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Abstract

Slow-wave sleep (SWS) is important for memory consolidation. During sleep, neural patterns reflecting previously acquired information are replayed. One possibility is that such replay exchanges information between hippocampus and neocortex, supporting consolidation. We recorded neuron ensembles in the rat medial prefrontal cortex (mPFC) to study memory trace reactivation during SWS following learning and execution of cross-modal strategy shifts. In general, reactivation of learning-related patterns occurred in distinct, highly synchronized transient bouts, mostly simultaneous with hippocampal sharp wave/ripple complexes (SPWRs), when hippocampal ensemble reactivation and cortico-hippocampal interaction is enhanced. mPFC neural patterns appearing during response selection replayed prominently coincident with hippocampal SPWRs taking place in sleep, following learning of a new rule. This was learning-

dependent, because it was not observed before rule acquisition. Thus, learning, or the resulting stable reward, influenced which patterns were most strongly encoded, and successively reactivated, in the hippocampal/prefrontal network.

INTRODUCTION

The acquisition of labile new memories can trigger processes spanning from molecular[1] to system-wide levels, gradually transforming and stabilizing memory traces. The system consolidation theory views the interaction between hippocampus and neocortex as instrumental for this[2-4]. While the hippocampus is vital in the initial acquisition and early storage of memories, the cerebral cortex, among other structures, play crucial roles later on[5]. The exchange between a fast-learning module (the hippocampus) and a slower one (the neocortex) would take place mainly after memory acquisition, allowing one-shot acquisition of new items without losses of older memories because of interference[2,3]. A further role of slow consolidation following acquisition would be to re-organize memories into more semanticized, de-contextualized representations[6-8].

A role for slow-wave sleep (SWS) in such exchange[3,5,9-11] would be the replay of neural patterns reflecting previously acquired information[12-18]. Such sleep replay would then instill a change in the neural substrate of memory traces, and ultimately favor memory consolidation. During sleep, the hippocampus and the neocortex engage in a dialogue which involves and affects the dynamical states of both[19-23]. Hippocampal sharp waves/ripple complexes (SPWR) are likely vectors for hippocampal-neocortical information exchange[24]: SPWRs[25] are brief (~50-150 ms), large bursts of hippocampal activity, mostly observed during SWS or immobility and correspond to increased hippocampal memory reactivation[14]. During SWS, neocortical activity displays periods of large, synchronous oscillations (0.1-4 Hz) of

membrane potentials and neural firing[26], and these are correlated with SPWRs[19-21]. Slow oscillations were recently found to coordinate episodes of visual cortical and hippocampal reactivation[16], but the precise temporal relationship between cortical and hippocampal replay remains unknown.

The prefrontal cortex (PFC) is often implicated in long-term memory consolidation [27] in particular for hippocampally-dependent spatial and contextual information. Indeed, the PFC shows detailed, time-compressed replay following initial acquisition of memory-related sequences of neural ensemble activation in rats[17] and increased coordination with the hippocampus during retrieval of sleep-consolidated memories in humans[28]. The PFC is one of the neocortical areas most closely associated to the hippocampus, both anatomically and physiologically because it has a unique afferent pathway from the hippocampus[29] endowed with synaptic plasticity[30]. Some functional imaging and immediate early genes expression data support the idea that, during consolidation, the hippocampus activity contributions decrease over time, with an opposite, increasing trend observed for the PFC[27,28,31]. However, the concerted function of PFC and hippocampus is also necessary for memory maintenance during task performance[32,33].

While the behavioral electrophysiology literature provides numerous examples of memory replay [12,14-18], the animals in these studies were over-trained, and little learning actually took place, or, no specific analysis of the evolution of replay with task performance was attempted[13]. The goal here is to investigate memory reactivation and hippocampal-neocortical interactions while new task-relevant information is actually being acquired in order to better characterize the link between learning and memory replay processes. Moreover, this study focussed on sub-second resolution of the time course of memory replay, in order to study of precise correlations between replay events and large-scale synchronization phenomena during SWS, such as SPWRs and slow oscillations. Indeed

learning-related changes in neural activity over brief time scales have been described in both the prefrontal cortex[34,35] and hippocampus, but the effects of these changes on the subsequent sleep activity has not yet been studied. We recorded neural activity in the PFC and the hippocampus in rats during a cross-modal rule shift task (known to implicate the medial PFC[36]), which allowed to introduce novel elements in the form of new rules, while leaving the perceptual aspects of the task unchanged.

RESULTS

mPFC ensemble patterns during a rule shift task

Multiple tetrodes recorded ensembles of medial PFC (mPFC; see Methods, Supplementary Fig. 2 online) neurons, together with mPFC and hippocampal local field potentials (LFP) in rats. The animals had to perform a task on a Y maze (Supplementary Fig. 1), where the animal had to select the rewarded arm using one of four possible rules (left arm, right arm, illuminated arm, non-illuminated arm; at each trial one of the two target arms was illuminated at random). This period will be referred to as the AWAKE epoch. Neural activity was monitored also during rest periods immediately before and after the AWAKE epoch (PRE and POST epochs). As soon as the rat achieved criterion performance (See Methods) according to the current rule, the rule was changed without any additional cue, and the rat had to again infer the new rule from the pattern of rewarded and non-rewarded arms. Because no pre-training was performed prior to the electrophysiological recordings, during the experiments the rats encountered novel rules, to which they had never been exposed before.

1692 cells were recorded in the mPFC (Supplementary Fig. 2) from four rats, during a total of 63 recording sessions (Rat 15: 16; Rat 18: 11; Rat 19: 12; Rat 20: 24). Only sessions with a minimum of 10 cells and at least four minutes of SWS in each rest epoch were analyzed.

