LETTER

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The hippocampus is crucial for forming non-hippocampal long-term memory during sleep

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There is a long-standing division in memory research between hippocampus-dependent memory and non-hippocampus-dependent memory, as only the latter can be acquired and retrieved in the absence of normal hippocampal function[1,2]. Consolidation of hippocampus-dependent memory, in particular, is strongly supported by sleep[3–5]. Here we show that the formation of long-term representations in a rat model of non-hippocampus-dependent memory depends not only on sleep but also on activation of a hippocampus-dependent mechanism during sleep. Rats encoded non-hippocampus-dependent (novel-object recognition[6–8]) and hippocampus-dependent (object–place recognition) memories before a two-hour period of sleep or wakefulness. Memory was tested either immediately thereafter or remotely (after one or three weeks). Whereas object–place recognition memory was stronger for rats that had slept after encoding (rather than being awake) at both immediate and remote testing, novel-object recognition memory profited from sleep only three weeks after encoding, at which point it was preserved in rats that had slept after encoding but not in those that had been awake. Notably, inactivation of the hippocampus during post-encoding sleep by intrahippocampal injection of muscimol abolished the sleep-induced enhancement of remote novel-object recognition memory. By contrast, muscimol injection before remote retrieval or memory encoding had no effect on test performance, confirming that the encoding and retrieval of novel-object recognition memory are hippocampus-independent. Remote novel-object recognition memory was associated with spindle activity during post-encoding slow-wave sleep, consistent with the view that neuronal memory replay during slow-wave sleep contributes to long-term memory formation. Our results indicate that the hippocampus has an important role in long-term consolidation during sleep even for memories that have previously been considered hippocampus-independent.

Since the description of the patient H.M., who underwent bilateral removal of large portions of the hippocampus and suffered from severe anterograde amnesia, the distinction between hippocampus-dependent and non-hippocampus-dependent forms of memory has been widely accepted[1,2,9]. Encoding and retrieval of hippocampus-dependent memories require the hippocampus, whereas this is not the case for non-hippocampus-dependent memory, which is otherwise comprised of rather heterogeneous kinds of memory (motor skills, cue fear conditioning and so on). The 'standard consolidation theory' and recent advances[2,9,10] assume that memory of episodes, and in particular the relations among their elements, are initially encoded into hippocampal networks, but that during consolidation the representations are redistributed over days, weeks, and months to neocortical networks that serve as long-term stores. In this way these memories may become independent of the hippocampus[9,11].

Sleep is known to support memory consolidation[3–5]. Sleep after memory encoding robustly enhances hippocampus-dependent memory, although there is also evidence that sleep enhances non-hippocampus-dependent forms of memory[12]. With regard to hippocampus-dependent memory, an active systems consolidation process has been proposed[10,13,14] on the basis of findings that neural representations of freshly encoded memories are replayed during subsequent slow-wave sleep (SWS)[15,16]. The neural replay originating from hippocampal networks, together with sharp-wave ripples and thalamic spindles, is likely to promote the transmission of memory information and, with repetitive occurrence, the gradual redistribution of the representation towards extrahippocampal networks[17,18].

The ability of sleep to consolidate non-hippocampus-dependent memory is less well understood[4,19,20]. Here, we compare the effects of post-encoding sleep with those of post-encoding wakefulness on consolidation of non-hippocampus-dependent and hippocampus-dependent forms of memory in rats, and examine the temporal evolution of consolidation effects. We used the novel-object recognition (NOR) task and an object–place recognition (OPR) task as tests of non-hippocampus-dependent and hippocampus-dependent memory, respectively (Fig. 1a). Performance on the NOR task relies on the perirhinal cortex, but normal hippocampal function is not necessary for encoding and retrieving NOR memory in rats[6–8,21].

After task encoding, rats either slept or remained awake during a 2-h interval. Retrieval was tested either immediately after the 2-h interval (recent test) or, in order to test long-term memory, 1 week or (for NOR only) 3 weeks later (remote tests; Fig. 1a). At the recent memory test, NOR memory did not differ between the sleep and wake conditions ($P = 0.43$), and exploration discrimination ratios indicated that there was significant NOR memory in both conditions ($P < 0.045$, Fig. 1b). By contrast, OPR memory at the recent test was enhanced in the sleep compared to the wake condition ($P = 0.034$) and was itself significant only after sleep ($P = 0.044$) and not in the wake condition ($P = 0.49$, $F_{1,20} = 4.70$, $P = 0.043$ for NOR/OPR × sleep/wake analysis of variance (ANOVA) interaction). That sleep benefits recent OPR but not NOR memory confirms previous findings in rats[22,23], and has been taken as evidence that sleep preferentially strengthens hippocampus-dependent memory. Total object exploration, total distance travelled and mean speed at retrieval were comparable between sleep and wake conditions (all $P > 0.194$, Extended Data Fig. 1a), excluding confounds by nonspecific changes, for example, in locomotion or motivation.

At the remote test performed after 1 week, NOR memory still did not differ between the sleep and wake conditions ($P = 0.45$), and in both conditions rats showed significant NOR memory ($P < 0.045$). Also, as at the recent test, at the 1-week test rats showed better OPR memory in the sleep than the wake condition ($P = 0.001$), and OPR memory was not significant in the wake condition ($P > 0.308$, $F_{1,15} = 17.26$, $P = 0.001$ for NOR/OPR × sleep/wake interaction; Fig. 1b).

NOR memory faded only when the retrieval delay was extended to 3 weeks. After 3 weeks, rats in the sleep condition but not in the wake condition showed significant NOR memory, and performance was significantly better for rats that had slept after encoding than for rats that had not ($P = 0.031$, $F_{1,17} = 4.696$, $P = 0.045$ for 1/3 weeks × sleep/wake interaction in analysis of NOR data). A supplementary experiment indicated that the decrease in NOR memory after post-encoding

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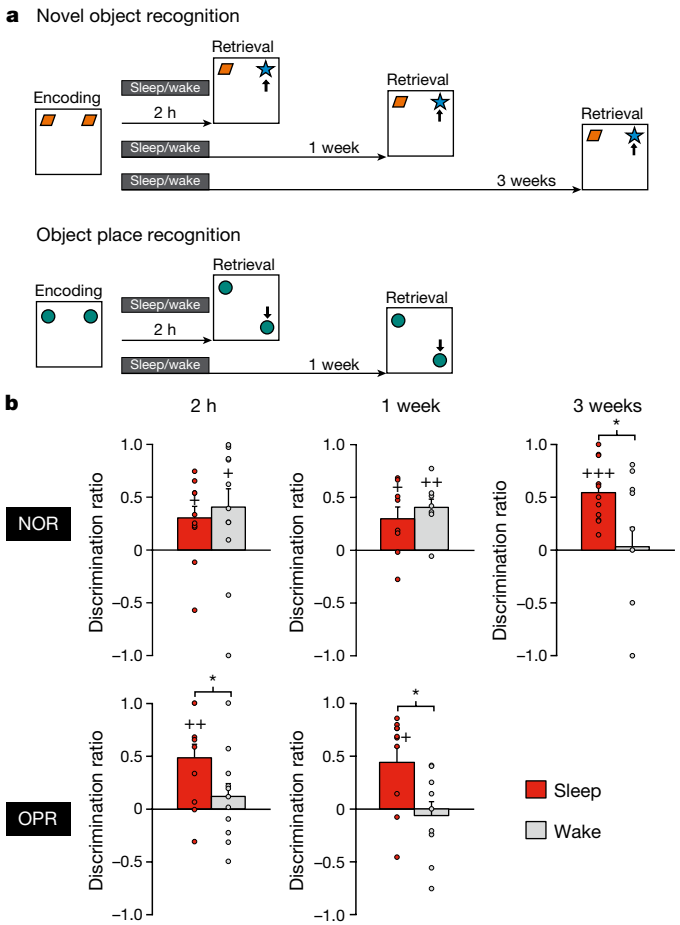

Fig. 1 | Effects of post-encoding sleep versus wakefulness on memory in the NOR and OPR tasks. a, During the encoding phase of both tasks, the rats explored (for 10 min) two identical objects in an arena. Encoding was followed by a 2-h interval in which the rat either slept or remained awake. Retrieval was tested immediately after the 2-h post-encoding interval (recent memory) and 1 week and (for NOR only) 3 weeks later (remote memory). At the retrieval test, the rat explored the arena for 5 min. To test NOR retrieval, one of the two objects (from the encoding phase) was replaced by a novel object (arrow); recognition memory was indicated when the rat spent more time exploring the novel object than the familiar object (discrimination ratio), with exploration during the first minute being most sensitive to exploration of novelty[6]. To test OPR retrieval, one of the objects was displaced (relative to its location at encoding, arrow) and memory for the place was indicated when the rat spent more time exploring the displaced object than the stationary object (which had not moved). **b**, Mean + s.e.m. discrimination ratios during the first minute of exploration for NOR and OPR at the recent (2 h) and remote (1 or 3 weeks) retrieval tests (dot plots overlaid). NOR memory benefited from post-encoding sleep (red bars; compared with wake, grey) only at the 3-week retrieval test, when NOR memory had decayed in the wake condition. By contrast, OPR memory benefited from sleep at both recent and remote testing. $n = 12$, 8 and 11 rats for NOR at 2 h, 1 week and 3 weeks; $n = 11$ and 9 rats for OPR at 2 h and 1 week, respectively. $^{+++}P < 0.001$, $^{++}P < 0.01$, $^{+}P < 0.05$ for one-sample t-tests against chance level; $^{*}P < 0.05$ for pairwise t-tests (two-sided) between sleep and wake (see Extended Data Fig. 2 for discrimination ratios during the entire retrieval phase).

wakefulness had already occurred 1 week earlier, at a 2-week retrieval test (Extended Data Fig. 2c). Overall, remote testing confirmed that NOR memory was maintained over time periods of up to one week, even if encoding is followed by a wake period[24]. However, the formation of more persistent long-term NOR memory requires sleep after encoding, with the sleep effect emerging only after 2–3 weeks, which corresponds to the time required for NOR memory in the wake condition to fade.

