shorter minimum spike intervals were associated with larger cofiring increases. Moreover, this effect was primarily observed for cell pairs that fired together most often (>50 cofiring events), whereas it was weak for cases with cofiring counts <50 (>50: novel, $r = –0.24$; familiar, $r = –0.181$; $P < 10^{-10}$; <50: novel, $r = –0.042$; familiar, $r = –0.062$; $P < 0.001$; **Fig. 5a**). Finally, the relationship between cofiring counts and firing association was strongest for cell pairs with a <50-ms minimum spike interval ($P < 0.00001$; **Fig. 5b**). This indicates that, during exploration, both the number of times that cell pairs fired together and the interval between their firing contribute to increased firing association in subsequent sleep.

Reactivation of CA3-CA1 region cell pairs

In a subset of sessions (ten session in four animals), we recorded the activity of CA3 cells (in one or two electrodes) and CA1 cells in the novel condition. This enabled us to examine whether the joint firing of CA3 and CA1 place cells are reactivated during sleep. We detected 107 CA3 pyramidal cells that were recorded simultaneously with 484 CA1 pyramidal cells, yielding a total of 1,440 possible CA3-CA1 cell pairs. However, not enough data (two sessions with a total of 12 CA3 cells) were available to repeat the analysis in the familiar condition. The waking patterns of CA3-CA1 pairs were significantly correlated with postsleep cofiring during detected SWR epochs (PFS versus postsleep SWR, $r = 0.33$; exploratory cofiring versus post sleep SWR, $r = 0.42$; $P < 0.000001$). However, no relationship was seen with presleep ($P > 0.1$). This suggests that CA3 and CA1 cells that encode similar regions are reactivated simultaneously. Finally, we examined whether activity-dependent changes determine firing association changes between CA3-CA1 cells. The number of joint firing events significantly predicted the change in firing association from pre- to postsleep ($r = 0.11$, $P < 0.0005$; **Fig. 6**).

DISCUSSION

We have shown that reactivated patterns seen in sleep-SWR are established through increased firing associations between cells that fire in similar locations during exploration. These changes in firing association are activity dependent, and the largest increases occurred between cells representing the most visited regions of the environment. These changes were dependent on the number of times that cells fired together with short latencies during exploration, so that the largest increases were seen between cells firing <50 ms apart. The modification of firing associations following repeated joint firing suggests that these processes follow a Hebbian leaning rule.

A past modeling study put forward two alternative models for reactivation, one that requires Hebbian modification and another that simply requires nonassociative changes in firing rates[26]. The latter model assumed that synaptic weights do not change between CA3 cells during exploration. In this case, increases in the firing rates of the spatially active cells, concomitant with a reduction in sleep rates for cells with low exploratory firing rates, could result in reactivated ensemble firing patterns. In support of the 'associative' model, our findings indicate that reactivation cannot be explained by firing-rate changes alone. We have shown that replay was behavior dependent; the cofiring was strongest during sleep for cells encoding frequently visited places. However, increased firing associations were significantly predicted by the number of cofiring events, even after controlling for both firing-rate changes and PFS. Thus, changes in firing associations are directly influenced by the joint-firing patterns of cells during exploration, which are in turn governed by behavior. Taken together, the most likely explanation for our findings is that reactivation is brought about by the potentiation of synapses between connected CA3-CA3 and CA3-CA1 cells that encode similar and frequently visited locations during exploration. However, it has yet to be determined whether such synaptic changes serve to establish new cell assemblies or to strengthen already existing ones.

We found that the strongest reactivation occurred during SWRs. Because hippocampal SWRs originate in the CA3 region, the reactivated patterns that are observed during SWRs are also likely to be initiated in this region[4,11,22]. Thus, our changes in firing associations in the CA1 region are most likely due to synaptic changes in upstream CA3-CA1 and CA3-CA3 connections. In support of this, we have seen reactivation between CA3-CA1 cell pairs, indicating that cells representing similar places are reactivated at the same time. Firing association changes between CA3-CA1 cell pairs also increased with the number of times that they fired together; indicating that Hebbian rules govern their changes as well as those of CA1 pairs.

Hebbian plasticity has been suggested to be required for the subsequent recall of newly formed place maps and for associative memory recall, as suggested by the NMDA dependence of these processes[33,34]. Moreover, it has been previously reported that blockade of NMDA receptors also prevents reactivation (J.J. Stanis *et al. Soc. Neurosci. Abstr.* 329.20, 2004). This too suggests that Hebbian plasticity underlies reactivation. However, NMDA blockade is also known to reduce SWR-occurrence frequency and to alter the frequency of ripple oscillations. Spike timing–dependent plasticity (STDP)[29,31] has been suggested as a possible Hebbian mechanism by which synaptic weights change during exploration. However, STDP may not explain the observed firing association increases in our procedure. We used 'open field' environments where the firing order of cell pairs with overlapping place fields is expected to be random, in which case STDP rules would suggest both potentiation and depression for subsequent pairings. A recent study suggested that potentiation between CA3-CA1 synapses requires fewer pairings, but in shorter time windows, than depression[35]. This suggests that cell pairs that frequently fire together in the same order (as is the case on the linear track) can undergo either potentiation or depression depending on their order, whereas cells that fire together in short time windows, but with a weaker sequence bias

(such as the case on an open field), may favor potentiation. Another line of work has shown that dendritic depolarization is sufficient for plasticity in CA1 cells; back-propagating action potentials (needed for STDP) are not required[36,37]. Place cells firing inside their place fields are expected to be depolarized. Thus, sufficient depolarization may enable the potentiation of connections between spatially active CA3 and CA3-CA1 cells that fire together.