Cells in the mPFC had diverse behavioral correlates, corresponding to one or more task phases, and in some neurons responses dynamically adapted as the rat acquired the current task rule (Battaglia et al, SfN abstract 2006). We used principal component (PC) analysis to extract the neural patterns characteristic of the AWAKE epoch (high rank principal components, associated with larger eigenvalues, or *encoding strengths*, will be referred to as *signal* components, while lower rank, or *non-signal* components, mostly reflect noise). (see Methods, Supplementary information, Supplementary Fig.32).

Signal components identified neuronal assemblies with reliable and consistent responses in the task. For example, they assigned same-signed weights to cells with similar behavioral correlates, and opposite-signed weights to cells with complementary correlates (Fig. 1A; Supplementary Fig. 4). Based on the eigenvalues associated with the PCs, and on a threshold value computed on the basis of the null hypothesis of random, uncorrelated spike trains, we could typically discriminate 1-6 signal components (and occasionally more) in each session (Fig. 1B). The patterns of activity detected by PCs are correlated with behavior: in this example session, the first PC (PC1) showed a positive peak activation right after trial onset (Fig. 1C), PC2 peaked later in the trial, and PC3 even later on. Moreover PC1 and PC2 increased their score and PC3 decreased its score as the rat, across trials, abandoned the strategy of always going to the right arm (Fig. 1C, trials indicated with a green background in the right panel), and instead chose, with a great probability to alternate between the two target arms. Thus, PC1, 2, and 3 extracted patterns of activity that correlated both with trial phase, and at a larger time scale, with the strategy that the rat followed in a block of trials.

FIGURE 1 near here

Transient, synchronized replay of AWAKE patterns

In order to assess the nature and extent of the interaction between prefrontal cortex and hippocampus in memory replay, we characterized the detailed time course of replay during rest episodes. For this, we computed the instantaneous *reactivation strength* (see Methods, Supplementary Information) of the signal components computed from the AWAKE epoch. At each moment (with a resolution of 100 ms, unless specified otherwise), reactivation strength assesses the similarity between reference AWAKE signal components and the rest period neural activity.

FIGURE 2 near here

During POST SWS, signal components reappeared more frequently and strongly than in PRE (e.g., Fig. 2A), confirming that experience-related patterns are reactivated in mPFC[17] in ensuing sleep. No such effects were observed in the rest periods that were not classified as SWS, as shown in Supplementary Fig. 5; thus further analyses were restricted to SWS. PRE and POST SWS did not differ in terms of average duration of the sleep episode, average population firing rates, rates of occurrence of delta waves and SPWRs, or local field potential power in the delta and spindle ranges (Supplementary Fig. 6). The average reactivation strength was greater during POST SWS than PRE SWS for signal components (Fig. 2B-C; $p < 0.005$ all comparisons. N = 10, 40, 273 for the three signal groups observed here, sorted according to their encoding strength; N = 811 for non-signal components). Reactivation strength correlated positively with encoding strength ($r^2 = 0.61$, $p<1e-30$ Pearson correlation test, N = 323; Fig. 2B; Supplementary Fig. 7). Thus, the patterns most active in the mPFC during AWAKE, are preferentially reactivated during the following SWS, similarly to

previous observations in the hippocampus[18]. During PRE SWS, this relationship was significantly weaker with respect to slope ($p<1e-20$; $N = 323$) and correlation ($p<1e-5$; $N = 323$, Supplementary Fig. 7). These observations were not likely to erroneously result from potentially faulty spike sorting, as they persisted when cell pairs discriminated from the same tetrode were ignored. Moreover, cell pairs from the same tetrodes, when considered alone, showed no replay effect, so that virtually all contributions to the replay results come from the correlations between neurons recorded with different tetrodes (Supplementary Fig. 8).

Strikingly, replay occurred in distinct events of strong signal reactivation in POST SWS (Fig 2A), denoting synchronous transient activation of the cell assemblies identified by the signal components. Histograms of the reactivation strengths for POST SWS were heavy-tailed (Fig. 2D right), with the tail constituting the main difference with PRE SWS (Fig. 2D left). The bulk of the distribution was similar in PRE and POST. This is reflected in the significant difference between POST and PRE in skewness of the signal reactivation strength histograms ($p<0.05$; t-test $N = 10$-40), most markedly for patterns with higher encoding strengths (Fig. 2E). The peaks in reactivation strength correspond to the transient, coordinated activation of the cells that are assigned a large weight in the relative principal component (Supplementary Fig 9). Those cells are the ones that have the greatest contribution to the total reactivation strength. Different PCs recruit different, rarely overlapping sets of high-weight strengths. Interestingly, during sleep, reactivation strengths for simultaneously recorded principal components tended not to peak at the same times, rather, concomitant activation of different principal component-related patterns was less than expected by chance, as can be inferred from the zero lag trough in their cross-correlograms (Supplementary Figs 9 and 10). Shuffled controls show that this cannot be explained by global fluctuations in the population firing rate alone (Supplementary Fig. 11).

To assess the prevalence of the strongest transient cell assembly activations, we computed the cumulative contribution to the epoch-wide reactivation of events with reactivation strengths up to certain values for POST SWS, and subtracted the same measure for PRE SWS. This cumulative contribution (Fig. 2F) increased steadily over two orders of magnitudes, and 40-50% of the net reactivation (difference between POST and PRE) came from events with reactivation strengths beyond the 99th percentile. Thus, rare events of elevated network synchronization or *network spikes*[37,38], while spanning only a small period of time, account for a substantial proportion of the total observed reactivation.