The consolidating effect of sleep on hippocampus-dependent spatial memory is mediated by repeated reactivations of the newly encoded hippocampal representations during subsequent SWS[15,16,25]. Moreover, hippocampus-dependent and non-hippocampus-dependent memory systems have been found to interact during consolidation[12,26]. Thus, we investigated whether hippocampal activity also critically contributes to the consolidation of non-hippocampus-dependent memory by reversibly inactivating hippocampal function by infusing muscimol into the dorsal hippocampus during sleep after encoding the NOR task.

At remote retrieval testing 3 weeks later, rats who had received muscimol injection into the hippocampus during sleep after learning did not show significant NOR memory ($P = 0.38$), whereas remote NOR memory was preserved in those injected with vehicle at the same time point ($P = 0.001$; $F_{1,14} = 8.99$, $P = 0.01$, for muscimol/vehicle main effect, Fig. 2a). Control parameters such as total object exploration did not differ between conditions, excluding nonspecific changes in motivation or vigilance (Extended Data Fig. 1). This result demonstrates that the hippocampus is crucial for the formation of persistent NOR memory during sleep. Previous studies that suppressed hippocampal activity after encoding in the NOR task had conflicting results[8,27–29], which fuelled a long-standing debate about the possible hippocampal dependency of NOR memory[21,30,31]. These discrepancies can be resolved by our results, which show that formation of persistent long-term NOR memory relies on a hippocampal mechanism that is specifically active during sleep.

To determine whether the hippocampus is specifically involved in sleep consolidation, in a control experiment hippocampi were inactivated during a 2-h post-encoding wake period and retrieval was tested 1 week later. In these rats, NOR memory tended to be enhanced when compared to control animals whose hippocampal function was intact during the wake period after encoding ($F_{1,13} = 4.492$, $P = 0.054$ for muscimol/control main effect; Fig. 2b and Extended Data Fig. 3b), suggesting that, during wakefulness, hippocampal activity normally interferes with NOR memory consolidation[8]. Overall, these results corroborate the notion that persistent long-term NOR memory formation relies on a hippocampal mechanism that is specifically active during sleep, whereas non-hippocampal mechanisms during post-encoding wakefulness enable NOR memory over a period of 1 week.

We investigated whether the hippocampus would also be required for retrieval of long-term NOR memory at 3 weeks. Hippocampal infusion of muscimol before the 3-week retrieval test (in rats that had slept for 2 h after encoding) did not abolish NOR memory, with the rats' performance being closely comparable to that of a vehicle-infused group ($P > 0.70$ for all comparisons, Fig. 2a). This result indicates that whereas the formation of long-term NOR memory during sleep requires the hippocampus, its retrieval is not dependent on hippocampal function. In two further control experiments, muscimol was infused either shortly before a retrieval test that took place 30 min after encoding, or shortly before the encoding phase, with retrieval tested 30 min later (Fig. 2c). The experiments confirmed that short-term retrieval of NOR memory and encoding per se likewise do not depend on hippocampal function ($P = 0.46$ and $P = 0.79$, respectively, for differences between vehicle and muscimol)[6,7]. Together, these results indicate that whereas the sleep-dependent formation of persistent long-term NOR memory requires the hippocampus, the retrieval of these memories is non-hippocampus-dependent at any time after encoding.

The architecture of post-encoding sleep was comparable to that reported in previous studies[23] (Extended Data Table 1). Correlation analyses revealed that remote NOR memory retrieval was strongly associated with measures of spindle activity during SWS, but not with rapid eye movement (REM)-sleep-related measures (Extended Data Table 2). Thus, NOR discrimination ratios at the 3-week retrieval test correlated with the number ($r = 0.719$, $P = 0.029$) and duration of spindles ($r = 0.705$, $P = 0.034$, Fig. 3a), with the latter correlation being most robust in an exploratory analysis focusing on the first 30 min of post-encoding sleep ($r = 0.888$, $P = 0.001$) in which neuronal replay in hippocampal networks, as a possible consolidation mechanism, is typically strongest[32] (see Extended Data Fig. 4 for related OPR data).

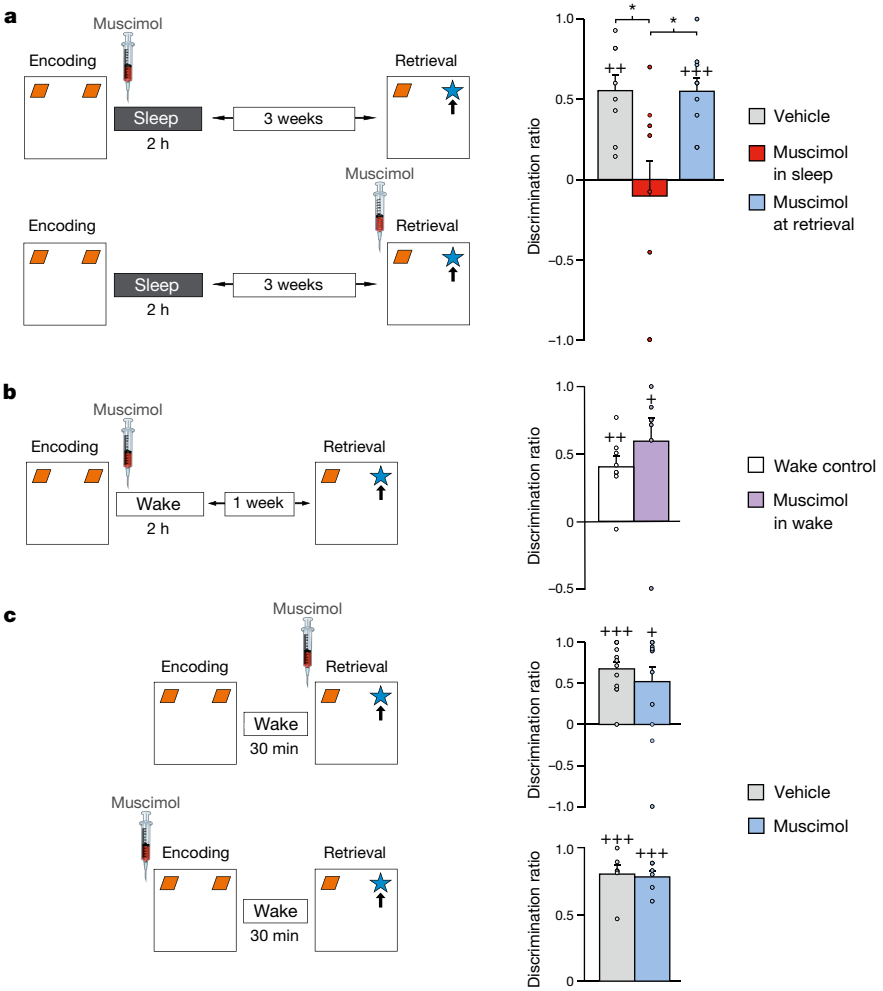

Fig. 2 | Effects of reversibly inactivating the hippocampus on NOR memory. Left, procedures; right, mean + s.e.m. discrimination ratios, with overlaid dot plots. **a**, To suppress hippocampal activity, muscimol was bilaterally infused (over 2 min) into the dorsal hippocampus, either during the post-encoding interval upon the first occurrence of continuous SWS (top; $n = 8$ rats each for muscimol and vehicle), or 15 min before remote retrieval testing 3 weeks after encoding (bottom; $n = 9$ rats). Hippocampal inactivation during post-encoding sleep (red bar) abolished remote NOR memory whereas inactivation before retrieval testing (blue bar) was ineffective. Grey bar, vehicle injection. **b**, Muscimol (purple bar) was infused shortly after encoding while the rats remained awake during the 2-h post-encoding interval ($n = 7$ rats). Retrieval was tested 1 week later. Compared with untreated wake control rats ($n = 8$ rats, empty bar), which had intact hippocampal function and stayed awake during the post-encoding interval, hippocampal inactivation did not disturb but rather tended to enhance NOR performance. Timing (with reference to encoding), dosage and procedures of muscimol infusion were the same as in **a**. **c**, Muscimol (or vehicle) was infused 15 min before retrieval testing of recent NOR memory (top, $n = 12$ rats each) or 15 min before the encoding phase (bottom, $n = 6$ rats each). Retrieval was tested 30 min after encoding (rats stayed awake during this interval). Hippocampal inactivation does not affect retrieval of recent NOR memory either during retrieval or during encoding. $^{+++}P < 0.001$, $^{++}P < 0.01$, $^{+}P < 0.05$ for one-sample t-test against chance level; $^{*}P < 0.05$ for pairwise tests (two-sided) between conditions.