The data presented here indicate that a single exploration session in a novel environment is sufficient for reactivation to be observed. A previous study observed a weak reactivation of novel environment–associated waking patterns during sleep[16]. The animal explored both the novel and familiar portions of the track before sleep, but the familiar portion was always the last to be explored. This may explain the weakened reactivation of the novel exploration patterns, considering that we found stronger negative changes in firing associations between nonoverlapping place cells in the familiar environments. In this case, the associations between cells on the novel half of the track may be weakened by independent firing in the familiar half of the environment.

It has been reported that average cofiring is higher during sleep after exposure to novel environments than to familiar ones[16]. This was also evident in our data. Moreover, our data indicate that reactivation was stronger following novel exploration; place cells with overlapping place fields showed stronger cofiring increases in sleep following exploration of novel, rather than familiar, environments. We observed that fewer cofiring events were required for an increase firing association following novel exploration. This suggests that some factor enhances the efficacy with which spike coincidence affects firing associations. Indeed, exploration in novel environments has been shown to lower the threshold for LTP in the CA1 region, an effect that was dopamine dependent[38]. In addition, the increased cofiring of place cells with overlapping fields during exploration in novel environments may also contribute to their stronger reactivation. Changes in neuromodulation and inhibitory tone during novelty[39,40] may promote an enhanced plasticity in novel environments, as well as increases in waking cofiring.

Synaptic efficacy could not increase unchecked without producing saturation, which may interfere with learning[41–43]. Our data show that increases in firing associations are coupled with a specific reduction in cofiring between those cells that fire in different locations. These changes were also activity dependent, as they were correlated with the number of times that cells fired separately, an effect that is stronger in the familiar condition. Thus, increases in cofiring during exploration for certain cells are balanced by decreases, particularly with repeated visits. Negative changes were weaker than positive changes; however, they affected a larger proportion of the cell pairs. Over time, these reductions in firing associations may also serve to separate assemblies representing nonoverlapping places.

In summary, our data indicate that firing association increases between cells followed a Hebbian learning rule[27]; that is, the changes in cofiring between sleep sessions are directly related to the number of times that cells fired together during exploration. The increases in firing associations were controlled by the spatial behavior of the animal. Cells that fired together in frequently visited places showed the strongest increases, implying that it was not the cognitive map *per se* that was reactivated during sleep. This suggests that behavioral experiences are reactivated during SWRs. This notion is also supported by recent studies showing that reactivation during SWRs recorded in waking immobility periods improves with experience[18,19]. Thus, SWR events may correspond with the replay of episodic-like memory traces of visited places[4]. Although place maps are thought to be stored in the hippocampus, the final repository of episodic memory traces is extrahippocampal[1,7]. Elevated firing during SWR epochs has been proposed as the mechanism by which hippocampal representations are transferred to the entorhinal cortex and, ultimately, the neocortex[4,20]. In this context, our data support the idea that SWRs facilitate the transfer of episodic-like memory traces to the cortex.

METHODS

Animals. All procedures involving animals were carried out in accordance with the Animals (Scientific Procedures) Act, 1986 (UK) and associated procedures under an approved project license. In total, 14 rats were implanted with independently moveable arrays of 4–16 tetrodes in the superficial layers of the neocortex above the right dorsal portion of the hippocampus, as previously described[11]. Following 7 d of postoperative recuperation, the tetrodes were lowered into the CA1 region of the hippocampus over a further period of around 7 d. Wide-band (0.1/1 Hz – 5 kHz) recordings of local field potentials and multiple-unit activity were amplified 1,000-fold (Sensorium Amplifier) and digitized at 20 kHz using a 64-channel AD converter (United Electronics Industries). Small light-emitting diodes mounted on the housing containing the electrode arrays were used to track the location of the animal.

Training and testing protocol. The recording apparatus used consisted of various environments, each located in different places in the same recording room. A variety of differently shaped open-field environments were used, including rectangular and more complex shapes (**Supplementary Fig. 5** online). The length and width of each environment ranged between ~40 and 150 cm long. Food and water were freely available for the animal before the recording procedures. The animal was familiarized with two of the environments for at least 30 min over several days while the electrodes were being lowered, and the remaining arenas were reserved for novel sessions. During the recording phase, the animals both explored and slept in the same recording apparatus. The speed of the animal was monitored throughout recording sessions. In waking sessions, the animal was encouraged to explore the whole environment (**Supplementary Fig. 5**) for at least 15 min using, as needed, appetitive food reward (chocolate sprinkles). Following exploration the rat was allowed to settle into sleep. After a successful recording of an exploration-sleep sequence, the animal was placed directly into a different environment for more exploration and subsequent sleep. In this way, sequences of sleep-exploration-sleep could be recorded, where the presleep was recorded after exploration of a different environment. For the familiar condition, the first and second exploration sessions were recorded in different familiar environments, whereas for the novel condition, the presleep was preceded by exploration of a familiar environment. In all, a total of 15 and 33 cycles of sleep-explore-sleep were analyzed for the familiar and novel conditions, respectively.

