Odor Cues During Slow-Wave Sleep Prompt Declarative Memory Consolidation

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to newly synthesized cell-wall components, form Ca^{2+} oxalate, or move into internal stores (*13*, *14*). Most sequestrated Ca^{2+} ions become immobile, and thus continuous Ca^{2+} supplies are needed and likely to be the regulated step. Third, the stomatal-conductance oscillations are regulated by photoperiod and the clock (*15*). Finally, soil Ca^{2+} is the primary source controlling the amplitudes of $[Ca^{2+}]_i$ oscillations. Our findings may also revise further the concept of resting $[Ca^{2+}]_i$ in plants. The constant remodeling includes oscillations at the basal concentration of ~0.1 μM (*6*), similar to that in specific neurons (*4*, *7*), and shifts of this level according to soil Ca^{2+} status and CAS activity. Clearly, transpiration-mediated soil Ca^{2+} uptake and transport synchronize the resting $[Ca^{2+}]_i$ throughout the plant. Because the transpiration rate is regulated by numerous factors (*26*) and because soil Ca^{2+} levels can fluctuate throughout the year in nature (*27*), this soil Ca^{2+}-CAS-IP_3 pathway may be physiologically relevant (*17*).

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Supporting Online Material

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Odor Cues During Slow-Wave Sleep Prompt Declarative Memory Consolidation

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Sleep facilitates memory consolidation. A widely held model assumes that this is because newly encoded memories undergo covert reactivation during sleep. We cued new memories in humans during sleep by presenting an odor that had been presented as context during prior learning, and so showed that reactivation indeed causes memory consolidation during sleep. Re-exposure to the odor during slow-wave sleep (SWS) improved the retention of hippocampus-dependent declarative memories but not of hippocampus-independent procedural memories. Odor re-exposure was ineffective during rapid eye movement sleep or wakefulness or when the odor had been omitted during prior learning. Concurring with these findings, functional magnetic resonance imaging revealed significant hippocampal activation in response to odor re-exposure during SWS.

Sleep facilitates the consolidation of newly acquired memories for long-term storage (*1*–*3*). The prevailing model assumes that this consolidation relies on a covert reactivation of the novel neuronal memory representations during sleep after learning (*3*–*6*). In rats, hippocampal neuronal assemblies implicated in the encoding of spatial information during maze learning are reactivated in the same temporal order during slow-wave sleep (SWS) as during previous learning (*7*, *8*). The consolidation of hippocampus-dependent memories benefits particularly from SWS (*9*–*11*), and reactivation of the hippocampus in SWS after spatial learning has also been seen in humans observed with positron emission tomography (*12*). However, none of these studies experimentally manipulated memory reactivation during sleep. Therefore, its causal role in memory consolidation is still unproven.

We used an odor to reactivate memories in humans during sleep, because odors are well known for their high potency as contextual retrieval cues not only for autobiographic memories, as delicately described in Marcel Proust's *Remembrance of Things Past*, but also for various other types of memory, including visuospatial memories (*13*, *14*). Notably, in the brain, primary olfactory processing areas bypassing the thalamus project directly to higher-order regions, including the hippocampus (*15*), which enables them to modulate hippocampus-dependent declarative memories (*16*). The use of olfactory stimuli for cueing memories during sleep is particularly advantageous because odors, in contrast to other stimuli, can be presented without disturbing ongoing sleep (*17*).

To establish a robust association between learning stimuli and a smell, we applied a purely olfactory stimulus (the smell of a rose) (*18*) repetitively while volunteers ($n = 18$) learned object locations in a two-dimensional (2D) object-location memory task in the evening before sleep. During the first two periods of subsequent SWS, the odor was presented again (in an alternating 30 s on/30 s off mode). In a control condition, odorless vehicle was delivered. The object-location task required visually learning the locations of 15 card pairs on a computer screen to a criterion of 60% correct responses (Fig. 1A). The task is sensitive to the memory-improving effect of sleep (*18*) and involves hippocampal function (*19*).