Intrahippocampal injection of muscimol during post-encoding sleep reduced electroencephalogram (EEG) theta activity ($P = 0.014$), which is thought to be generated in septal–hippocampal circuitry, and accordingly reduced time spent in both REM (0.83 ± 0.83 versus 6.04 ± 1.07 min after vehicle) and preREM sleep (1.97 ± 0.51 versus 5.80 ± 0.70 min after vehicle, both $P < 0.003$). Muscimol did not influence surface EEG activity during SWS (all $P > 0.410$, Extended Data Table 1). However, intrahippocampal local field potential (LFP) recordings from additional rats showed a distinct reduction in the number and density of hippocampal ripples, hippocampal spindle power and slow oscillation amplitude following muscimol infusion during post-encoding sleep ($P = 0.005$, 0.025, 0.013, and 0.007, respectively; Fig. 3b). These changes are consistent with the view that muscimol prevents formation of long-term NOR memory by suppressing hippocampal ripples and associated reactivation of representations during SWS[33], although our findings do not rule out contributions of REM-sleep-related mechanisms[34].

There is ample evidence that the hippocampus is involved in the consolidation of memory classified as hippocampus-dependent, as it can be acquired and retrieved only with normal hippocampal function[9]. We have now shown that normal hippocampal function is also required for the formation of persistent long-term representations on a task that, based on the same criterion, is classified as non-hippocampus-dependent[6,7]. How does the hippocampus contribute to the formation of long-term NOR memory? In the NOR task, representation of the object resides mainly in the perirhinal cortex, whereas the hippocampus encodes spatial context features[7,35]. Accordingly, retrieval in the NOR task also involves hippocampal function—making the task seemingly hippocampus-dependent—when it is performed in a context that is novel to the rat[8,36]. Along this line, we propose that, during sleep, the hippocampus is likely to boost object representation through activation of context-related representations, rather than directly enhancing perirhinal object memory. The observed correlation of long-term NOR performance with post-encoding sleep spindle activity corroborates this view: neuronal reactivations of spatial context representations during sleep occur in the hippocampus, in conjunction with ripples and thalamic spindles[25,32]. Spindles, moreover,

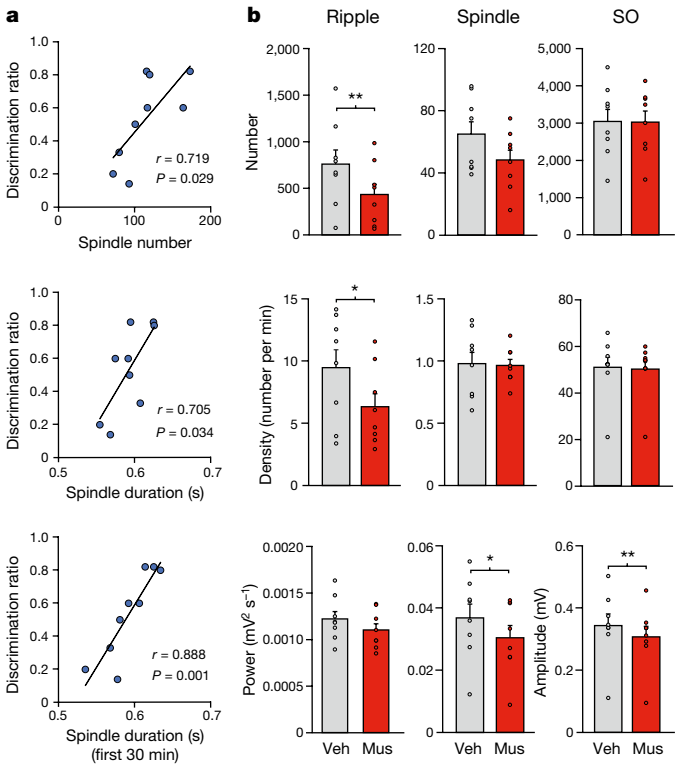

Fig. 3 | Contribution of post-encoding slow wave sleep to remote NOR memory. a, NOR performance (discrimination ratio) at the 3-week retrieval test was correlated with the number of sleep spindles during SWS (top) and spindle mean duration during the 2-h post-encoding interval (middle), as well as with spindle mean duration during the first 30 min of post-encoding sleep (bottom; Pearson's product–moment correlations, $n = 9$ rats). **b**, Intrahippocampal LFPs were recorded in additional rats to examine the effects of bilateral intrahippocampal infusion of muscimol (Mus) (versus vehicle, Veh) on (from left to right) ripples, spindles, and slow oscillations (SO) in hippocampal networks during SWS ($n = 8$ tests per condition). Muscimol decreased the total number and density of ripples, as well as spindle power and slow oscillation amplitude. Data shown as mean + s.e.m. with overlaid dot plots. $^{**}P < 0.01$, $^{*}P < 0.05$ for pairwise two-sided t-tests.

have been identified as a mechanism that favours the spreading of reactivations to extrahippocampal networks[17,37], thereby promoting plastic synaptic changes that can ultimately strengthen these extrahippocampal representations[18,38].

In conclusion, our findings suggest that a common hippocampal mechanism boosts consolidation in both hippocampus-dependent and non-hippocampus-dependent memory systems through the reactivation of contextual features. Indeed, in humans, hippocampal activity during training predicts sleep-dependent consolidation of a motor skill that is considered to be non-hippocampus-dependent[12,26]. From this perspective, the formation of long-term representations during sleep, whether hippocampus-dependent or not, critically depends on their being encoded within a spatiotemporal context—that is, as episodic memories. Because such a mechanism puts the hippocampus-dependent episodic memory system into a supra-ordinate position to organize long-term memory, it has strong implications for current theorizing about interacting 'parallel memory systems'[39]. However, non-hippocampus-dependent memory is heterogeneous, and other memories of this kind need to be studied to scrutinize the proposed general hippocampal mechanism of long-term memory formation.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-018-0716-8.

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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. Moscovitch, M., Cabeza, R., Winocur, G. & Nadel, L. Episodic memory and beyond: the hippocampus and neocortex in transformation. *Annu. Rev. Psychol.* **67**, 105–134 (2016).
3. Stickgold, R. Sleep-dependent memory consolidation. *Nature* **437**, 1272–1278 (2005).
4. Rasch, B. & Born, J. About sleep's role in memory. *Physiol. Rev.* **93**, 681–766 (2013).
5. Tononi, G. & Cirelli, C. Sleep and the price of plasticity: from synaptic and cellular homeostasis to memory consolidation and integration. *Neuron* **81**, 12–34 (2014).
6. Winters, B. D., Forwood, S. E., Cowell, R. A., Saksida, L. M. & Bussey, T. J. Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: heterogeneity of function within the temporal lobe. *J. Neurosci.* **24**, 5901–5908 (2004).
7. Winters, B. D., Saksida, L. M. & Bussey, T. J. Object recognition memory: neurobiological mechanisms of encoding, consolidation and retrieval. *Neurosci. Biobehav. Rev.* **32**, 1055–1070 (2008).
8. Oliveira, A. M., Hawk, J. D., Abel, T. & Havekes, R. Post-training reversible inactivation of the hippocampus enhances novel object recognition memory. *Learn. Mem.* **17**, 155–160 (2010).
9. Eichenbaum, H. A cortical-hippocampal system for declarative memory. *Nat. Rev. Neurosci.* **1**, 41–50 (2000).
10. 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).
11. Frankland, P. W. & Bontempi, B. The organization of recent and remote memories. *Nat. Rev. Neurosci.* **6**, 119–130 (2005).
12. King, B. R., Hoedlmoser, K., Hirschauer, F., Dolfen, N. & Albouy, G. Sleeping on the motor engram: the multifaceted nature of sleep-related motor memory consolidation. *Neurosci. Biobehav. Rev.* **80**, 1–22 (2017).
13. Diekelmann, S. & Born, J. The memory function of sleep. *Nat. Rev. Neurosci.* **11**, 114–126 (2010).
14. Lewis, P. A. & Durrant, S. J. Overlapping memory replay during sleep builds cognitive schemata. *Trends Cogn. Sci.* **15**, 343–351 (2011).
15. Wilson, M. A. & McNaughton, B. L. Reactivation of hippocampal ensemble memories during sleep. *Science* **265**, 676–679 (1994).
16. Rasch, B., Büchel, C., Gais, S. & Born, J. Odor cues during slow-wave sleep prompt declarative memory consolidation. *Science* **315**, 1426–1429 (2007).
17. Latchoumane, C. V., Ngo, H. V., Born, J. & Shin, H. S. Thalamic spindles promote memory formation during sleep through triple phase-locking of cortical, thalamic, and hippocampal rhythms. *Neuron* **95**, 424–435.e6 (2017).
18. Seibt, J. et al. Cortical dendritic activity correlates with spindle-rich oscillations during sleep in rodents. *Nat. Commun.* **8**, 684 (2017).
19. Ramanathan, D. S., Gulati, T. & Ganguly, K. Sleep-dependent reactivation of ensembles in motor cortex promotes skill consolidation. *PLoS Biol.* **13**, e1002263 (2015).
20. Li, W., Ma, L., Yang, G. & Gan, W. B. REM sleep selectively prunes and maintains new synapses in development and learning. *Nat. Neurosci.* **20**, 427–437 (2017).
21. Brown, M. W. & Aggleton, J. P. Recognition memory: what are the roles of the perirhinal cortex and hippocampus? *Nat. Rev. Neurosci.* **2**, 51–61 (2001).
22. Inostroza, M., Binder, S. & Born, J. Sleep-dependency of episodic-like memory consolidation in rats. *Behav. Brain Res.* **237**, 15–22 (2013).
23. Oyanedel, C. N. et al. Role of slow oscillatory activity and slow wave sleep in consolidation of episodic-like memory in rats. *Behav. Brain Res.* **275**, 126–130 (2014).
24. Broadbent, N. J., Gaskin, S., Squire, L. R. & Clark, R. E. Object recognition memory and the rodent hippocampus. *Learn. Mem.* **17**, 5–11 (2009).
25. van de Ven, G. M., Trouche, S., McNamara, C. G., Allen, K. & Dupret, D. Hippocampal offline reactivation consolidates recently formed cell assembly patterns during sharp wave-ripples. *Neuron* **92**, 968–974 (2016).
26. Albouy, G. et al. Maintaining vs. enhancing motor sequence memories: respective roles of striatal and hippocampal systems. *Neuroimage* **108**, 423–434 (2015).
27. de Lima, M. N., Luft, T., Roesler, R. & Schröder, N. Temporary inactivation reveals an essential role of the dorsal hippocampus in consolidation of object recognition memory. *Neurosci. Lett.* **405**, 142–146 (2006).
28. Rossato, J. I. et al. On the role of hippocampal protein synthesis in the consolidation and reconsolidation of object recognition memory. *Learn. Mem.* **14**, 36–46 (2007).
29. Kim, J. M., Kim, D. H., Lee, Y., Park, S. J. & Ryu, J. H. Distinct roles of the hippocampus and perirhinal cortex in GABAA receptor blockade-induced enhancement of object recognition memory. *Brain Res.* **1552**, 17–25 (2014).
30. Cohen, S. J. & Stackman, R. W. Jr Assessing rodent hippocampal involvement in the novel object recognition task. A review. *Behav. Brain Res.* **285**, 105–117 (2015).
31. Squire, L. R., Wixted, J. T. & Clark, R. E. Recognition memory and the medial temporal lobe: a new perspective. *Nat. Rev. Neurosci.* **8**, 872–883 (2007).
32. O'Neill, J., Pleydell-Bouverie, B., Dupret, D. & Csicsvari, J. Play it again: reactivation of waking experience and memory. *Trends Neurosci.* **33**, 220–229 (2010).