Unit isolation and selection. Unit isolation and clustering procedures have been described previously[11,44]. Only units with clear refractory periods in their autocorrelation and well-defined cluster boundaries[45] were used for further analysis; an isolation distance (based on Mahabalonis distance[45]) was calculated to ensure that the selected spike clusters did not overlap during the course of the recordings. Pyramidal cells and interneurons were discriminated by their autocorrelations, firing rates and wave forms, as previously described[46]. Because our goal was to analyze changes in the joint-firing patterns of pyramidal cell ensembles over different time points, we needed to ensure that our sample of cells was taken from clusters with stable firing. We therefore clustered together periods of waking spatial exploration and pre/postsleep sessions and verified the stability of the recorded cells over time, so that cells could be selected by their wave-form stability. We calculated the first principle component of the recorded wave forms for each cell. We only included cells with mean first principle components differing by less than one s.d. between the first and last quarter of the recording. Additionally, the firing rates in the first and last recorded sleep sessions were calculated. Only those cells that fired in both sleep sessions and had rates that did not deviate between the sessions by more than 70% were included. In total, 613 (in 33 sessions) pyramidal cells in the novel and 309 (in 15 sessions) in the familiar conditions were included in the analysis.

Coactive firing pattern analysis. Only cell pairs recorded from different tetrodes were compared, yielding an available combination of cell pairs of 19,054 and 4,865 in the novel and familiar conditions, respectively. To measure

the degree to which cells fire together, we took spike counts in consecutive 100-ms windows during theta exploratory epochs and in the sleep periods as described above, and calculated the Pearson's correlation coefficient of spike counts. Only those correlations in which both cells fired at least 50 times in the measurement time windows were considered. A cofiring event during exploration was defined as a window in which spikes were detected from both cells in the pair, and the sum of these events during exploration provided the cofiring count. The average minimum cofiring spike interval (CSI) for each cell pair was calculated during exploration (theta epochs only, speed >2 cm s^{-1}) as follows. The spike times of the first cell in the pair were used as a reference and the closest action potential fired by its partner was detected within 200 ms before or after the reference spike. These CSIs were measured across the exploration session and their mean was calculated for each pair.

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

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AUTHOR CONTRIBUTIONS

J.O. carried out the data analysis. T.J.S., J.O., J.C. and K.A. conducted the experiments and spike clustering. J.C., J.O. and J.R.H. wrote the manuscript. J.C. supervised the project.