At retrieval testing after sleep, memory of the card locations was distinctly enhanced when the odor had been presented during SWS as compared to presentation of the vehicle alone. After the odor night, participants remembered 97.2 ± 4.1% of the card pairs they had learned before sleep, but they remembered only 85.8 ± 3.8% after the vehicle night ($P = 0.001$; Fig. 2A,

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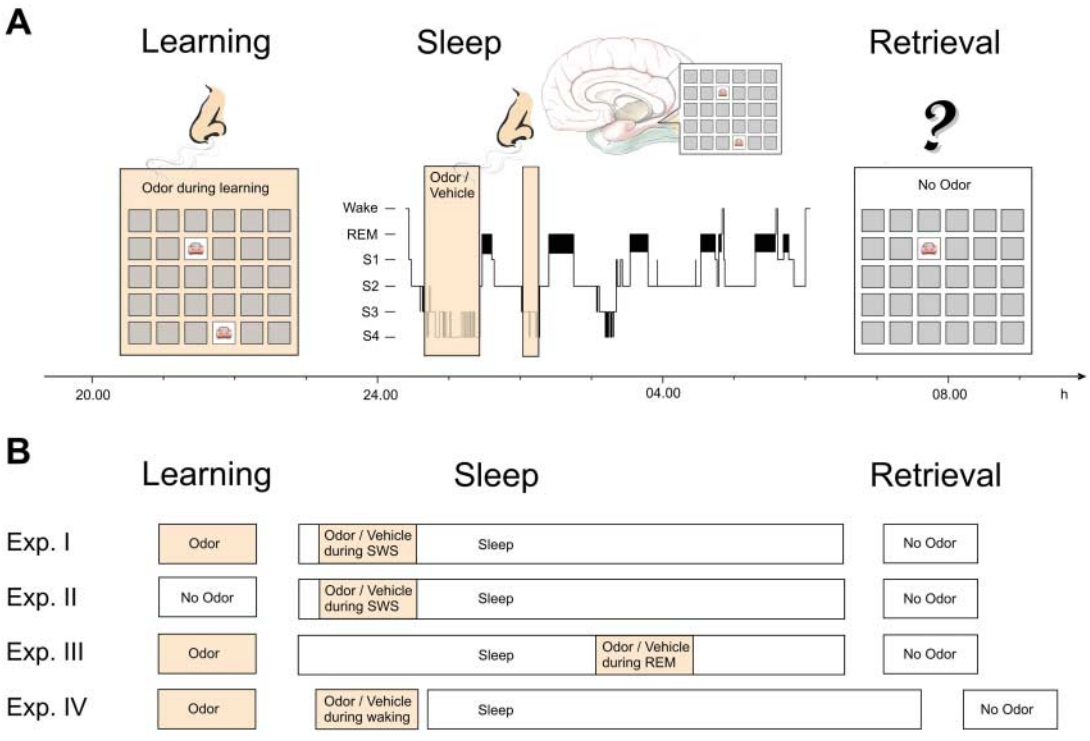

Fig. 1. (**A**) Volunteers learned a visuospatial 2D object-location task (and a procedural finger-tapping task, not illustrated) between 21.30 and 22.30 hours (Learning). The odor was administered time-locked to the learning stimuli. During subsequent sleep (lights off at 23.00 hours, awakening at 6.30 hours), the same odor (versus vehicle) was delivered during the first two periods of SWS in an alternating 30 s on/30 s off mode (to prevent habituation). Stimulation started with the first occurrence of SWS and was interrupted whenever the sleep stage changed. Retrieval was tested between 7.00 and 7.30 hours in the absence of odor (*18*). (**B**) Experiments I and II were identical except that in experiment I, the odor was presented at learning and again contingent upon SWS, whereas in experiment II, the odor was not presented at learning. In experiment III, the odor was presented at learning and again during post-learning REM sleep. In experiment IV, the odor was presented at learning, but post-learning re-exposure took place while participants were awake. Experiments were conducted according to a double-blind crossover design.