Preferential mPFC replay during hippocampal SPWRs

The standard systems consolidation theory holds that, following acquisition, experience-related information flows from the hippocampus towards the neocortex[24]. Conversely, neocortical influences may contribute to selecting the pattern reactivated in the hippocampus[22]. We tested the relationship between mPFC cell assembly reactivation and hippocampal SPWRs, the most prominent pattern of hippocampal activation during SWS (which, like reactivation strength peaks, occur irregularly). Indeed, cortical assembly reactivation events occurred in concert with hippocampal SPWRs. Examining data from entire POST sessions (Fig. 3) reveals that virtually all reactivation peaks occur concomitantly with a SPWR event (and also with an increase in synchronous activity of those cells with large positive weights in this signal component). In the example in Fig. 4, the ensemble spike trains corresponding to a reactivation peak are shown (red ticks in Fig. 4E): at the time of the peak, virtually all cells with large positive weights in this signal component were active (and negative weight cells reduced their activity). In this example, the two largest peaks (Fig 4A) coincided with SPWR events (red asterisks in Fig 4B), and one preceded a delta wave (Fig. 4C).

FIGURES 3, 4 near here

The average reactivation strength in POST SWS was considerably greater for bins coinciding with SPWRs than for non-SPWR bins (all comparisons $p<0.005$; N = 8, 37, 225; including only sessions with reliable discrimination of ripple signals; Fig. 5A). The effect was stronger for components with higher encoding strengths (Pearson's correlation test, $p<1e-20$, Supplementary Fig. 7). The SPWR-triggered average (Fig. 5B) showed that during POST (but not PRE), SWS reactivation strength for signal components increased by ~70% at the time of the sharp waves with respect to baseline ($p<1e-10$, N=270). Reactivation in mPFC declined to baseline values within 1 s before and after the peak of the SPWR events. No such effect was found for non-signal components. A similar analysis at higher time resolution (Fig. 5C) showed that reactivation peaked ~40 ms after SPWR occurrences, which is compatible with the transmission delay measured for prefrontal responses to hippocampal stimulation[39] (the second peak in the event triggered histogram is likely due to the frequent occurrence of sharp wave "doublets"). On the other hand, overall ensemble mPFC activity (of all recorded neurons, including those not involved in signal components) showed a qualitatively different, sharply asymmetric profile with respect to SPWR occurrences (Fig. 5D): on average, prefrontal population activity transiently increased with the SPWRs, and maintained sustained activity thereafter[20,21] (with no difference between PRE and POST). This sustained post-SPWR activity contrasts with the faster decay of signal reactivation, arguing against an explanation of the latter solely in terms of general population activity fluctuations. Furthermore, autocorrelograms of both reactivation strength and SPWR occurrences (Fig. 5E) decay with very similar time constants (respectively 150 and 160 ms for exponential fits), suggesting that the clustering in time of SPWRs is reflected by a similar grouping of reactivation events.

FIGURE 5 near here

relation of slow oscillations with SPWRs and mPFC replay

A hallmark of cortical activity during SWS is slow oscillations[26], which trigger and orchestrate LFP waves in the delta (2-4 Hz) and spindle (10-20 Hz) ranges.Reactivation episodes in the hippocampus and neocortex coincide with the slow oscillation phase with high neural activity [16] (UP state) and are correlated with hippocampal SPWRs, but little is known about the precise temporal relation between cortical oscillatory phenomena, hippocampal activity and neocortical reactivation. In Fig. 2A the relation between mPFC reactivation and SWS oscillations is shown: episodes of strong replay were significantly concentrated into periods of elevated prefrontal LFP oscillatory activity in the delta (2-4 Hz) and spindle (10-20 Hz) ranges ($p < 1e\text{-}5$ for all, t-test; Supplementary Fig. 5). Thus, we tested the correlation between reactivation strength and LFP markers of slow oscillations. First, we considered delta waves, large positivities of the depth cortical LFP, associated with states of reduced cortical activity (DOWN states), and with the K-complex phase characterized by absence of spindles[40]. During POST SWS, reactivation strength for signal components showed a significant ($p < 0.001$, t-test) increase ~ 400 ms prior to the peak of the delta wave (Fig. 6A top). This was experience-dependent and possibly memory related, since the modulation was smaller for PRE SWS and null for non-signal components. The timing of hippocampal SPWRs relative to delta peaks closely resembled that of mPFC reactivation (Fig. 6A middle). In contrast, mPFC ensemble activity showed a different profile (Fig. 6A bottom) with a minimum immediately prior to the peak of the delta wave, but symmetric peaks before and after (with a return to baseline in 500-1000 ms). The second peak was not associated to an increase in reactivation.

To further investigate this relationship, the same analysis was performed with times of putative DOWN to UP state transitions (Fig. 6B) (putative DOWN states were defined as a decrease of neural activity in windows of at least 80 ms). The relations between reactivation SPWR occurrence with these transitions were comparable to the delta wave results.

Because spindles (bouts of 10-20 Hz oscillations) appear at the onset of UP states[41], we examined their correlation with reactivation strength. In general, signal reactivation tended to occur before spindle episodes. Reactivation event-triggered averages centered on spindles troughs are asymmetric, with an increase in reactivation in the ~1 s preceding spindles compared to the period thereafter ($p < 0.001$, t-test; Fig. 6C top). As is the case for delta waves, the increased pre-spindle reactivation over a broad time scale echoes the increased probability for hippocampal sharp waves preceding spindles (Fig. 6C middle). In contrast, the population activity modulation showed a symmetrical time course peaked at the time of spindle events (Fig. 6C bottom).

The respective cross-correlograms and Event-Time Averages of reactivation relative to these three cortical events were strongly correlated (Fig. 6D). Thus, coupling between reactivation and sharp waves primarily structured the relationship between the reactivation time course and cortical slow oscillations.