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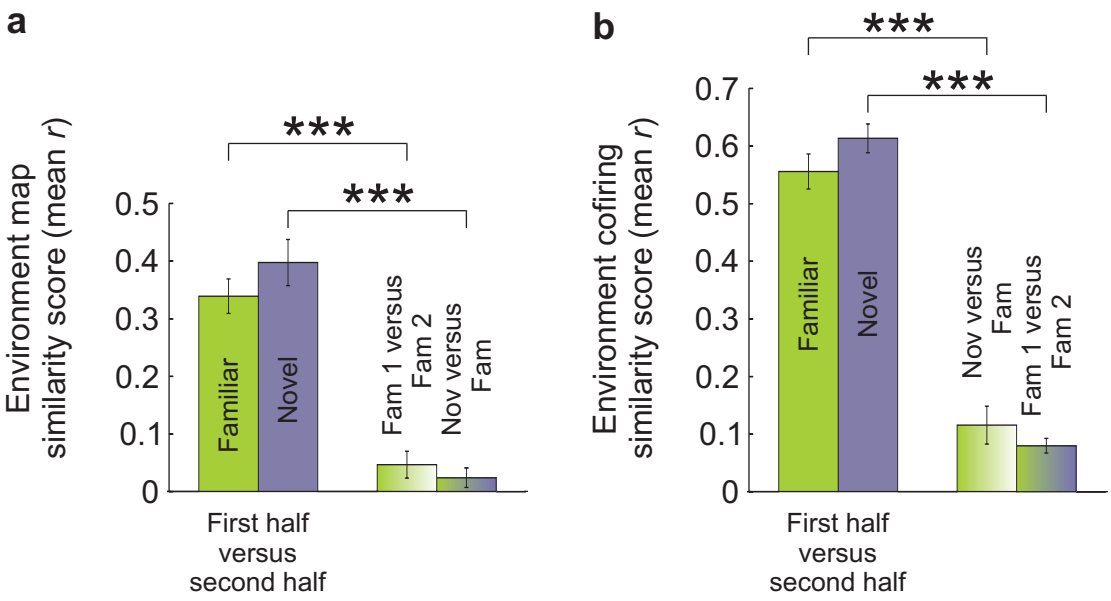
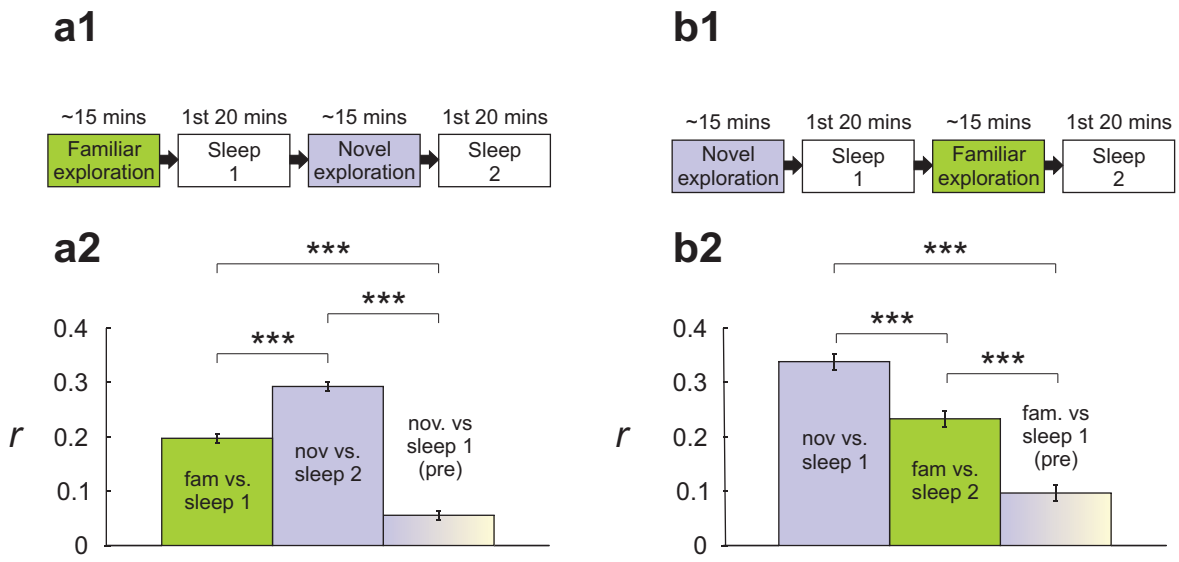
1. Squire, L.R. Memory and the hippocampus: a synthesis from findings with rats, monkeys and humans. *Psychol. Rev.* **99**, 195–231 (1992).
2. O'Keefe, J. & Nadel, L. *Hippocampus as a Cognitive Map* (Oxford University Press, Oxford, 1978).
3. Morris, R.G., Garrud, P., Rawlins, J.N. & O'Keefe, J. Place navigation impaired in rats with hippocampal lesions. *Nature* **297**, 681–683 (1982).
4. Buzsaki, G. Two-stage model of memory trace formation: a role for 'noisy' brain states. *Neuroscience* **31**, 551–570 (1989).
5. McClelland, J.L., McNaughton, B.L. & O'Reilly, R.C. 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.* **102**, 419–457 (1995).
6. Wilson, M.A. & McNaughton, B.L. Reactivation of hippocampal ensemble memories during sleep. *Science* **265**, 676–679 (1994).
7. Morris, R.G. Elements of a neurobiological theory of hippocampal function: the role of synaptic plasticity, synaptic tagging and schemas. *Eur. J. Neurosci.* **23**, 2829–2846 (2006).
8. Pavlides, C. & Winson, J. Influences of hippocampal place cell firing in the awake state on the activity of these cells during subsequent sleep episodes. *J. Neurosci.* **9**, 2907–2918 (1989).
9. Hirase, H., Leinekugel, X., Czurko, A., Csicsvari, J. & Buzsaki, G. Firing rates of hippocampal neurons are preserved during subsequent sleep episodes and modified by novel awake experience. *Proc. Natl. Acad. Sci. USA* **98**, 9386–9390 (2001).
10. Battaglia, F.P., Sutherland, G.R., Cowen, S.L., Mc Naughton, B.L. & Harris, K.D. Firing rate modulation: a simple statistical view of memory trace reactivation. *Neural Netw.* **18**, 1280–1291 (2005).
11. O'Neill, J., Senior, T. & Csicsvari, J. Place-selective firing of CA1 pyramidal cells during sharp wave/ripple network patterns in exploratory behavior. *Neuron* **49**, 143–155 (2006).
12. Skaggs, W.E. & McNaughton, B.L. Replay of neuronal firing sequences in rat hippocampus during sleep following spatial experience. *Science* **271**, 1870–1873 (1996).
13. Louie, K. & Wilson, M.A. Temporally structured replay of awake hippocampal ensemble activity during rapid eye movement sleep. *Neuron* **29**, 145–156 (2001).
14. Lee, A.K. & Wilson, M.A. Memory of sequential experience in the hippocampus during slow wave sleep. *Neuron* **36**, 1183–1194 (2002).
15. Nadasdy, Z., Hirase, H., Czurko, A., Csicsvari, J. & Buzsaki, G. Replay and time compression of recurring spike sequences in the hippocampus. *J. Neurosci.* **19**, 9497–9507 (1999).
16. Kudrimoti, H.S., Barnes, C.A. & McNaughton, B.L. Reactivation of hippocampal cell assemblies: effects of behavioral state, experience and EEG dynamics. *J. Neurosci.* **19**, 4090–4101 (1999).
17. Foster, D.J. & Wilson, M.A. Reverse replay of behavioural sequences in hippocampal place cells during the awake state. *Nature* **440**, 680–683 (2006).
18. Jackson, J.C., Johnson, A. & Redish, A.D. Hippocampal sharp waves and reactivation during awake states depend on repeated sequential experience. *J. Neurosci.* **26**, 12415–12426 (2006).
19. Csicsvari, J., O'Neill, J., Allen, K. & Senior, T. Place-selective firing contributes to the reverse-order reactivation of CA1 pyramidal cells during sharp waves in open-field exploration. *Eur. J. Neurosci.* **26**, 704–716 (2007).
20. Chrobak, J.J. & Buzsaki, G. Selective activation of deep layer (V–VI) retrohippocampal cortical neurons during hippocampal sharp waves in the behaving rat. *J. Neurosci.* **14**, 6160–6170 (1994).
21. Buzsaki, G. Hippocampal sharp waves: their origin and significance. *Brain Res.* **398**, 242–252 (1986).
22. Csicsvari, J., Hirase, H., Mamiya, A. & Buzsaki, G. Ensemble patterns of hippocampal CA3–CA1 neurons during sharp wave–associated population events. *Neuron* **28**, 585–594 (2000).