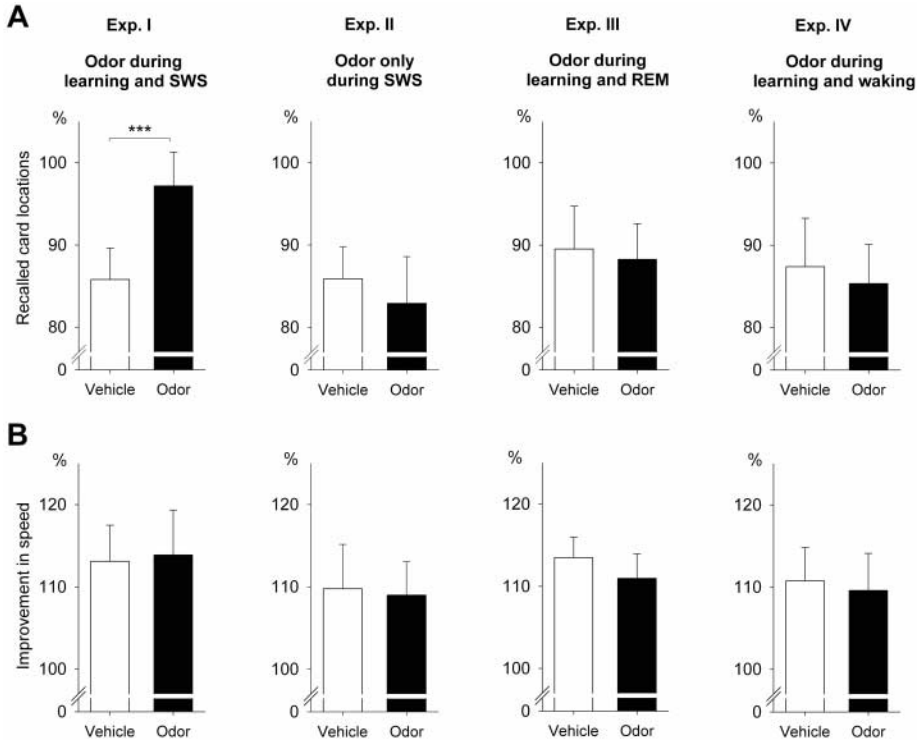

Fig. 2. Retention performance on the visuospatial 2D object-location task (upper panels) and the procedural finger sequence tapping task (lower panels) is shown for four different experiments, each comparing the effects of stimulation with odor and vehicle. (**A**) Only when memory was cued by the context odor during SWS (experiment I) was declarative memory of card locations enhanced. The interaction contrast between experiment I and all other experiments was significant ($P = 0.01$). (**B**) Overnight gains in procedural finger-tapping speed were not affected by odor cueing. Memory performance on both tasks is calculated as percentage of retrieval performance, with performance at learning before sleep set to 100%. Means ± SEM are shown. ***$P = 0.001$.

experiment I). No difference in initial learning performance between conditions was observed ($P > 0.4$; table S1). There was no awareness of the nocturnal odor treatment in the morning after sleep ($P > 0.6$). Odor cueing affected neither sleep architecture (table S2) nor electroencephalogram spectral power in comparisons of odor-on and -off periods ($P > 0.1$) (*18*).

We also tested volunteers on a procedural memory task (finger sequence tapping) that does not require hippocampal function but is likewise sensitive to the enhancing effect of sleep (*20*). During learning, participants repeatedly tapped a five-element finger sequence on a keyboard as fast and accurately as possible for 12 30-s periods while the odor was applied. At retrieval testing after sleep, tapping speed was improved after the presentation of both odor and vehicle during SWS ($P < 0.02$) (*2*, *20*). However, in contrast to performance on the object-location task, sleep-associated gains in tapping skill were not changed by re-exposing the participants to the odor during sleep after training ($P > 0.7$; Fig. 2B and table S3).