33. Girardeau, G., Benchenane, K., Wiener, S. I., Buzsáki, G. & Zugaro, M. B. Selective suppression of hippocampal ripples impairs spatial memory. *Nat. Neurosci.* **12**, 1222–1223 (2009).
34. Chen, L., Tian, S. & Ke, J. Rapid eye movement sleep deprivation disrupts consolidation but not reconsolidation of novel object recognition memory in rats. *Neurosci. Lett.* **563**, 12–16 (2014).
35. Brown, M. W., Barker, G. R., Aggleton, J. P. & Warburton, E. C. What pharmacological interventions indicate concerning the role of the perirhinal cortex in recognition memory. *Neuropsychologia* **50**, 3122–3140 (2012).
36. Piterkin, P., Cole, E., Cossette, M. P., Gaskin, S. & Mumby, D. G. A limited role for the hippocampus in the modulation of novel-object preference by contextual cues. *Learn. Mem.* **15**, 785–791 (2008).
37. Bergmann, T. O., Mölle, M., Diedrichs, J., Born, J. & Siebner, H. R. Sleep spindle-related reactivation of category-specific cortical regions after learning face-scene associations. *Neuroimage* **59**, 2733–2742 (2012).
38. Chauvette, S., Seigneur, J. & Timofeev, I. Sleep oscillations in the thalamocortical system induce long-term neuronal plasticity. *Neuron* **75**, 1105–1113 (2012).
39. Lisman, J. et al. Viewpoints: how the hippocampus contributes to memory, navigation and cognition. *Nat. Neurosci.* **20**, 1434–1447 (2017).
40. Miller, K. J., Botvinick, M. M. & Brody, C. D. Dorsal hippocampus contributes to model-based planning. *Nat. Neurosci.* **20**, 1269–1276 (2017).
41. Pack, A. I. et al. Novel method for high-throughput phenotyping of sleep in mice. *Physiol. Genomics* **28**, 232–238 (2007).
42. Neckelmann, D., Olsen, O. E., Fagerland, S. & Ursin, R. The reliability and functional validity of visual and semiautomatic sleep/wake scoring in the Møll-Wistar rat. *Sleep* **17**, 120–131 (1994).

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Competing interests The authors declare no competing interests.

Additional information
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METHODS

Animals. Ninety-one adult male Long Evans rats (Janvier, 260–310 g, 10–12 weeks) were used for the experiments. Rats were housed in groups of 2–4 rats per cage, except during the post-surgery recovery period, when they were kept individually on a 12-h light/12-h dark cycle (lights on at 06:00), and had unrestricted access to water and food throughout the experiments. All experimental procedures were performed in accordance with the European animal protection laws and policies (Directive 86/609, 1986, European Community) and were approved by the Baden-Württemberg state authority.

No statistical methods were used to predetermine sample size. In all experiments, rats were randomly assigned to experimental groups and conditions before the experiment. The experimenters were not blinded to the experimental group or condition during data collection. However, all behavioural and electrophysiological recordings were analysed offline, with the experimenters blinded to the experimental groups and conditions.

Design and general procedures. Different groups of rats were tested on either the NOR task or the OPR task, using post-encoding retention intervals of 2 h, 1 week and, only for the NOR task, of 3 weeks. Each group of animals was tested on a sleep condition (allowed to sleep during the 2-h post-encoding interval) and a wake condition (stayed awake during this interval). The order of sleep and wake conditions was counterbalanced across animals of a group. For an individual rat, the conditions were separated by an interval that was at least 2 weeks and twice as long as the tested retention interval. Encoding and the subsequent 2-h post-encoding interval took place in both the sleep and wake conditions during the animal's rest phase (between 08:00 and 13:00). In the sleep condition, during the 2-h post-encoding interval, the animals were left undisturbed in a 'post-encoding' box (35 × 35 cm, height: 45 cm) that was made of plastic and contained some bedding materials. Sleep was assessed using video recorded behaviour using standard procedures (see below). In the wake condition, wakefulness was enforced using gentle handling[22,23]. This procedure minimizes stress and confounding influences of locomotion. It involved tapping on the retention box and, if necessary, gently shaking the box. No intense stimulation was used, and video records ensured that signs of startle or freezing behaviour did not occur. In the remote groups tested after 1 and 3 weeks, animals were brought to their home cages after the 2-h post-encoding interval and kept under routine conditions until testing.

Habituation and memory tasks. After handling daily for five consecutive days for 5–10 min, the rats were brought into the test room once every day on three consecutive days for a habituation session. For object familiarization, the rat was placed into an empty cage with an object (not used for the experiments) positioned in the centre of the cage. The rat was allowed to freely explore the object for 10 min. For arena familiarization, the rat was then placed into an empty open field, facing a different wall of the open field at each session to facilitate allocentric navigation, and allowed to explore for 10 min. Immediately afterwards, the rat was left undisturbed in the post-encoding box for 2 h.

On the day after the habituation phase, the experiment started with the encoding phase of the memory task. The encoding phase was identical for the NOR and OPR task, and comprised a 10-min interval during which the rats were allowed to explore two identical objects in the open field. For testing retrieval on the NOR task, one of the two objects of the encoding phase was replaced by a novel object. For testing retrieval on the OPR task, one of the two objects of the encoding phase was moved to a different location. At each test, the rat had 5 min to explore the arena.

The tasks were performed in a room with a noise-generator providing masking noise. The open field (80 cm × 80 cm, height of walls: 40 cm) was made of grey PVC. Through the open upper side of the arena the rat could perceive distal cues (two rectangles at the north wall, two other rectangles at the east wall, and a square at the west wall). Objects for exploration were made of glass, with different colours and shapes, and heavy enough not to be moved by the rat (height: 15–30 cm; base diameter: 7–12 cm). They were positioned at least 10 cm equidistant from the walls to ensure that the animal's preference to stay in corners did not bias exploration times. Pilot studies ensured that the rats could discriminate among the different objects and did not show any preference for one of the objects. The locations of objects during the encoding and retrieval phases were randomized across rats. Each rat's exploration behaviour was monitored by a video camera and analysed offline by an experienced researcher using ANY-maze software (Stoelting Europe). After each phase, the apparatus and objects were cleaned with water containing 70% ethanol.

Inactivating the hippocampus during sleep. To reversibly inactivate the dorsal hippocampus during sleep, we infused the GABA-A receptor agonist muscimol, according to standardized procedures[8,40]. After 5 days of handling, guide cannulae were surgically implanted bilaterally into the dorsal hippocampi, and at least 8 days were allowed for recovery. Muscimol (Sigma, 0.5 μg dissolved in 0.5 μl saline solution, per hemisphere) or an equivalent volume of vehicle (saline solution) was infused bilaterally over 2 min by an automated syringe pump. (In pilot studies with this dosing, no spread of the substance to extrahippocampal regions occurred; Extended Data Fig. 5b.) For substance administration, two 30-gauge injection cannulae were connected to two 10-μl Hamilton microsyringes (Hamilton), with 1-m polyethylene cannula tubing. The injection cannulae protruded 1 mm beyond the tip of the guide cannulae. The injection cannulae were kept in the bilateral guide cannulae for a further 2 min to prevent backflow. The procedure enabled substance administration into freely moving rats without disturbing ongoing sleep. Rats were killed at the end of the experiments for histological confirmation of the infusion sites (Extended Data Fig. 5).