23. Ylinen, A. *et al.* Sharp wave–associated high-frequency oscillation (200 Hz) in the intact hippocampus: network and intracellular mechanisms. *J. Neurosci.* **15**, 30–46 (1995).
24. Bostock, E., Muller, R.U. & Kubie, J.L. Experience-dependent modifications of hippocampal place cell firing. *Hippocampus* **1**, 193–205 (1991).
25. Muller, R.U. & Kubie, J.L. The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells. *J. Neurosci.* **7**, 1951–1968 (1987).
26. Shen, B. & McNaughton, B.L. Modeling the spontaneous reactivation of experience-specific hippocampal cell assembles during sleep. *Hippocampus* **6**, 685–692 (1996).
27. Hebb, D.O. *The Organization of Behavior: a Neuropsychological Theory* (Lawrence Erlbaum Associates, New York, 1949).
28. Huerta, P.T. & Lisman, J.E. Bidirectional synaptic plasticity induced by a single burst during cholinergic theta oscillation in CA1 *in vitro*. *Neuron* **15**, 1053–1063 (1995).
29. Markram, H., Lubke, J., Frotscher, M. & Sakmann, B. Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science* **275**, 213–215 (1997).
30. Magee, J.C. & Johnston, D. A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. *Science* **275**, 209–213 (1997).
31. Bi, G.Q. & Poo, M.M. Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength and postsynaptic cell type. *J. Neurosci.* **18**, 10464–10472 (1998).
32. Paulsen, O. & Sejnowski, T.J. Natural patterns of activity and long-term synaptic plasticity. *Curr. Opin. Neurobiol.* **10**, 172–179 (2000).
33. Nakazawa, K. *et al.* Requirement for hippocampal CA3 NMDA receptors in associative memory recall. *Science* **297**, 211–218 (2002).
34. Kentros, C. *et al.* Abolition of long-term stability of new hippocampal place cell maps by NMDA receptor blockade. *Science* **280**, 2121–2126 (1998).
35. Wittenberg, G.M. & Wang, S.S. Malleability of spike timing–dependent plasticity at the CA3–CA1 synapse. *J. Neurosci.* **26**, 6610–6617 (2006).
36. Golding, N.L., Staff, N.P. & Spruston, N. Dendritic spikes as a mechanism for cooperative long-term potentiation. *Nature* **418**, 326–331 (2002).
37. Lisman, J. & Spruston, N. Postsynaptic depolarization requirements for LTP and LTD: a critique of spike timing–dependent plasticity. *Nat. Neurosci.* **8**, 839–841 (2005).
38. Li, S., Cullen, W.K., Anwyl, R. & Rowan, M.J. Dopamine-dependent facilitation of LTP induction in hippocampal CA1 by exposure to spatial novelty. *Nat. Neurosci.* **6**, 526–531 (2003).
39. Nitz, D. & McNaughton, B. Differential modulation of CA1 and dentate gyrus interneurons during exploration of novel environments. *J. Neurophysiol.* **91**, 863–872 (2004).
40. Giovannini, M.G. *et al.* Effects of novelty and habituation on acetylcholine, GABA and glutamate release from the frontal cortex and hippocampus of freely moving rats. *Neuroscience* **106**, 43–53 (2001).
41. Castro, C.A., Silbert, L.H., McNaughton, B.L. & Barnes, C.A. Recovery of spatial learning deficits after decay of electrically induced synaptic enhancement in the hippocampus. *Nature* **342**, 545–548 (1989).
42. Moser, E.I., Krobert, K.A., Moser, M.B. & Morris, R.G. Impaired spatial learning after saturation of long-term potentiation. *Science* **281**, 2038–2042 (1998).
43. Brun, V.H., Ytterbo, K., Morris, R.G., Moser, M.B. & Moser, E.I. Retrograde amnesia for spatial memory induced by NMDA receptor–mediated long-term potentiation. *J. Neurosci.* **21**, 356–362 (2001).
44. Csicsvari, J., Hirase, H., Czurko, A. & Buzsaki, G. Reliability and state dependence of pyramidal cell–interneuron synapses in the hippocampus: an ensemble approach in the behaving rat. *Neuron* **21**, 179–189 (1998).
45. Harris, K.D., Hirase, H., Leinekugel, X., Henze, D.A. & Buzsaki, G. Temporal interaction between single spikes and complex spike bursts in hippocampal pyramidal cells. *Neuron* **32**, 141–149 (2001).
46. Csicsvari, J., Hirase, H., Czurko, A., Mamiya, A. & Buzsaki, G. Oscillatory coupling of hippocampal pyramidal cells and interneurons in the behaving Rat. *J. Neurosci.* **19**, 274–287 (1999).