Our results support the hypothesis that once an odor has become associated as the context of learned object locations, reapplication of the odor during subsequent SWS acts as a context cue that reactivates the new memories and thereby boosts their consolidation. However, it can be argued that odor presentation during SWS exerted a non-specific effect on the ongoing consolidation of memories that was independent of any previously formed association between odor and learning

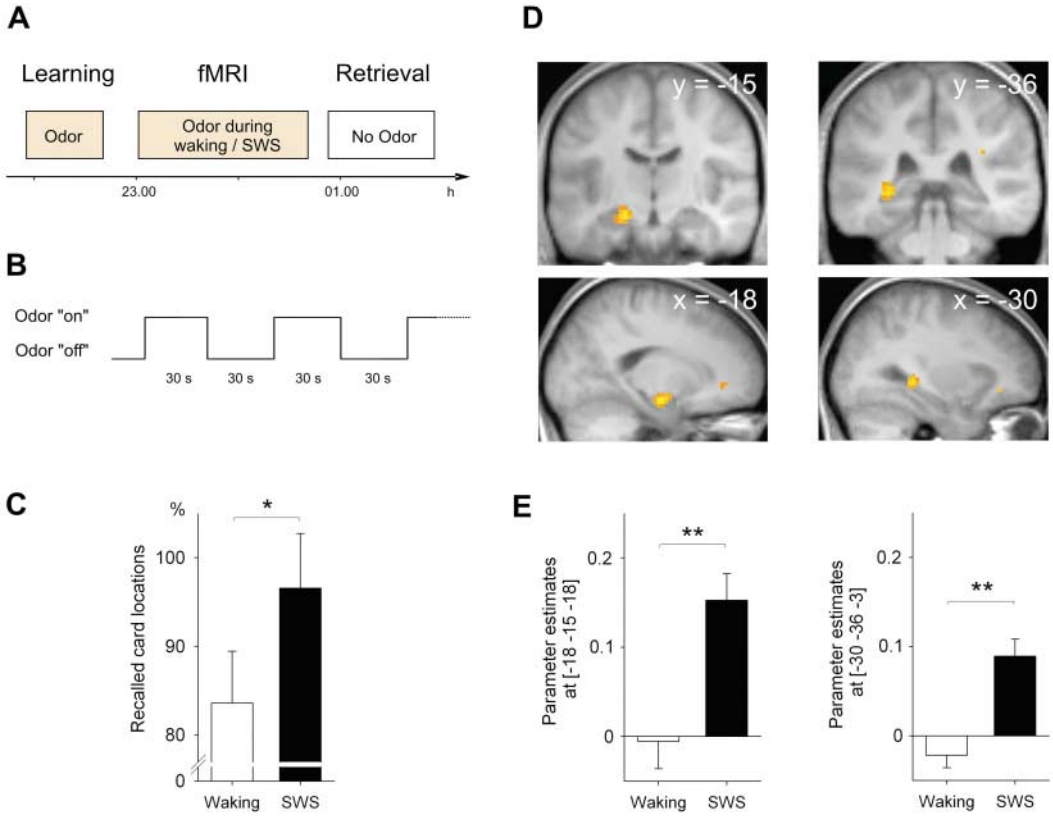

Fig. 3. (**A**) Procedure. The odor was presented during learning and again during post-learning SWS, or at a corresponding time after learning (~45 min) while participants were awake. (**B**) fMRI scans were obtained every 5.61 s during re-exposure to the odor presented, as in the behavioral studies, in an alternating 30 s on/30 s off pattern. (**C**) Retrieval tested after fMRI scanning revealed better retention on the 2D object-location task when participants had slept (for 55.0 ± 4.9 min) than after the corresponding wake interval (*$P < 0.05$, one-tailed test). Note that the shorter retention interval (2 hours) renders the comparison of performance with experiments I to IV difficult. (**D**) Brain activation in response to odor presentation during SWS (threshold set at $P < 0.005$ uncorrected; superimposed on the average structural MRI of all volunteers). BOLD responses to odor-on periods indicate activation in the left anterior hippocampus (left panels) and in the left posterior hippocampus (right panels). (**E**) Parameter estimates (regression coefficients with arbitrary units) for waking and SWS conditions, at the coordinates of local maxima observed during SWS in the left anterior (left panel) and left posterior (right panel) hippocampus. Means ± SEM are indicated. **$P < 0.01$.