The effects of muscimol and vehicle were compared in a between-subjects comparison in 16 rats (8 per group). To test the effects of hippocampal inactivation during sleep in the 2-h post-encoding interval, substance administration started immediately upon (visual) online detection of continuous SWS for at least 10 s. On average, substance administration took place after 38.30 ± 2.16 min of the post-encoding interval in the muscimol condition and after 40.35 ± 1.33 min in the vehicle condition ($P = 0.42$).

Surgery in experiments with reversible inactivation of the hippocampus. Guide cannulae were implanted under general isoflurane anaesthesia (induction: 1–2%, maintenance: 0.8–1.2% in 0.35 l/min O_2). Preoperatively, fentanyl (0.005 mg/kg), midazolam (2 mg/kg) and medetomidine (0.15 mg/kg) were administered intraperitoneally. Rats were placed in the stereotaxic frame and the skull was exposed. Two stainless steel guide cannulae (7 mm long, 23 gauge, Plastics One) were bilaterally implanted into the dorsal hippocampi (anterior–posterior (AP): −4.3 mm, mediolateral (ML): ±2.8 mm, dorsoventral (DV): −1.3 mm under skull surface, relative to bregma). The cannulae were introduced to this position laterally tilted by 9° with respect to the vertical axis and were affixed to the skull with four bone screws and cold polymerizing dental resin. Dummy cannulae (7 mm long, Plastics One) were inserted into the guide cannulae and removed only for infusions.

For simultaneous EEG recordings in the animals, four screw electrodes were implanted: two frontal electrodes (AP: +2.6 mm, ML: ±1.8 mm, relative to bregma) and two occipital electrodes (AP: −10.0 mm, ML: ±1.8 mm), with the latter serving (for all recordings) as reference and ground, respectively. Additionally, in a subgroup of animals, two platinum electrodes were attached to the guide cannulae to record hippocampal LFP signals (AP: −4.3 mm, ML: ±2.8 mm, DV: −2.3 mm, relative to bregma). Two stainless steel wire electrodes were implanted bilaterally in the neck muscles for electromyography (EMG) recordings. Electrodes were connected to a Mill-Max pedestal and fixed to the skull with cold polymerizing dental resin and the wound was sutured. After the surgery, the rats received a subcutaneous 1-ml injection of saline solution to prevent dehydration, and carprofen (5 mg/kg). Rats were allowed to recover for at least 8 days.

Correct placement of the cannulae and of electrodes for LFP recordings was confirmed by histology after completion of the experiments. For this, the rats were perfused intracardially with 0.9% saline followed by 4% paraformaldehyde (PFA). After decapitation, the brains were removed and immersed in the 4% PFA for at least two days. Coronal sections of 50–70 μm were cut on a vibratome, stained with toluidine blue and examined under a light microscope (Extended Data Fig. 5).

Analysis of memory performance. Exploration was defined by the rat being within 2 cm of an object, directing its nose towards the object and engaging in active exploration behaviours such as sniffing. For each task, the time a rat spent exploring each object during the retrieval test was converted into a discrimination ratio according to the general formula: (time spent at novel − time spent at familiar)/(time spent at novel + time spent at familiar), where 'novel' on the NOR task refers to the novel object and on the OPR task refers to the displaced object. A value of zero indicates no exploration preference, whereas a positive value indicates preferential exploration of the novel configuration, thus indicating memory of the familiar configuration. Additionally, the total time of object exploration (across both objects), distance travelled and mean speed on each task were determined. Statistical comparisons concentrated on cumulative discrimination ratios for the first 1 min and 3 min of the retrieval phase.

Analysis of sleep, EEG, and hippocampal LFP recordings. Sleep during the retention interval was assessed using video recordings and tracking software (ANY-Maze, Stoelting Europe) using standard visual procedures[41]. In brief, sleep was scored whenever the rat showed a typical sleep posture and stayed immobile for at least 10 s. If brief movements interrupted sleep epochs by <5 s, continuous sleep was scored. The agreement of the procedure with EEG-based scoring of sleep in the present (see below) and previous studies was >92%[22,41]. Scores indicated an average of 46.97 ± 2.86 min spent asleep during the 2-h post-encoding retention interval, with the first bout of sleep occurring 41.24 ± 2.99 min after the encoding phase. There were no significant differences in sleep parameters between NOR and OPR task conditions or retention intervals tested (Extended Data Table 1).

In the experiments testing the effects of reversible inactivation of the hippocampus, sleep was additionally analysed using EEG and EMG recordings. For the recordings, electrodes were connected through a preamplifier headstage (Model HS-18MM, Neuralynx) to a Digital Lynx SX acquisition system (Neuralynx),

amplified, filtered (EEG: 0.01–300.0 Hz; EMG: 30.0–300.0 Hz), and sampled at a rate of 1,000 Hz. Sleep stages (SWS, preREM and REM sleep) and wakefulness were scored offline by visual inspection using 10-s epochs according to standard criteria[42]. In brief, the wake stage was characterized by predominant low-amplitude fast activity associated with increased EMG tonus. SWS was characterized by predominant high-amplitude delta activity (<4.0 Hz) and reduced EMG activity, and REM sleep by predominant theta activity (4.0–8.0 Hz), phasic muscle twitches and minimal EMG activity. PreREM sleep was identified by a decrease in delta activity, a progressive increase in theta activity and the presence of sleep spindles (10.0–16.0 Hz). Sleep stage classification was performed by an experienced experimenter.

EEG signals in these experiments were also used to identify slow oscillations and spindles during SWS. Identification of slow oscillations followed procedures as described[17]. In brief, the EEG signal during all SWS epochs for an animal was filtered between 0.3 and 4.5 Hz. A slow oscillation event was then identified if the following criteria were fulfilled: (i) two consecutive negative-to-positive zero crossings of the signal occurred at an interval between 0.4 and 2.0 s; (ii) of these events in an individual rat, the 35% with the highest negative peak amplitude between both zero crossings were selected; and (iii) of these events the 45% with the highest negative-to-positive peak-to-peak amplitude were selected. These criteria resulted in the detection of slow oscillations with negative peak amplitudes exceeding −80 μV and peak-to-peak amplitudes exceeding 120 μV. For spindle detection, the EEG signal was filtered between 10.0 and 16.0 Hz. The Hilbert transform was calculated for the filtered signal and smoothed with a moving average (window size 200 ms). A spindle was identified when the absolute value of the transformed signal exceeded 1.5 s.d. of the mean signal during the animal's SWS epochs, for at least 0.4 s and not more than 2.0 s.

The same procedures were applied to identify slow oscillations and spindles in the hippocampal LFP recordings. To identify ripples in these LFP recordings, the signal was filtered between 150.0 and 250.0 Hz. As for spindle detection, the Hilbert transform was calculated and the signal was smoothed using a moving average (window size 200 ms). A ripple event was identified when the Hilbert transform value exceeded a threshold of 2.5 s.d. from the mean signal during an animal's SWS epochs, for at least 25 ms (including at least 3 cycles) and for not more than 500 ms.

Statistical analyses. Statistical analyses were performed using SPSS 21.0 for Windows. To evaluate the discrimination ratios determined for each task, we used ANOVAs that included group factors for the task (NOR/OPR) or the retention interval (1/3 weeks), and repeated-measures factors representing the sleep/wake conditions and discrimination ratios after 1 and 3 min of the retrieval phase. (ANOVAs separately run on 1-min and 3-min values yielded almost identical results and are not reported here.) Muscimol/vehicle comparisons were introduced as group or repeated-measures factors, depending on the experiment. ANOVAs indicating significance for main or interaction effects of interest were followed by post hoc *t*-tests (two-sided). Discrimination ratios were also compared with chance level performance (zero) using one-sample *t*-tests. To analyse the relationship between post-encoding retention sleep and memory performance, Pearson product–moment correlation coefficients were calculated. $P < 0.05$ was considered significant.