Supplementary figure 1

Supplementary Figure 1: Firing patterns of place cells remap in different environments

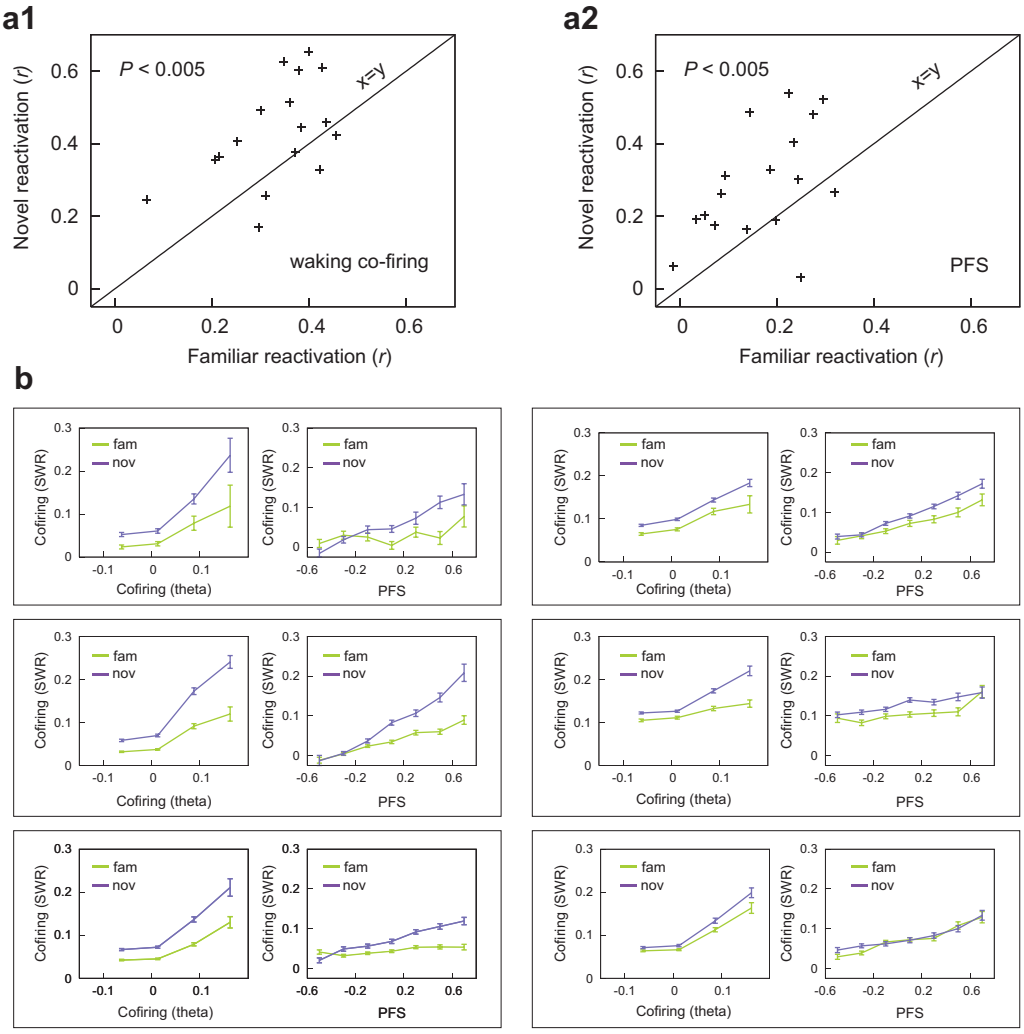
Place field similarity (**a**) and cofiring (**b**) were established from the population of recorded cells for each environment on the same recording day. Spatial firing patterns were weakly or uncorrelated between environments, when either two different familiar, or a familiar and novel environment were compared. However, the spatial firing patterns established in the first half of the exploration were strongly correlated with that in the second half of each environment, in both the novel and familiar exploration sessions. Across all the recording days, the spatial firing patterns were more similar within than between different environments (ANOVA, both familiar and novel, $P < 0.00001$). Error bars: ± SEM.