Table 1. Brain regions showing significant activity during odor-on periods. Based on a priori hypotheses, significances are corrected for small volumes of interest (SVCs) covering the left (L.) and right hippocampus. Results of an exploratory whole brain analysis with a threshold set at $P < 0.001$ (minimal voxel size $k = 3$) are also indicated where applicable. Post-hoc comparisons between SWS and waking were restricted to the left hippocampus. No significant activations were observed for waking > SWS × on > off. MNI, Montreal Neurological Institute.

	MNI coordinates (mm)				
	x	*y*	*z*	*Z* score	*P*
SWS; on > off					
L. anterior hippocampus	−18	−15	−18	3.59	$<0.05_{SVC}$
L. posterior hippocampus	−30	−36	3	3.39	$<0.05_{SVC}$
L. inferior frontal gyrus	−24	33	−9	3.24	<0.001
Waking; on > off					
L. anterior hippocampus	−33	−12	−18	3.39	$<0.1_{SVC}$
SWS > waking × on > off					
L. posterior hippocampus	−33	−38	−3	3.32	$<0.05_{SVC}$
L. anterior hippocampus	−18	−9	−15	3.03	$<0.05_{SVC}$

stimuli. This possibility was examined in a control experiment (experiment II, $n = 17$ participants) using a design and procedures identical to those of the main experiment, with the only exception being that no odor was presented during learning before sleep. In contrast to odor that had been linked to the learning stimuli (experiment I), odor presentation during SWS alone proved ineffective in enhancing memory. The percentage of recalled locations was 82.9 ± 5.7% in the odor condition and 85.9 ± 3.9% after vehicle presentation ($P > 0.6$; Fig. 2A and table S1). Restricting odor presentation to post-learning sleep also did not affect finger-tapping skill ($P > 0.4$; Fig. 2B and table S3).

Is the effect of odor cueing on object-location memory specific to SWS? In a third experiment (experiment III, $n = 17$ participants), we tested the effect of odor-stimulated memory reactivation during rapid eye movement (REM) sleep, which predominates during late nocturnal sleep (*9*). Experimental procedures (including the duration of odor stimulation; table S4) were again the same as in the main experiment, except that the timing of odor re-exposure was shifted to the first two periods of REM sleep occurring after 3 hours of sleep. Despite prior coupling of the odor to the learned stimuli, odor re-exposure during post-learning REM sleep failed to affect the memory of card locations (88.3 ± 4.4% versus 89.5 ± 5.2% after vehicle presentation; $P > 0.8$; Fig. 2A and table S1). Overnight gains in finger tapping skill did not differ between conditions either ($P > 0.4$; Fig. 2B and table S3). The lack of odor-related memory effects during REM sleep is unlikely to be ascribed to the longer time between acquisition and re-exposure, because experimental odors remain effective retrieval cues for days (*13*, *14*). Also, it cannot result from reduced olfactory processing, because sensitivity to olfactory stimuli is enhanced during REM sleep as compared with SWS (*17*). Whether the memory-enhancing effect of odor cueing extends to lighter forms of non-REM sleep (stage 2 sleep) remains to be tested.