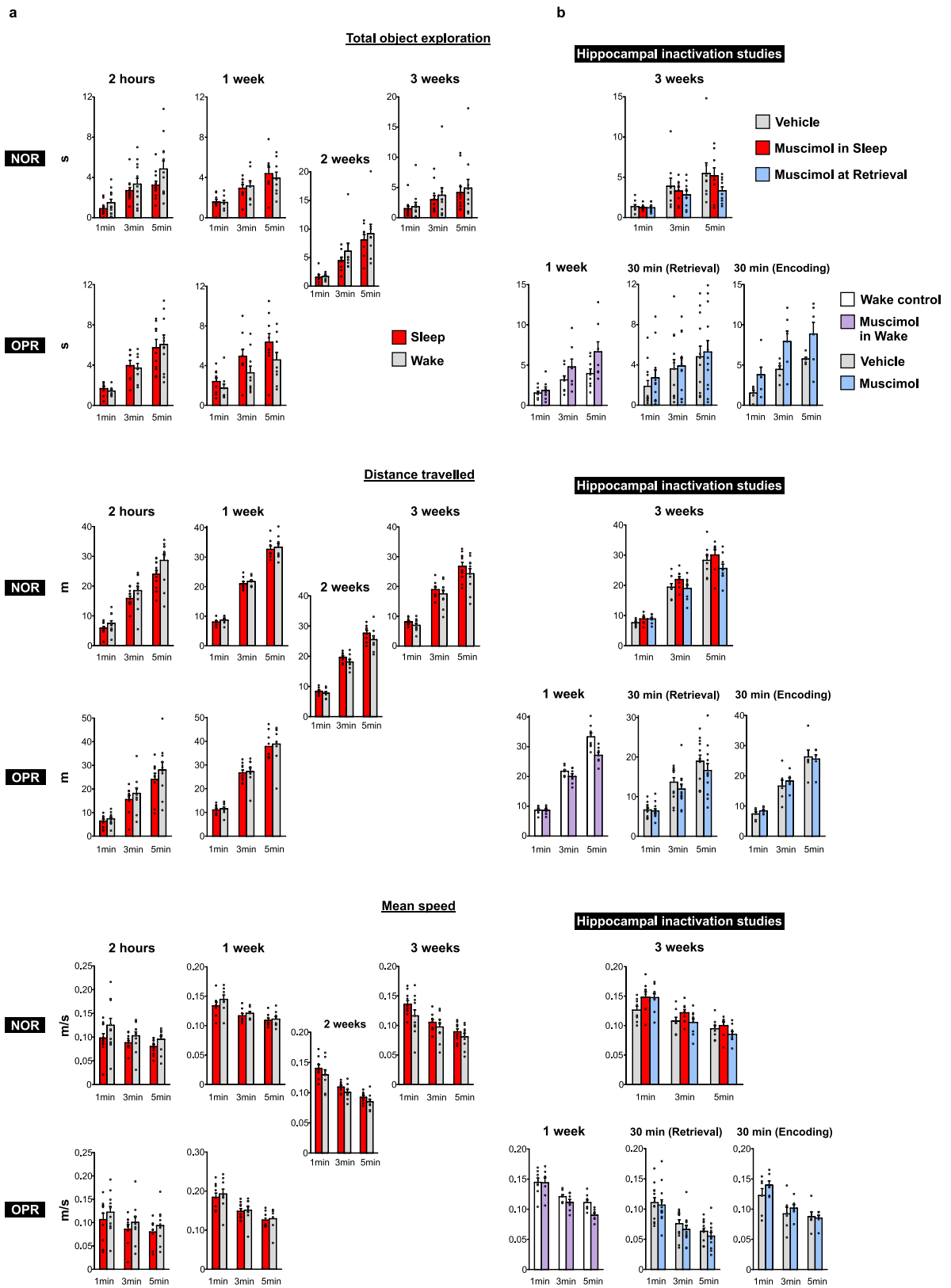
Code availability. The codes used in this study are available from the corresponding authors on reasonable request. MATLAB scripts used for analyses of EEG and LFP signals are available at https://github.com/MedPsych/LongTermMemory_Sleep.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

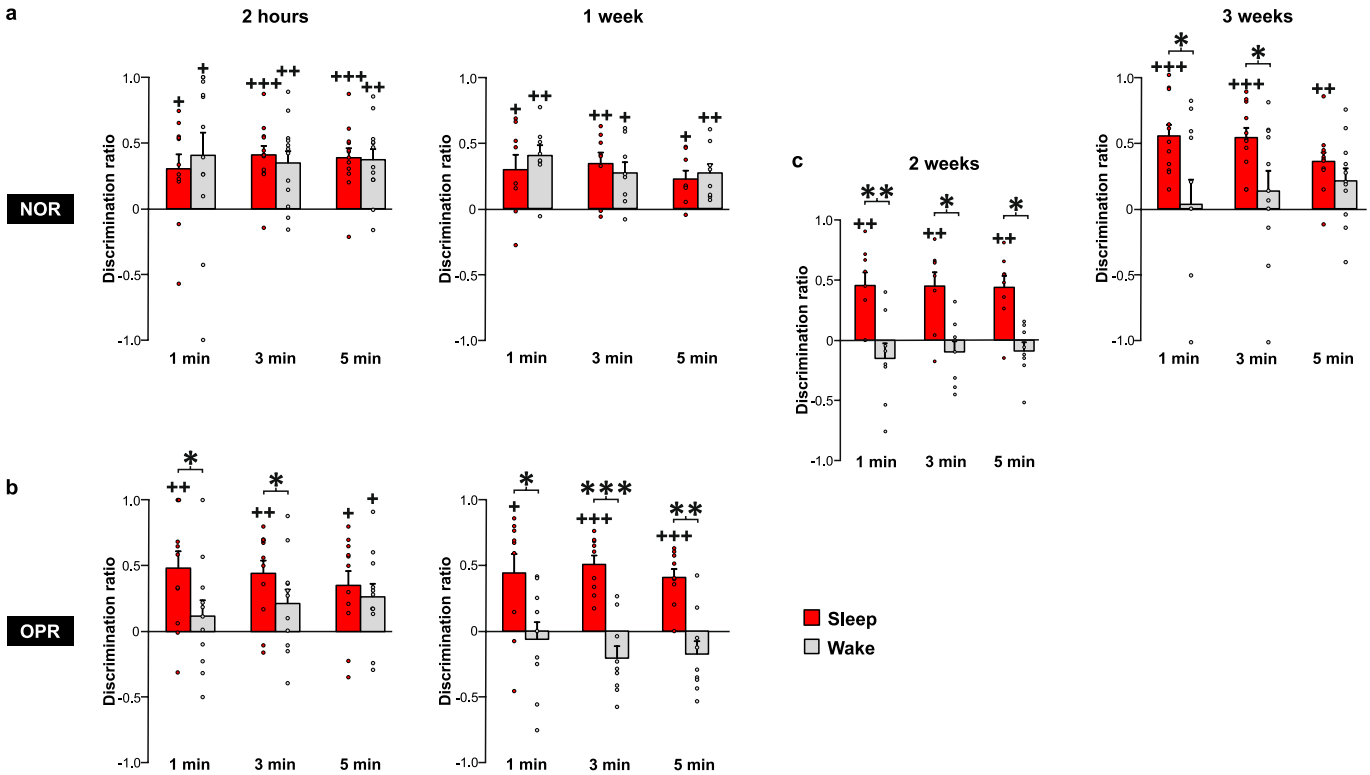
The data that support the findings of this study are available from the corresponding authors on reasonable request. Source Data for graphs shown in Figs. 1–3 and Extended Data Figs. 1–4 are available in the online version of the paper.

43. Dix, S. L. & Aggleton, J. P. Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. *Behav. Brain Res.* **99**, 191–200 (1999).
44. Chambon, C., Wegener, N., Gravius, A. & Danysz, W. A new automated method to assess the rat recognition memory: validation of the method. *Behav. Brain Res.* **222**, 151–157 (2011).
45. Ozawa, T., Yamada, K. & Ichitani, Y. Long-term object location memory in rats: effects of sample phase and delay length in spontaneous place recognition test. *Neurosci. Lett.* **497**, 37–41 (2011).
46. Goshen, I. et al. Dynamics of retrieval strategies for remote memories. *Cell* **147**, 678–689 (2011).
47. Allen, T. A. et al. Imaging the spread of reversible brain inactivations using fluorescent muscimol. *J. Neurosci. Methods* **171**, 30–38 (2008).
48. Bonnevie, T. et al. Grid cells require excitatory drive from the hippocampus. *Nat. Neurosci.* **16**, 309–317 (2013).