Supplementary figure 2

Supplementary Figure 2: Reactivation in novel environments is stronger than familiar, irrespective of the order in which they occur during the recording day
Exploration and sleep sessions were performed throughout each recording day, with exploration performed alternately in familiar and novel environments, each followed by a sleep session. Two sets of 4 session sequences were selected; where familiar exploration occurred first (**a**) and where novel exploration occurred prior to familiar (**b**). Of the 33 sessions in the novel condition, 27 were analysed with the preceding familiar exploration session (**a**), and a further 14 sequences novel-sleep-familiar-sleep were analysed together (**b**). In both sets of sessions, the correlation between place field similarity and sleep SWR cofiring was significant ($P < 0.00001$) and stronger in the novel condition ($P < 0.00001$), irrespective of the sequence order. Error bars: ± SE of the correlation coefficient.

Note: In both (**a2**) and (**b2**) the correlation between firing patterns in the second exploration and sleep 1 (i.e. 'pre-sleep') is significantly weaker than that of either familiar or novel exploration sessions in subsequent sleep ($P < 0.00001$). Pre-sleep appears to be larger in (**b2**) than (**a2**), which may be due to the fact that the animal was exposed to the same familiar environment earlier in the recording day (10 out of the 14 sets of sessions shown in **b2** were recorded after a previous exposure to the familiar environment on the same day).

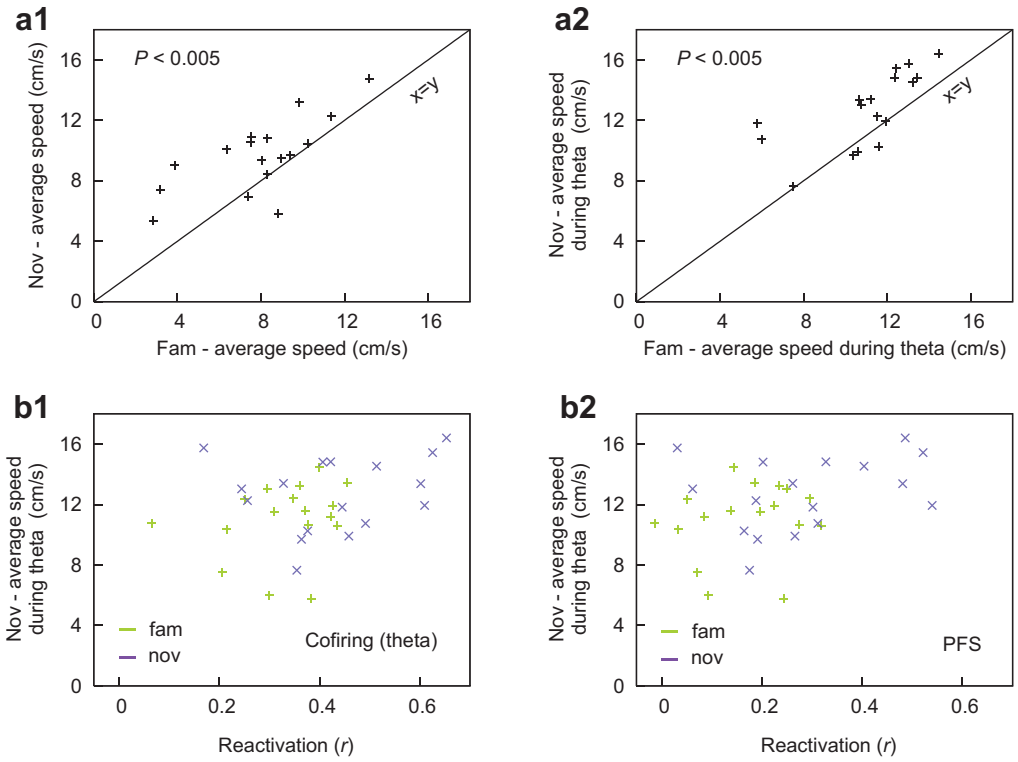


Supplementary figure 3

Supplementary Figure 3: Reactivation in novel and familiar environments
The correlation between waking patterns and SWR cofiring in subsequent sleep was established for each recording day, for both novel and familiar sessions. On each day, the similarity between cell pair waking firing patterns were characterised by place field similarity (PFS) and cofiring during exploratory theta epochs, as before.

a1 & a2: The correlation between waking firing patterns and SWR cofiring in subsequent sleep was stronger in the novel condition, when either waking cofiring (a1) or PFS (b1) was considered (paired t-test, both $P < 0.005$).

b: Post sleep cofiring as a function of waking activity is shown separately for 6 individual recording days, each taken from 6 different animals.