FIGURE 6 near here

Salient behavioral events, rule learning increase replay

The PC analysis that characterized the time course of experience-related pattern reactivation during sleep may conversely be employed to find out which aspects of the neural assembly coactivations during task performance are replayed during sleep. For this, PCs were computed from the ensemble neural activity during sleep PRE and POST, separately for SPWR and inter-SPWR time bins. Those templates were matched to the activity during the AWAKE period. For a significant number of components extracted from SPWR bins (Fig. 7A-B; Supplementary Fig. 12), co-activations became stronger as the rat started a run of correct trials, signaling rule acquisition. This difference was not significant for PCs computed from the in-

ter-SPWR intervals in POST, or from PRE. This was not simply due to the elapsed time during the session, as there was no such difference between the two halves of those sessions where no rule learning occurred.

Furthermore, the PCs computed from POST SPWR appeared primarily when the rat was on the central platform of the Y maze, that is, the point where it was required to select the behavioral response (Fig. 7A,C). A significant effect of learning on the spatial distribution of PCs from POST SPWR appeared only in the part of the maze going from the central platform to the end of the target arm (Fig. 7C). Moreover, a factor analysis of these spatial distributions revealed that the two most important factors (which ones?) are concentrated on the platform and on the target arm respectively (Fig. 7D; see also Supplementary information).

Rule acquisition was not accompanied by a change in principal measures of the rat behavior, including terms of (in term of) trial duration, of trajectories (which followed the same stereotyped paths before and after rule acquisition), and running speed at each point of the trajectory (Supplementary Fig. 12). Moreover, the greater contribution to reactivated patterns during SPWRs from trials occurring right after rule acquisition is not likely to be due to changes in the general sensory experience other than reward. To test this hypothesis, we compared trials occurring before and after spontaneous strategy shifts operated by the rat which did not lead to acquisition of the rewarded strategy, in days in which (when) no learning took place. Rats operated this sort of shifts while seeking the correct rule by trial and error. In these cases, no difference was observed in contribution to reactivated patterns during SPWRs (Fig. 7B; Supplementary Fig. 13; Supplementary Information).

FIGURE 7 near here

DISCUSSION

This work shows new links between learning and the dynamics of replay in the mPFC and hippocampus with 3 main results. First, mPFC replay occurs in transient episodes, apparently corresponding to the activation of distinct cell assemblies. Second, prefrontal replay, while occurring throughout the following sleep episode, was most likely coincided with hippocampal sharp wave events, and therefore with increased hippocampal replay and hippocampal/neocortical interactions. Third, and most importantly, mPFC replay during hippocampal sharp waves principally concerned neural activity patterns emerging only after the acquisition of a new rule.

The results demonstrated the relation between memory replay, the task phase where the activity patterns originated, and task performance level in a dynamic setting, wherein the rat were obliged to continually adapt to new rules. Interestingly, when the rat behaved according to the newly acquired rule, the patterns that contributed the most to memory replay in mPFC during hippocampal SPWRs were those appearing when the rat commits to choose one target arm. During the trial, the contribution to hippocampal-related replay in the mPFC climbed steadily on the departure arm (where activity may predict the choice[42], to peak at the arms intersection. Furthermore, preferentially replayed patterns arose just when rats began a series of correct trials at criterion with respect to the new rule. Prefrontal cortical activity reflecting a newly learned associations has been found to emerge only after that the associations have been acquired[34]. The time course that we observe for replay is similar: co-activations preferentially replayed in SPWRs arise after the rat starts to perform at criterion. These effects were likely not a consequence of different neural activity statistics at the choice point, as they remain specific for POST SPWR patterns and are not found for patterns extracted from PRE or from inter-SPWR intervals.

The hippocampal involvement in the formation of these novel cell assemblies is likely to be critical: strikingly, times in the task linked to preferential replay were also marked by in-

creased coherence between the hippocampus and mPFC LFP in the theta range (Benchenane et al. *Soc. Neurosci. Abs* 690.15, 2008). Thus, upon learning, the choice point is the site of an increase hippocampal-prefrontal coherence. Cell assemblies activated at those times are prominently replayed in the mPFC, during SPWRs, that is, when the hippocampal-neocortical interaction is at its peak[19-22]. Coactivations may reflect prefrontal (or prefrontal-hippocampal) cell assemblies representing new information. The nature of this new information could be two-fold: on one hand, it could represent an emerging representation of the newly learned rule; on the other hand, it might reflect processes that take place *after* learning has taken place, for example, a representation that is activated by consistent stream of reward. The reward signal may "tag" the representations making them more likely to be replayed. In support of this hypothesis, we analyzed those periods in which rats were searching for the correct rule by trial and error. We showed that when the rat,makes spontaneous strategy shifts to strategies different from the rewarded one, patterns from before or after the shift are indifferently replayed in sleep (Supplementary Fig. 13). Thus, the consequent changes in the general sensory experience, do not cause the resulting neural patterns to be replayed more or less strongly. Also, this seems to rule out interpretation of our result in terms of repetitive experience resulting in stronger replay[18]. Because preferential replay only occurs during SPWRs, we speculate that regulation of hippocampal-prefrontal interactions could result from dopaminergic, reward-related signals[43]. The replay of reward tagged patterns, in concert with the hippocampal replay during SPWRs[14,44] would mark the initial period of systems consolidation.

The high temporal resolution of our coactivation measures revealed another remarkable feature of prefrontal replay, its detailed time course. Replay is largely accounted for by brief events, with durations on the order of 100 ms or less (e.g. Fig. 2). In each event (similar to 'network spikes'; see also Supplementary Discussion), a substantial number of cells is co-

activated. The observed highly irregular time course of reactivation strength suggests that replay is not simply due to changes in probability of co-firing for cell pairs (a straightforward consequence of a change in the efficacy of the connection between the two cells[18]), but is more likely generated by global network effects, induced for example by the excitatory feed-back connections within the mPFC.