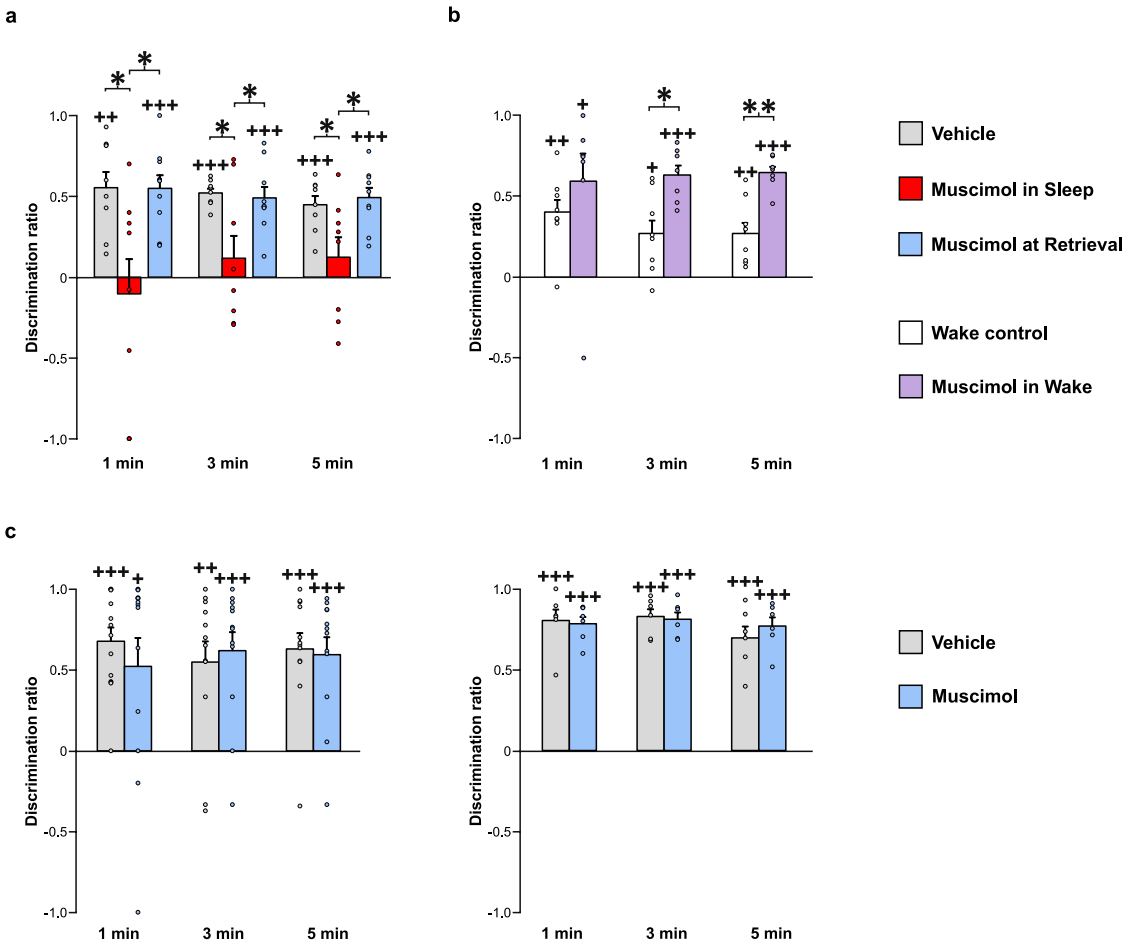


Extended Data Fig. 1 | See next page for caption.

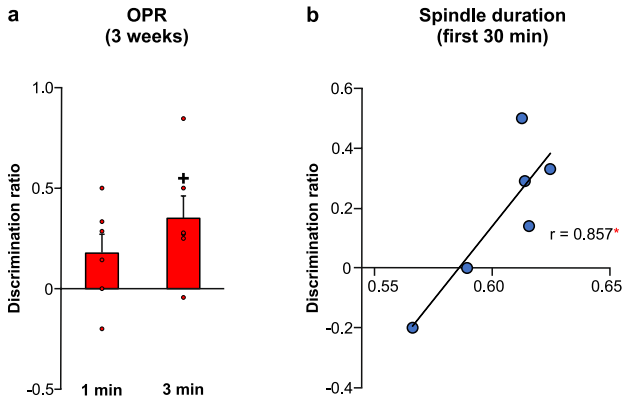
Extended Data Fig. 1 | Control measures for NOR and OPR task performance. Total object exploration (s), total distance travelled (m) and average speed (m s^{-1}) at retrieval testing. Mean values (± s.e.m., dot plots overlaid) for the first 1 and 3 min and for the entire 5 min of the retrieval phase are shown. **a**, Results from main experiments of NOR and OPR memory as illustrated in Fig. 1. Retrieval was tested either immediately after the 2-h retention interval (recent) or 1 week or (for the NOR task only) 3 weeks later (remote). In a supplementary experiment, NOR was tested 2 weeks after encoding (offset downwards). Red, sleep; grey, wake; $n = 12$, 8, 8 and 11 rats for NOR testing after 2 h and 1, 2 and 3 weeks, and $n = 11$ and 9 rats for OPR testing after 2 h and 1 week, respectively. **b**, Results from experiments after bilateral intrahippocampal infusion of muscimol as in Fig. 2. Top, muscimol (versus vehicle, grey bars, $n = 8$ rats) was infused either during the 2-h post-encoding interval (upon first occurrence of SWS, red bars, $n = 8$ rats) or 15 min before retrieval (blue bars, $n = 9$ rats) with the retrieval phase taking place 3 weeks after encoding. Bottom, control studies. Left, muscimol (purple, $n = 7$ rats) was infused shortly after encoding while the rats remained awake during the 2-h post-encoding interval, compared with untreated wake control rats ($n = 8$ rats, empty bars). Retrieval was tested 1 week after encoding (corresponding to Fig. 2b). Right, muscimol (blue bars, versus vehicle, grey bars) was infused either 15 min before retrieval testing ($n = 12$ rats) or 15 min before encoding ($n = 6$ rats) with the retrieval phase taking place 30 min after encoding (corresponding to Fig. 2c). There were no significant differences between sleep and wake or between muscimol and vehicle conditions ($P > 0.194$, for all comparisons based on ANOVA and two-sided post hoc t-tests, see Methods and Figs. 1, 2 for further details).



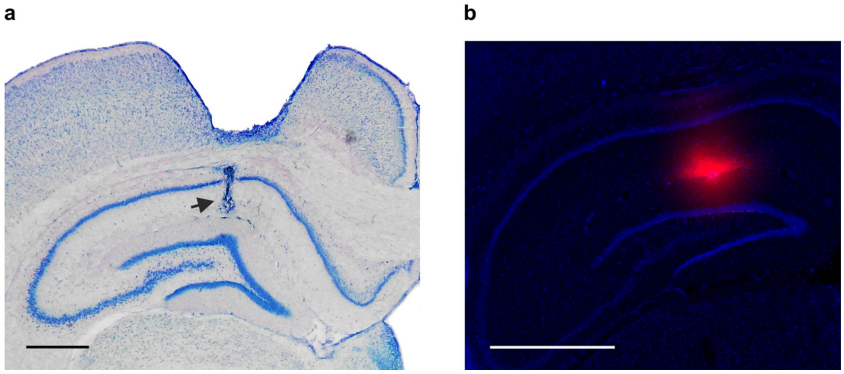
Extended Data Fig. 2 | Performance in recent and remote tests for NOR and OPR tasks. Memory is indicated by mean ± s.e.m. discrimination ratios during the first 1 min, first 3 min, and entire 5 min of the retrieval phase on the NOR and OPR tasks (dot plots overlaid). **a**, NOR was tested with 2-h (recent) and with 1-week and 3-week (remote) retrieval tests. **b**, OPR was tested with 2-h (recent) and 1-week retrieval tests. Whereas OPR memory benefited from sleep (red bars; compared to wake, grey) at both recent and remote (1 week) retrieval tests, NOR benefited from sleep only at the 3-week retrieval test, when NOR memory had decayed in the wake condition. **c**, A supplementary experiment with NOR retrieval tested 2 weeks after post-encoding sleep and wake intervals showed that NOR memory in the wake condition had already faded at this 2-week point, whereas it was preserved in the sleep condition ($F_{1,7} = 14.997$, $P = 0.006$, for sleep/wake main effect; $F_{1,14} = 18.151$, $P = 0.01$ and $F_{1,14} = 0.82$, $P = 0.382$, for 1 versus 2-week comparisons in the wake and sleep conditions, respectively, $F_{1,14} = 12.073$, $P = 0.005$, for 1/2 weeks × sleep/wake interaction; $P > 0.222$ for all comparisons between 2- and 3-week retrieval). In all experiments, recognition memory was assessed by the discrimination ratios during the first 1 and first 3 min of the retrieval period, which typically cover exploration of novelty most sensitively on both the NOR and OPR tasks[6,43–45]. With extended exploration periods, the novelty response often decreases and is thought to become more noisy. Hence, here, the 5-min values were not used for the assessment of recognition memory. $n = 12$, 8, 11 and 8 rats for NOR testing at 2 h, 1 week, 3 weeks and 2 weeks; $n = 11$ and 9 rats for OPR testing at 2 h and 1 week, respectively. $^{+++}P < 0.001$, $^{++}P < 0.01$, $^{+}P < 0.05$ for one-sample t-test against chance level; $^{***}P < 0.001$, $^{*}P < 0.05$ for pairwise t-tests (two-sided) between sleep and wake conditions.



Extended Data Fig. 3 | Performance on NOR task for hippocampal inactivation studies. Memory is indicated by mean ± s.e.m. discrimination ratios during the first 1 min, first 3 min, and entire 5 min of the retrieval phase on the NOR task in experiments involving reversible inactivation of the hippocampus (dot plots overlaid). **a**, Muscimol (red bars, $n = 8$ rats, versus vehicle, grey bars, $n = 8$ rats) was infused into the hippocampus in the post-encoding interval upon the first occurrence of continuous SWS, or 15 min before retrieval testing (blue bars, $n = 9$ rats). Retrieval was tested 3 weeks after encoding. **b**, Control study in which muscimol (purple bars, $n = 7$ rats) was infused shortly after encoding while the rats remained awake during the 2-h post-encoding interval, compared with untreated wake control rats ($n = 8$ rats, empty bars). Retrieval was tested 1 week after encoding. Infusion of muscimol during post-encoding wakefulness tended to enhance NOR performance, which suggests that during wakefulness hippocampal activity normally interferes with NOR memory consolidation[8]. It might also reflect compensatory plasticity occurring in extrahippocampal regions upon hippocampal suppression[46]. **c**, Control studies in which muscimol (blue bars, versus vehicle, grey bars) was infused 15 min before retrieval testing of recent NOR memory (left, $n = 12$ rats for each substance condition) or 15 min before the encoding phase (right, $n = 6$ rats for each substance condition). Retrieval was tested 30 min after encoding, with the rats staying awake during this interval. $^{+++}P < 0.001$, $^{++}P < 0.01$, $^{+}P < 0.05$ for one-sample t-test against chance level; $^{**}P < 0.01$, $^{*}P < 0.05$ for pairwise t-tests (two-sided) between conditions. See Fig. 2 for further details.



