nature neuroscience

Selective suppression of hippocampal ripples impairs spatial memory

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Sharp wave–ripple (SPW-R) complexes in the hippocampus-entorhinal cortex are believed to be important for transferring labile memories from the hippocampus to the neocortex for long-term storage. We found that selective elimination of SPW-Rs during post-training consolidation periods resulted in performance impairment in rats trained on a hippocampus-dependent spatial memory task. Our results provide evidence for a prominent role of hippocampal SPW-Rs in memory consolidation.

Memory consolidation refers to the stabilization of labile memory traces, possibly including intrahippocampal synaptic reinforcement and the transfer of information initially encoded in the hippocampal system to the neocortex for long-term storage[1–3]. The consolidation process has been proposed to occur during post-learning rest or sleep[3,4] by reactivation of memory traces in short bouts of neuronal activity associated with SPW-R events[3,5–7], which can be temporally biased by neocortical slow oscillations[8–10]. Although numerous studies provide compelling correlative links between hippocampal SPW-Rs and memory consolidation[3,5–7], a causal relationship has not yet been demonstrated. To examine the consequences of SPW-R elimination on performance in a hippocampus-dependent, spatial-reference memory task[11] (**Supplementary Methods** and **Supplementary Fig. 1**), we selectively suppressed SPW-Rs during post-learning sleep. All of our experiments were conducted in accordance with institutional (CNRS Comité Opérationnel pour l'Ethique dans les Sciences de la Vie) and international (US National Institutes of Health guidelines) standards and legal regulations (certificat no. 7186, Ministère de l'Agriculture et de la Pêche).

The onset of SPW-Rs was detected online by filtering the signal in the ripple-band and thresholding it. Threshold crossing triggered single-pulse stimulation of the ventral hippocampal commissure[12] ($n = 17$ rats). This