Transient replay is concentrated during SWS (Supplementary Fig. 5). This differs from studies of hippocampal reactivation which showed non-zero restful, non-sleep periods[14]. This may be an intrinsic difference between the hippocampus and the mPFC, or it could be due to a different sensitivity of our analysis methods. Within SWS, replay occurs principally in proximity to hippocampal SPWRs (Fig. 3-5). Thus, besides playing a special role for the hippocampal replay of newly formed memories[14], SPWRs also coincide with an increase of overall reactivation in hippocampal output structures. Moreover, sharp-waves are related to increases in cortical activity and transitions to UP states[20,21]. It is therefore possible that the correlation between cortical replay and SPWRs observed here may be due to a hippocampal influence on mPFC. At present, though, it is difficult to speculate whether reactivation events originate in the hippocampus or the neocortex: our data show that prefrontal replay peaks about 40 ms after SPWR occurrences, a latency which is compatible with observations of prefrontal responses to hippocampal stimulation[39], and thus with hippocampus triggering this extra-hippocampal replay. On the other hand, it is known that the neocortical slow oscillations[45] influence the membrane potential of hippocampal cells[23] and the probability of emission of SPWR[22]; such an influence would allow the neocortex to contribute to selecting the information reactivated in the hippocampus. As a third alternative, the concurrent prefrontal cortical replay and hippocampal SPWRs may be the result of particular neocortical network states that simultaneously regulate when hippocampal reactivation takes place and facilitate

the transfer of reactivated patterns between brain regions[20]. In a more radical version of this hypothesis, the representation of a memory item could integrally involve both hippocampus and neocortex already at the time of initial encoding, and thereafter this would reactivate as a whole and manifest itself simultaneously in both structures. In fact, analyzing data from these same experiments we show that signs of hippocampal/neocortical interplay were already present when the replayed representation was likely formed, at the level of theta-band coherence.

In conclusion, the acquisition of new rules involves the hippocampo-cortical network; during the ensuing sleep, the PFC activity patterns during hippocampal SPWRs reflect the neural patterns that occurred during the task phase, particularly when a rule has been learned, just at the time when hippocampo-cortical coherence is enhanced. Whether the predominating causal influence in this dialogue is cortical or hippocampal, or rather this corresponds to an emergent system-wide representation of information, our results show a possible mechanism by which task-relevant learned information can be expressed and reactivated in the prefrontal cortex. This would be contingent to SPWRs for newly formed memories, but more uncoupled to the hippocampus for more distant memory traces.

METHODS

Animals Four Long-Evans (pigmented) male rats (René Janvier, Le Genest-St-Isle, France) weighing 250-300g at arrival, were handled and pre-trained as described in the Supplementary Information. All experiments were in accord with institutional (CNRS Comité Opérationnel pour l'Ethique dans les Sciences de la Vie), international (NIH guidelines) stan-

dards and legal regulations (Certificat no. 7186, French Ministère de l'Agriculture et de la Pêche) regarding the use and care of animals.

After pre-training, rats were anesthetized with intramuscular Xylazine (Rompun 0.1 ml), and intra-peritoneal pentobarbital (35 mg/kg). A drive containing 7 tetrodes (six recording, plus one reference) was implanted on the skull above the right medial prefrontal cortex (AP 3.5-5 mm, ML 0.5-1.5 mm). Each tetrode was contained in a 30 ga hypodermic tube, with the tubes arranged in two parallel, adjacent rows. Tetrodes were twisted bundles of 13 m diameter polymide-coated nichrome wire (Kanthal, Palm Coast, FL); the drive allowed independent adjustment of tetrode depth. After dura retraction, the rows of cannulae were implanted parallel to the sagittal sinus, so that they targeted, respectively, the superficial and deep layers of the medial bank of the cortex. A separate micro-drive containing three tetrodes was targeted to the ventral hippocampus (AP -5.0 mm ML 5.0 mm). The tetrodes were electrically connected in a single-electrode configuration (all channels shorted together) and used for local field potential (LFP) recordings. For all LFP recordings, a screw implanted on the occipital bone above the cerebellum was used as a reference. The hippocampal tetrodes were lowered to the CA1 pyramidal layer; the depth was adjusted with the help of LFP signs (flat sharp waves, maximum amplitude of ripple oscillations) After surgery, rats were allowed to recover for at least 2 weeks, while the tetrodes were gradually lowered to reach the Prelimbic area (PL; main drive), and the CA1 pyramidal layer (hippocampal micro-drive). During the experiment, they were gradually lowered to probe, in different sessions, different dorso-ventral levels of the prelimbic cortex.

Behavioral task Four rats performed an attentional set shift task on a Y-maze (see Supplementary information for a description of the apparatus). Such extradimensional set shift tasks were shown to require the function of the medial prefrontal cortex (mPFC) in rats[36]. This parallels the involvement of the human prefrontal cortices in the Wisconsin Card Sorting

Task, which inspired the present experimental design. Each recording session consisted of a 20-30 minutes sleep or rest epoch (PRE epoch) in which the rat remained undisturbed in a padded flowerpot placed on the central platform of the maze, followed by an AWAKE epoch, in which the rat performed the behavioral task described below for 20-40 minutes, and by a second sleep or rest epoch (POST epoch; same situation as in PRE) of 20-30 minutes.

The first recording sessions corresponded to the first time rats encountered the behavioral task. Rats started each trial in the same arm (the departure arm). One of the two other (choice) arms was illuminated at random (pseudo-random schedule: runs of more than 4 consecutive trials with the same illuminated arm were avoided, as were repeated bouts of imposed alternation between the two arms). After that, the central platform was lowered, allowing the rat to access the choice arms.