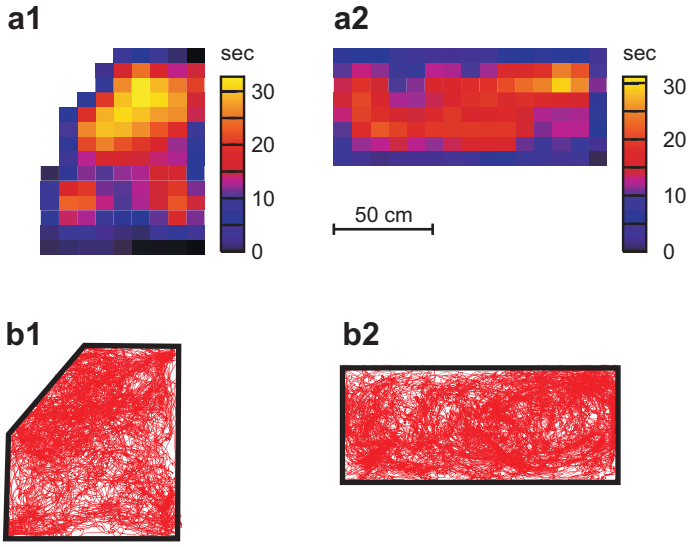


Supplementary figure 4

Supplementary Figure 4: Running speed in familiar and novel environments

a1 & a2: The average speed of the animal was higher in novel environments than familiar, when all waking activity (a1) was analysed or when only exploratory theta epochs (b2) were considered (paired t-test, both $P < 0.05$).

b1 &b2: The correlation between waking firing patterns (either place field similarity - PFS-b1, or waking cofiring –b2) and SWR cofiring is plotted against average running speed during theta epochs, for the novel and familiar conditions, on each recording day. Average running speed did not correlate with reactivation strength in either familiar or novel environments (both $P > 0.3$).



Supplementary figure 5

Supplementary Figure 5: Examples of the animal's movement path and time spent in different regions of the environment during exploration of typical familiar and novel enclosures

a1 & **a2**: Intensity plot to show the distribution of time spent within different regions of a novel (a1) and familiar (a2) environment, during theta epochs with a running speed > 2 cm/s (23 minutes, in each condition). Each pixel represents a 9.2 cm^2 region of the open-field environment.

b1 and **b2**: Movement paths for the environments shown in (a). Border represents the walls of the open field.

Supplementary Methods

Field detection: Both theta and SPW detection were performed as previously described[46,47] . To identify periods of theta activity, the theta/delta power ratio was measured in 1600ms segments (800ms steps in between measurement windows), using Thomson's multi-taper method[48,49]. For the detection of SWRs, local field potentials were band pass filtered (150 – 250 Hz). The power (root mean square) of the filtered signal was calculated for each electrode and summed across electrodes designated as being in the CA1 pyramidal cell layer. The threshold for SWR detection was set to 7 standard deviations (SD) above the background mean.

Definition of exploration and sleep periods: For analysis, recordings were segregated offline onto periods of exploratory activity, immobility and sleep. For each session, the theta/delta ratio was plotted against speed so that the behavioural state could be manually identified. Only theta periods during locomotion (>2 cm/sec) from the selected waking epochs were considered for analysis while waking immobility periods were not included. Sleep epochs were identified by extended periods of continuous immobility (at least 7min) and the presence of REM-theta periods and slow-wave oscillations. All sleep analysis was performed during the first 20 minutes of the slow wave component of sleep during either the SWR or non SWR sleep states (mean duration: 16.9 minutes ± 3.7 SD, from the first to the last detected SWR). A substantially greater proportion of slow wave sleep was formed by non SWR periods than SWR[16]. In order to equalize the time periods analyzed from both states[16], unit activity during SWR was assessed in 100 ms windows centred on the peak of ripple power, whilst the analysis of non SWR periods was confined to an equal number of 100 ms windows taken from randomly selected non-overlapping time points within the inter SWR periods.

Place field and cofiring field analysis. Place rate-maps were calculated as described before[11,45] by a kernel-based method in which both the firing rate and occupancy maps were smoothed with a Gaussian kernel function (SD = 3cm). A Pearson's correlation coefficient of the place rate-maps was calculated to compare place fields (Place Field Similarity –*PFS*). *Cofiring fields* were established between cell pairs with similar fields (PFS $r > 0.1$). The product of spike-count pairs measured in 100ms windows was summed separately at different locations to calculate the cofiring rate maps, which were then divided by the occupancy map. Cofiring maps were smoothed

as described above for the place-rate maps. Empty (covering < 5 percent of the environment) and spatially unselective cofiring maps (covering > 70 percent of the environment) were excluded from further analysis. The boundary of the cofiring map was regarded as areas with rate bins within 80% of the peak rate bin.

Regressions were compared using a Fisher's *z*-transform of the Pearson correlation coefficients[50]. Partial correlations and their significance were calculated as described by Zar (1998). Firing rates were log transformed when assessing whether the relationship between cofiring counts and the change in firing-associations during sleep can occur independently of firing rate changes[10].