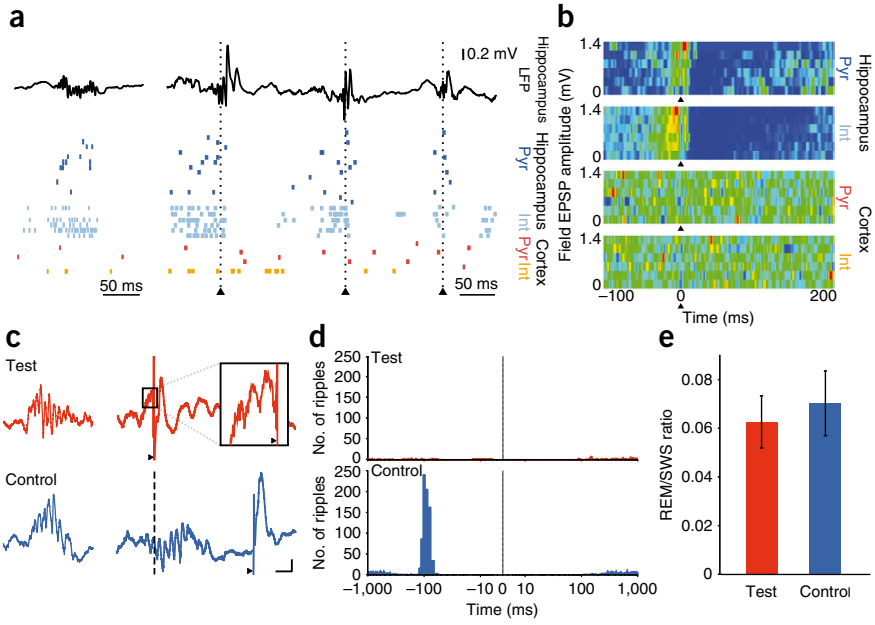

Figure 1 Ventral hippocampal commissural stimulation interrupts SPW-Rs and hippocampal cell discharges without changing global sleep architecture. (**a**) Interruption of SPW-R and spiking activity in the hippocampus. Local field potential (LFP, black) in the hippocampus and spiking activity (vertical ticks) of pyramidal cells (pyr; hippocampus, dark blue; sensorimotor cortex, red) and interneurons (int; hippocampus, light blue; sensorimotor cortex, orange). Left, an intact ripple and the associated spiking activity. Vertical dashed lines and arrowheads represent stimulation times. (**b**) Duration of unit activity suppression as a function of the magnitude of the evoked field response. Pseudo-color plots show the *z* scores of multiple unit activity with increasing levels of stimulation (ordinate). Note the transient, evoked response magnitude–dependent suppression of spiking activity in the hippocampus with no observable effect on global neocortical activity. The increased activity before the stimulus is a result of the buildup of ripple-associated discharge. (**c**) SPW-R blocking by ventral hippocampal commissural stimulation (arrowheads). Example SPW-R in a test rat and a control rat (left). SPW-R was blocked after a few cycles in the test rat (upper right). For illustration purposes, the SPW-R–detection threshold was set higher for this example than in sleep sessions (inset). In the control rat (lower right), stimulation was triggered after a delay. Scale bars represent 20 ms and 0.2 mV. (**d**) Cross-correlograms of stimulations and offline-detected SPW-Rs in test and control rats. Virtually all SPW-Rs were suppressed in test rats, but were preserved in control rats, as a result of the 80–120-ms delay introduced between the ripples (blue peak) and the stimulations (time zero). (**e**) Average random eye movement (REM) sleep/slow-wave sleep (SWS) ratios in a random subset of test and control sessions ($n = 24$ and $n = 27$, respectively; *t* test, not significant ($P > 0.05$), error bars represent s.e.m.).

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Received 20 May; accepted 9 July; published online 13 September 2009; doi:10.1038/nn.2384

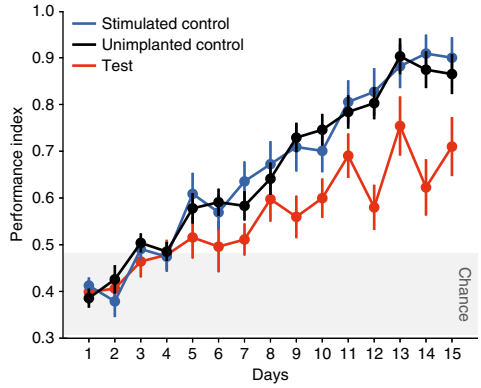

Figure 2 Suppression of SPW-Rs interferes with memory consolidation. Test rats (red: $n = 7$) were significantly impaired in the radial maze task compared with control rats (blue, $n = 7$ stimulated controls; black, $n = 12$ unimplanted controls; error bars represent s.e.m.). Grey shading indicates the chance zone. Although performance increased in the three groups, rats with ripple suppression took more days to perform above upper chance level (*t* tests) and their performance remained consistently below that of the control groups. The average s.d. of the performance index were 0.18 (unimplanted controls), 0.18 (stimulated controls) and 0.19 (tests).

blocked further development of the oscillation and transiently silenced hippocampal spiking activity[12] (**Fig. 1a**,**b** and **Supplementary Fig. 2**), thus preventing potential replay of place-cell[13] sequences[5,6] previously activated during waking. In contrast with hippocampal cells, firing of neocortical neurons was not interrupted at the stimulus intensities that we used for abolishing ripples (**Fig. 1a**,**b** and **Supplementary Fig. 3** shows data from anterior cingulated and prelimbic/infralimbic prefrontal cortices, two major candidates of hippocampal-neocortical information transfer during spatial memory consolidation[2]).

Next, we tested the role of SPW-Rs on memory consolidation. Three groups of rats (test group, $n = 7$; stimulated controls, $n = 7$; unimplanted controls, $n = 12$) were trained to find food rewards on an eight-arm radial maze in which the same three arms were baited every day (**Supplementary Fig. 1**). The rats performed three trials per day, after which they were allowed to sleep for 1 h.