Extended Data Fig. 4 | Remote 3-week OPR testing. OPR memory was tested in $n = 6$ rats, 3 weeks after a 2-h post-encoding sleep interval. These supplementary experiments followed the same procedures as described for the 1-week sleep condition on the OPR task, but included sleep EEG recordings. **a**, OPR memory is indicated by the mean ± s.e.m. discrimination ratio during the first 1 min and 3 min of exploration. $^{+}P = 0.034$, for one-sample *t*-test against chance level. Rats displayed significant OPR memory after 3 min (as well as for the whole 5-min exploration period). **b**, OPR performance (discrimination ratio at 1 min) at the 3-week retrieval test was correlated with sleep spindle duration during the first 30 min of post-encoding sleep ($^{*}P = 0.029$, Pearson's product–moment correlation). A similar correlation with NOR performance at the 3-week retrieval (Fig. 3a) points towards a similar mechanism underlying the formation of long-term NOR and OPR memory during sleep.



Extended Data Fig. 5 | Verification of cannula location and muscimol spreading. a, Coronal brain section showing location of cannula in the dorsal hippocampus (black arrow) together with position of guide cannula in overlying cortex. **b**, Coronal brain section showing spread of muscimol (red) after infusion into the hippocampus. Experiments were repeated in $n = 3$ rats with similar results. The infusion protocol was the same as in the behavioural experiments. In brief, after implantation of the guide cannula in the dorsal hippocampus, animals were infused using the injection cannulae with 0.5 μl fluorophore-conjugated muscimol[47,48]. After infusion, animals were intracardially perfused and brains were post-fixed with PFA 4% for 24 h. Brains were cut on a vibratome to obtain 70-μm-thick sections and stained with DAPI (1:5,000 μl in PBS) for 15 min. Fluorescent images were acquired by epifluorescence microscopy (Axio imager Zeiss, Germany). Scale bars, 1 mm.

Extended Data Table 1 | Sleep parameters

a

Sleep parameter	NOR			OPR	
	2 hours	1 week	3 weeks	2 hours	1 week
Duration (min)	56.87 ± 6.36	53.12 ± 8.92	41.85 ± 7.10	46.94 ± 5.63	40.05 ± 5.01
Latency (min)	31.46 ± 5.77	29.78 ± 8.06	43.24 ± 6.35	44.57 ± 6.73	43.24 ± 4.06

b

Sleep parameter	Latency (min)	Duration (min)		
	SWS	SWS	PreREM	REM
Vehicle	20.80 ± 5.71	47.13 ± 5.44	5.80 ± 0.71	6.04 ± 1.07
Muscimol	17.57 ± 6.16	53.63 ± 11.15	1.97 ± 0.51**	0.83 ± 0.83**

c

SWS parameter	SO density (number/min)	SO amplitude (mV)	Spindle density (number/min)	Spindle power (mV^2/s)	Spindle mean duration (s)
Vehicle	32.61 ± 4.06	0.189 ± 0.021	2.81 ± 0.15	0.026 ± 0.002	0.591 ± 0.011
Muscimol	31.62 ± 3.47	0.187 ± 0.015	2.86 ± 0.10	0.025 ± 0.002	0.598 ± 0.022

a, Sleep duration and latency during the 2-h post-encoding interval for the sleep groups of the main experiments (Fig. 1). In these experiments retrieval was tested either immediately after the 2-h retention interval (test of recent memory) or 1 week or (for the NOR task only) 3 weeks later (tests of remote memory). There were no significant differences between NOR and OPR task conditions or retention intervals. $n = 12, 8$, and 11 rats for NOR testing after 2 h, 1 week and 3 weeks, and $n = 11$ and 9 rats for OPR testing after 2 h and 1 week, respectively. **b**, Post-encoding sleep in the experiments after bilateral intrahippocampal infusion of muscimol (Fig. 2a). Sleep latency, time in SWS, preREM sleep, and REM sleep are indicated ($n = 8$ rats for each condition). **c**, For the same experiments, density and amplitude of slow oscillations (SO) and density, power, and mean duration of spindles identified during SWS are indicated for the vehicle and muscimol conditions. Substances were infused during the 2-h post-encoding interval (upon the first occurrence of SWS). **PreREM $P = 0.002$, REM $P = 0.003$, for pairwise t-tests (two-sided) with vehicle condition. Data shown as mean ± s.e.m.

Extended Data Table 2 | Correlations between NOR after 3 weeks and sleep parameters

	SWS	Spindles				Slow Oscillations			
	Duration	Number	Duration	Density	Power	Number	Duration	Density	Power
r	0.536	0.719	0.705	0.654	-0.192	0.259	-0.215	-0.132	-0.319
p	0.137	0.029*	0.034*	0.056	0.620	0.501	0.578	0.735	0.402

	REM Sleep	
	Duration	Theta power
r	0.251	0.081
p	0.514	0.836

Summary of correlations between NOR performance at the 3-week retrieval (1 min discrimination ratio) and sleep parameters during the 2-h post-encoding interval ($n = 9$ rats). Pearson's correlation coefficients and *P* values are indicated. *$P < 0.05$ level (uncorrected).

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☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
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☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

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Software and code

Policy information about availability of computer code

Data collection	ANY-Maze software (Stoelting Europe, Dublin, Ireland) was used for collecting behavioral data. Electrophysiological data were acquired using Cheetah data acquisition software version 5 (Neuralynx, USA).
Data analysis	ANY-Maze software (Stoelting Europe, Dublin, Ireland) was used for tracking and analyzing animal behavior, i.e., for scoring exploratory behavior, tracking animal movements, measuring speed and distance travelled in the maze, and confirming behavioral sleep. Electrophysiological analyses were performed using custom-made scripts created in MATLAB 2015b. All software and data are available from the corresponding author upon request. SPSS 21.0 (IBM, Armonk,USA) was used for all statistical analyses.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	We did not carry out formal power analyses. However, in all cases, we aimed to keep sample sizes similar to or larger than those used in previous rodent studies on the effects of sleep on memory consolidation, from our own (e.g., Oyanedel et al. 2014) or other labs (e.g., Oliveira et al. 2010).
Data exclusions	Regarding retrieval performance, we limited our analysis of discrimination ratios to the first and to the first 3 min of the Retrieval phase which lasted 5 min. The restriction to the overall first 3 min was done to take into account the typical dynamics of memory driven exploratory behavior on the NOR and OPR tasks (Dix and Aggleton, Behav Brain Res, 1999). The criterion is consistent with the procedures of most previous studies in the field. For the analysis of EEG recordings in rats with post-encoding intrahippocampal infusions, data from 3 post-encoding intervals were excluded because of technical problems causing recording artefacts. The artifacts prevented the classification of sleep stages according to standard criteria (Neckelmann et al., Sleep, 1994).
Replication	The central finding of the main experiments of a sleep-induced enhancement of remote NOR memory at the 3-week Retrieval test was successfully replicated in a different sample of rats undergoing intrahippocampal infusion of saline during post-encoding sleep. We also successfully replicated previous findings from our and other labs as to the effects of post-encoding sleep on recent NOR and OPR memory (Binder et al., 2012, Inostroza et al., 2013, Oyanedel et al., 2014).
Randomization	In all experiments, rats were randomly assigned to experimental groups and conditions before the experiment. In the experiments testing the effects of post-encoding sleep vs. wakefulness on the retention of NOR and OPR memories, with the retrieval test taking place either 2 hours, 1 week or 3 weeks after encoding, different groups of rats were used to test NOR and OPR memories. Different groups were also used for testing retrieval at the different time points after encoding. The comparison between the effects of post-encoding sleep vs. wakefulness was done in a within-subject design, i.e., each rat was tested on both Sleep and Wake conditions. For these within-subject comparisons the order of experimental conditions was counterbalanced across animals. Additionally, control ANOVA including an additional Order factor (Sleep-Wake vs. Wake-Sleep) were run which did not reveal any significant main or interaction effects for this factor (all $p > 0.172$), thus excluding any substantial effects of the order in which Sleep and Wake conditions were performed. Effects of muscimol vs. vehicle were compared using a between-groups design to assess NOR memory at the 3-week retrieval test, and using a within-subject design to assess the immediate effects of muscimol on hippocampal local field potential recordings.
Blinding	The experimenter was not blind to the experimental group or condition during data collection. However, all behavioral and electrophysiological recordings were analyzed offline, with the experimenter blind to the specific experimental groups and conditions.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Subjects were adult male Long Evans rats (Janvier, Le Genest-Saint-Isle, France, 260–310 g, 10-12 weeks). Rats were housed in groups of 2-4 rats per cage on a 12-h light/12-h dark cycle (lights on at 6:00 h), except during the post-surgery recovery period when they were kept individually. Animals had unrestricted access to water and food throughout the experiments. All experimental procedures were performed in accordance with the European animal protection laws and policies (Directive 86/609, 1986, European Community) and were approved by the Baden-Württemberg state authority.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.