Only one of the choice arms was rewarded, according to one of 4 contingency rules. Two contingency rules were spatially guided (always go to the right arm, or to the left arm), the other two were cue guided (go to the illuminated arm, or to the dark arm). The rule employed at any given moment in time was not signaled to the rat in any way, so that the animal had to learn the rule by trial and error. Once the rat reached a criterion of 10 consecutive correct trials, or only one error out of 12 trials, the rule was changed with no further cue warning to the rat. Rule changes were extra-dimensional, that is, from a spatially-guided rule to a cue-guided rule, and vice versa. Because of an operator mistake, an intradimensional shift was operated once. Typically, the trials necessary for acquisition of one rule spanned more than one session, which implies that rule shifts occurred only in a fraction of the sessions.

All four rats learned the Right and Light rules (at least 10 consecutive correct trials), whereas only two learned the Left task, one learned the Dark task.

Electrophysiology Tetrode recordings were obtained from 6 tetrodes implanted in the Prelimbic and Infralimbic areas of the rat neocortex, hippocampal local field potentials were obtained from two further tetrodes implanted in the CA1 pyramidal layer. The tetrodes were gradually lowered in the cortical tissue during the course of the experiment. Because they were not moved after each recording session, it is possible that the same cells were recorded more than once, however, usually in different behavioral situations (rewarded strategy, performance level, etc.).

For electrophysiological recordings, signals from all electrodes were fed into a unit-gain headstage pre-amplifier (HS-54; Neuralynx, Bozeman, MT) and then, through a tether cable, to programmable amplifiers (Lynx-8, Neuralynx, Bozeman, MT). All signals were there amplified 2000x, Signals for single unit recordings were bandpass filtered between 600 and 6000 Hz, signals for LFP recordings were filtered between 0.1 and 475 Hz (cortex) and 1 and 475 Hz (hippocampus). Data were digitized and stored on hard disk by a Power 1401 (CED, Cambridge, UK) acquisition system, controlled by the Spike 2 software (also by CED). Single unit data were sampled at 25 kHz, and a 1.3 ms sample was timestamped and stored for all the channel in a tetrode whenever any of the 4 channels exceeded a threshold. Local field potentials were sampled and stored at a 2 kHz sampling rate.

Learning: The first trial of a block of at least three consecutive correct ones and from which the performance until the end of the session was above 80% was defined as the learning point (i.e. the rule was acquired).

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Histology At the end of the experiments, a small electrolytic lesion was made by passing a small cathodal DC current (20 μA, 10 s) through each recording tetrodes to mark the location

of its tip. The rats were then deeply anesthetized with pentobarbital. Intracardial perfusion with saline was followed by 10 % formalin saline. Histological sections were stained with cresyl violet. The electrode tracks were reconstructed, verifying that the recording sites were in the Prelimbic cortex, or in exceptional cases, in the dorsal end of the infralimbic cortex.

Slow Wave Sleep Detection Slow Wave Sleep (SWS) was automatically detected. Power spectrograms of cortical and hippocampal LFPs were computed for each sleep sessions with bins of 1 seconds. Power in the cortical delta band (1-4 Hz), hippocampal theta (5-10 Hz), cortical spindles (10-20 Hz) and speed of head motion were clustered with a K-means algorithm. Clusters corresponding to high values of delta and spindle powers, and to a low degree of head movement, were considered as corresponding to SWS. Successive SWS clusters occurring within intervals of less than 1 minute were merged and finally, resulting time intervals of SWS smaller than 10s were dropped.

Data Preprocessing. For single unit activity discrimination, the first three principal components of the energy-normalized waveforms were computed from spike waveforms for the 4 tetrodes, generating a 12-dimensional vector describing each spike. Those vectors were the input of the KlustaKwik[46] clustering program. The resulting classification was manually refined using the Klusters[47] interface.

For ripple detection, the hippocampal LFP from the CA1 pyramidal layer was bandpass filtered in the ripples frequency range (100-300 Hz) to detect SPWRs. Then, the RMS signal was convolved with a 20 ms gaussian window. Only intervals of time for which the resulting envelope was 2 SD above the raw filtered signal were kept. Ripples events were time stamped with the times of the deepest LFP troughs in these intervals if the latter were at or below 5 SD from the RMS signal baseline. Finally, discrimination of SPWRs were considered reliable when at least 40 ripples were detected. In most sessions, at least 200 SPWRs were detected.

For spindles detection, cortical LFP signal were filtered between 10 and 20 Hz. The RMS was low-pass filtered with a gaussian window of 100 ms. Then time intervals 1 SD above the filtered signal, which were closer than 100 ms apart were merged. Of the resulting intervals, only those at least 500 ms long were kept. Spindles troughs are the minima of the filtered signal during those intervals. For delta waves detection, cortical LFP signal was filtered between 0.1 and 4 Hz. Delta waves peaks were the minima below 2 SD of the filtered signal.

Computational methods. Reactivation strengths describe the instantaneous replay at each time, during rest sessions, of neural co-activation patterns that were characteristic of the AWAKE epoch. Briefly, the spike trains from all cells recorded in the AWAKE epoch were binned (in 100 ms bins) and z-transformed; then, principal component analysis (See SOI; Supplementary Fig. 3) was used to extract the characteristic activity patterns. PRE and POST epoch spike trains were similarly binned and transformed, and reactivation strengths for each component at any given time during the rest epoch were computed from the projection of the binned activity vector on each principal component, which measures the instantaneous reactivation of that activity pattern. By summing over all components and all times, one obtains a measure of similarity between the correlation matrices in the AWAKE period and in the PRE and POST periods analogous to previously published reactivation measures[12,14]. High rank (which we will refer to as *signal*) principal components (associated to large eigenvalues), capturing fundamental processes , are defined as those associated to an eigenvalue greater than a threshold value. As a threshold, we took the maximum theoretical value (λ_{max}) for eigenvalues of principal components for random uncorrelated spike trains[48]. (see SOI, Fig. 1C). The normalized eigenvalues, or *encoding strengths* $\Phi = \lambda/\lambda_{max,}$ quantify the weight of each component during the AWAKE epoch in a uniform way across sessions.