During post-training rest and sleep, all of the online-detected ripples were suppressed by commissural stimulations in test rats (average online detection rate was 86.0 ± 1.3% (s.e.m.) of *post hoc* detected SPW-Rs; **Fig. 1c**,**d** and **Supplementary Methods**). Stimulated control rats underwent the same protocol, except that a random delay (80–120 ms) was introduced between SPW-R detection and stimulation, ensuring that the stimulations occurred mainly outside of the ripple episodes (**Fig. 1c**,**d**). Thus, these control rats received the same number of stimulations as test rats (*t* test, not significant, $P > 0.05$), but their hippocampal ripples were left largely intact. The global architecture of sleep and the local field potential power in distinct sleep stages were not modified by the suppression of SPW-Rs (**Fig. 1e** and **Supplementary Fig. 4**). Because reactivations of previously active cell assemblies occur preferentially during the first half-hour of sleep after exploration[5], we blocked SPW-Rs for 1 h following training sessions. As stimulation outside SPW-Rs had no detectable effect on task performance (no significant difference between stimulated control and unimplanted rats, two-factor ANOVA, day × group, $P > 0.05$; **Fig. 2**), the two control groups were pooled and compared with test rats. Performance of the test rats was significantly impaired compared with control rats (two-factor ANOVA day × group, $P < 0.001$ for main factors, $P < 0.01$ for interaction; **Fig. 2**). In control rats (stimulated and unimplanted groups combined), performance exceeded the upper chance level after 5 d of training, whereas test rats continued to perform at chance level until the eighth day of training (*t* tests, $P < 0.05$). Test rats did not develop stereotyped turning strategies (**Supplementary Fig. 5**) and working memory errors remained very low (less than one error per trial on average) in the three groups (**Supplementary Fig. 6**).

Suppression of SPW-Rs and associated neuronal discharges resulted in deterioration of memory consolidation. The behavioral effect was specifically related to suppression of SPW-Rs, rather than to non-specific consequences of the stimulation, as SPW-R–yoked control stimulation had no detectable effect on behavior. The observed deficit is all the more notable, as we suppressed the SPW-Rs for only 1 h and ripple incidence returned to normal levels after the stimulation period (**Supplementary Fig. 4**). The magnitude of impairment in the ripple-suppressed rats was comparable to that reported in a previous study on hippocampus-lesioned rats[11]. The slight performance improvement in the test group could be the result of the spared small-amplitude SPW-Rs, of the SPW-Rs occurring after the stimulation period or of other, nonhippocampal learning mechanisms, as has been reported previously[11]. Our findings therefore indicate that SPW-Rs are critical for memory consolidation, possibly because, by temporally compressing reactivations of waking firing sequences[3,5,6] in the hippocampus, they allow spikes to occur in a time window that is compatible with activation of the NMDA receptors and spike timing–dependent plasticity. In addition or alternatively, they would enable the reactivated ensembles to exert a strong effect on downstream target neurons[7]. Moreover, hippocampal SPW-Rs are coordinated in time with neocortical unit firing, slow oscillations and sleep spindles[8–10,14,15], suggesting that they have a widespread effect on cortical function underlying long-term memory consolidation.

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

ACKNOWLEDGMENTS

We thank S. Sara, A. Larrieu, L. Hazan, A. Fruchart, P. Ruther, S. Kisban, S. Herwik, S. Doutremer, M.-A. Thomas, Y. Dupraz, M. Ehrette and S. Rateau for advice and technical support. This work was supported by the International Human Frontiers Science Program Organization (CDA0061/2007-C), the US National Institutes of Health (NS34994), European Projects NeuroProbes (IST-027017) and Integrating Cognition, Emotion and Autonomy (FP6-IST 027819), and a Collège de France Visiting Professor Fellowship.

AUTHOR CONTRIBUTIONS

G.B. and M.B.Z. designed the study. G.G. carried out the majority of the experiments, K.B. and M.B.Z. carried out the remaining experiments. M.B.Z., G.G. and K.B. analyzed the data. All of the authors contributed to writing the manuscript.

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