The failure of REM sleep–contingent odor stimulation to enhance procedural memory for finger-tapping skill might be unexpected, because previous findings indicated a reactivation of skill memories during post-learning REM sleep (*21*, *22*) as well as REM sleep–related benefits for this type of memory (*2*, *9*, *23*). However, there is little evidence that procedural skills can be effectively conditioned to context cues such as odors (*14*), and olfactory processing areas may not have the same immediate access to the structures subserving skills (the motor cortex, striatum,

cerebellum, etc.) as they have to hippocampal structures (*24*). Therefore, the sleep-associated administration of odor stimuli seems to be an approach suitable only for reactivating hippocampus-dependent memories.

We also tested whether odor re-exposure depends on application during sleep or whether the same memory consolidation effect could be also observed during wakefulness (experiment IV, $n = 18$ participants), as suggested by previous studies (*25*, *26*). Re-exposure to odor (versus vehicle) took place during a 1-hour interval starting 45 min after learning, while participants performed a vigilance task (Fig. 1B). Thereafter, participants slept normally and retrieval was tested the next morning. Unlike SWS-contingent odor presentation, odor re-exposure during wakefulness did not affect the retention of visuospatial memories (85.3 ± 4.7% versus 87.4 ± 5.9% recalled locations after vehicle presentation; $P > 0.7$; Fig. 2A and table S1) or tapping skill ($P > 0.5$; Fig. 2B and table S3).

Central to our hypothesis is the notion that the odor-induced reactivations boosting the consolidation of hippocampus-dependent declarative memories are indeed related to hippocampal activity during SWS (*5*, *6*). Consequently, we used functional magnetic resonance imaging (fMRI) to examine whether odor cues that were previously associated with learning stimuli are capable of activating the hippocampus during post-learning SWS (experiment V, $n = 12$ participants). Odor stimulation was applied, as in the main experiment, during learning and again during subsequent SWS or during wakefulness (Fig. 3). In the sleep condition, all of these participants reached SWS averaging 15.5 ± 4.1 min (*18*). As hypothesized, re-exposure to the odor cue during SWS activated the hippocampus (Table 1). Significant blood oxygenation level–dependent (BOLD) responses to odor-on periods were revealed in the left anterior and posterior hippocampus [P small volume–corrected (P_{SVC}) < 0.05; Fig. 3D]. A trend for activation in the left anterior hippocampus was also observed in response to odor stimulation presented during wakefulness ($P_{SVC} < 0.08$). Direct comparisons between the waking and sleep conditions revealed an even stronger activation in response to odor presentation during SWS than during wakefulness in both the anterior and posterior part of the left hippocampus ($P_{SVC} < 0.05$; Fig. 3E). The data fit well with previous findings on brain imaging during wakefulness, indicating no or only short-lived hippocampal activation in response to passive smelling of experimental odors (*15*, *27*), whereas substantial activation in the left anterior and posterior hippocampus was reported by Herz *et al*. (*28*) in response to odors (personal perfume) that were strongly associated with autobiographic episodes. However, it is noteworthy that odor cues activate the hippocampus during SWS to a much greater extent than during wakefulness. Beyond showing that memory-associated odors have access to the hippocampus during SWS, this observation points to a particular sensitivity of hippocampal networks in this sleep stage to stimuli that are capable of reactivation.

Currently, two diverging concepts are discussed regarding how sleep might enhance the consolidation of hippocampus-dependent memories. One assumes that enhanced memory is an indirect consequence of a global downscaling of synaptic connectivity that is induced by slow oscillatory activity during SWS, which basically increases the signal-to-noise ratio for newly encoded information (*29*, *30*). The other concept, examined here, assumes that memory consolidation evolves from repeated covert reactivation of newly encoded hippocampal representations during SWS, which takes place in a synchronized dialogue between thalamocortical and hippocampal circuitry and which eventually leads to the transfer of the representations to neocortical regions for long-term storage (*3*, *5*). Although our results do not exclude processes of synaptic downscaling during SWS, they support the latter concept, indicating that covert reactivations are a causative factor for the consolidation of hippocampal memories during sleep.

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Supporting Online Material

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