Conversely, in order to establish which moments during the task expressed the patterns that account for most of the sleep replay, principal components can be computed by the same

procedure from the activity in the POST SWS epoch (PRE SWS as a control), and reactivation strength can be computed as defined above from the AWAKE activity.

An in-depth description and a schematic (Supplementary Fig. 3) of the mathematical method can be found in the Supplementary information

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

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Author Contributions SIW, FPB and MK designed the experiment, FPB, MK, and AP performed the experiment, AP, FPB and KB designed the analysis techniques, AP analyzed the data, FPB, AP and SIW wrote the paper.

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FIGURE LEGENDS

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Figure 1

Signal components and their behavioral correlates

A: Signal components and behavioral correlates of their component mPFC cells: Peri-event time histograms of all cells from a recording session, aligned with trial initiation (vertical dashed line). Cells are sorted by their weight in the first signal component (scale above), showing that cells with similar weights tended to have similar behavioral correlates and cells with opposite signed large weights had complementary behavioral correlates. B: AWAKE epoch correlation matrix eigenvalues from the same session as Figs. 2A and 3A. Dotted line: the signal threshold, defined as the theoretical upper bound for eigenvalues in the random spike trains case (see SOI). Filled squares: eigenvalues associated with the six (suprathreshold) signal components. Hollow squares: non-signal components. C Trial-by-trial time scores of the first three principal components over the activity during the awake epoch, plotted as a function of the linearized position of the rat on the maze. The right panels

summarize the rat behavior: a beige background color indicated trials in which the rat reliably chose the right arm, the green background indicated trials in which the rat, to a large extent alternated between the two arms (with no other discriminable strategy). The black dots in the first column ("Arm") denote trials in which the rat chose the *left* arm. The black dots in the second column ("Correct") indicate *rewarded* trials. (Here the Light rule was imposed and neither strategy was successful.) Note that PC scores are displayed instead of reactivation strength (see below) because in this case the sign is important, since large positive PC scores denote activation of those cells with positive PC weights (see fig. 2A) and large negative PC scores denote activation of those cells with negative PC weights.

Figure 2

Time course of memory replay

A: Reactivation strength (black/white traces; right axis) of the signal component shown in Fig. 2A during sleep PRE (*left*) and POST (*right*) epochs superimposed on the mPFC LFP spectrogram (left axis). White traces: SWS periods; black trace: non SWS. The spectrogram shows periods of elevated slow/delta and spindle oscillations often coinciding with SWS. Reactivation strengths show high peaks during POST epoch SWS, usually concomitant with periods of strong oscillations. B: Bar plot of the epoch-wide average reactivation strengths, grouped by their encoding strength (normalized eigenvalue), $\Phi = \lambda/\lambda_{max}$, for the PRE and POST epochs (data from 63 sessions and 1692 recorded cells; error bar: S.E.M.). C: Average difference in reactivation strength between PRE and POST, for PCs grouped by their encoding strengths (error bars: SD). D: Incidence of reactivation strengths during rest periods (left, PRE; right, POST) for the same session as Figs. 2A and 3A. Black filled zones: during SWS; gray trace: non SWS. The POST SWS histogram has a heavy tail, reflecting strong transient reactivation events. E: Average difference between POST and PRE of skewness of

reactivation strength incidence histogram (3C) for components grouped by their encoding strength, showing that POST epoch histograms were generally more skewed than the PRE. Error bars: SEM. F: Cumulative contribution to the difference between total reactivation strength in POST and PRE for signal components (black; grouped by encoding strength) and for non-signal components (gray). Red diamonds: 99th percentile of reactivation strength distribution (in POST). About half of the reactivation was accounted for by reactivation strengths over the 99th percentile.

Figure 3

Memory replay, SPWRs and cell activity in a rest session

A: Reactivation strength for one signal component. B: Occurrences of SPWRs. C: Averaged firing rate of the cells that contributed the highest weights to the signal component. Note that peaks correspond to events in A and B. D: Average firing rate for all cells recorded in this session.

Figure 4

Example of reactivation strength peaks coinciding with hippocampal SPWR. A: Reactivation strength (white/gray traces; right axis) of the signal component superimposed on the mPFC LFP spectrogram (left axis). This is expanded from the zone in Figure 3A (POST) delimited by the dashed line. Black dashed line: normalized population firing rate. B: The bandpass filtered hippocampal LFP (100-300 Hz) shows ripple events (red asterisks); signal is normalised by its SD. C: bandpass filtered (0-5 Hz) PFC LFP. Delta waves are denoted by green asterisks. D: Raster plot of spike trains from the PFC cells sorted by principal component weight magnitude (as in Fig. 1A). E: Expansion of the 300 ms surrounding the peak indicated by an arrow in A. Red rasters represent spikes occurring in the bin of peak reactivation strength. The example also show two delta waves in the cortical LFP, with pre-

ceding and following increases in population activity (or UP states[41]). Note that, in the absence of SPWRs only a smaller reactivation peak was obtained (see Supplementary Fig. 7 for statistical analysis of reactivation/slow oscillations interactions).

Figure 5

A: Average reactivation strength for non-signal ($\Phi < 1$) and signal components (grouped by encoding strength) during PRE and POST SWS for SPWR bins (left) and non-SPWR bins (right); error bars: S.E.M. B: Event Triggered Average (ETA) of reactivation strength centered on hippocampal SPWR for all analyzed signal components during PRE SWS (blue) and POST SWS (red) and for non-signal components (respectively black and gray), showing an increase around SPWRs during POST for signal components only; error bars: S.E.M; Grey bars indicate significantly higher reactivation strengths ($p < 0.001$, t-test) for signal components during POST SWS with respect to baseline (defined as the average reactivation strength from -4 s to -2 s and from 2 s to 4 s from delta wave peak). C: Expanded view of the central portion of C for POST SWS (time bins of 20 ms) with increased reactivation peak 40 ms after SPWRs. D: ETA of spiking probability density of multi-unit activity relative to SPWR occurrences and averaged over all recording sessions. grey: PRE SWS; black: POST SWS. No difference was observed between PRE and POST. E Autocorrelogram for POST SWS reactivation strengths (grey) and SPWR occurrences (black), showing similar decay time constants.

Figure 6. Reactivation strength relative to mPFC LFP events.

A: Cortical delta wave peaks. Top: ETA of signal components' reactivation strength centered on cortical delta wave peaks, for PRE (grey) and POST SWS (black). Error bars: S.E.M.; grey bars indicate significantly higher reactivation strengths ($p < 0.001$, t-test) as defined in Fig. 5Bf. Reactivation had a significant peak preceding the delta wave. Middle: Cross-

correlogram of SPWR occurrences relative to delta peaks. SPWRs tended to occur more frequently just before delta peaks, similarly to reactivation. Bottom: Spiking probability density of multi-unit activity relative to delta waves and averaged over all recording sessions. grey: PRE SWS; black: POST SWS. Prefrontal cells exhibited a strong decrease in firing at the time of the delta peak, preceded and followed by activity increases. B: Same plot as in A but centered on DOWN to UP transition (as defined by population average firing rate). Results are comparable with A except for spiking probability which only shows a dramatic deflection during the DOWN state . C: Same plots as in A but centered on spindle troughs. Top: Reactivation strength was significantly higher for over 1 s before spindles. Middle:, Hippocampal SPWR ETA centered on spindles showed a similar profile Bottom: Spike activity increased at the time of spindles. D: Comparison between time relationship of reactivation strength and SPWR occurrence relative to the three cortical events presented in A-C showing a high correlation in each cases.

Figure 7

Replayed activity during SPWRs is correlated to rule acquisition

A: The activation strength of principal components computed from POST SPWR activity is displayed in color peri-event rasters, for two examples of sessions with rule learning (in different rats). The maze was linearized and divided into 25 equal bins. Rewarded trials are marked with white dots at right. The arrows indicate the trial where learning is considered to be achieved. B: Incidences of PCs computed from PRE or POST SPWRs or inter-SPWR epochs during sessions with learning (n = 10, 4 rats). (black bar is after learning occurred in the session, grey bars are before). Significantly more POST SPWR activity patterns were positively correlated with activity after learning than with activity before learning ($p < 0.05$,

two-ways ANOVA followed by t-test). No such difference was observed for PRE SPWR patterns or for inter-SPWR patterns, or when a comparison was made between the first and second half of non-learning sessions or when the rat switched between two erroneous, unrewarded strategies. Error bars: 95% confidence interval defined for multinomial distributions (see Methods). C: Average spatial distribution of all fifteen principal components computed from POST SPWR which were significantly higher on average after learning (black trace) than before (grey trace). The grey bar (above) marks significantly different bins ($p < 0.05$, t-test, n=15). Dashed lines displays SEM. D: Factorial analysis scores of data from C exhibits two profiles with peaks at the goal arms and the central choice point respectively (see also supplementary information).

SUPPLEMENTARY DISCUSSION

Memory replay and network spikes.

The transient co-activations which make up most of replay resemble *network spikes,* transient activations of groups of cells, which have been described in mathematical models of recurrent neural networks, in which dynamics is governed by synaptic facilitation and depression[38]. These events are an intrinsic feature of network dynamics in several neural systems, e.g. organotypic cultures[49] and in neocortical slices[50]. From a theoretical viewpoint, network spikes may be triggered by small fluctuations in activity, activating a subset of cells encoded in the synaptic matrix, then terminating because of synaptic depression. Network spike firing may also, through synaptic facilitation, increase the likelihood of a successive activation of the same group of cells[38] in a self-sustained, repetitive process, favoring synaptic plasticity and a more permanent encoding of that cell assembly in the connectivity matrix. Thus, by themselves, network spikes do not denote learning or information processing. However, they provide a mechanism for the expression of learning in neural activities: patterns of activity may be "tagged", for example by synaptic facilitation, or long-term synaptic plasticity, and be more likely to re-emerge later under the form of a network spike. Moreover, several groups of cells likely to give rise to network spikes may co-exist, and activate at different moments during sleep. Conceivably, the sleep activity of the mPFC could be largely composed of network spikes, each involving a different cell group. Of these groups, our technique can detect but a few among those that are related to the immediately preceding experience. Another interesting point is that different reactivating cell groups are unlikely to be active at the same time, as shown by the cross-correlograms for reactivation strengths relative to different PCs (Supplementary Figure 10). This suggests that some sort of pattern separation mechanism (possibly through feedback inhibition), takes place specifically during sleep. This result does not contradict studies[16,17] demonstrating the replay of neuronal ensemble activation in sequential or-

der, spanning up to several hundreds of ms: our technique will search for the patterns of co-activation accounting for the largest fraction of the activity variance in the AWAKE epoch. With respect to sequences, two situations may arise. First the sequence is shorter than the bin size we used (100 ms), then it would be completely contained in one of our co-activation patterns. For longer sequences, or in any case sequences that are cut in two at the bin boundaries, nothing ensures that all parts of the sequence will be detected as co-activation patterns by our analysis, so that the sequential character of replay can be analyzed. In general this will not be the case, because here PCs are computed by AWAKE analysis without reference to precise, repetitive behavioral templates, as it is the case for analyses of sequential activation of ensembles. Thus, the present method and sequence-based methods highlight complementary aspects of sleep replay.