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# Labile or stable: opposing consequences for memory when reactivated during waking and sleep

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**Memory consolidation is a dynamic process. Reconsolidation theory assumes that reactivation during wakefulness transiently destabilizes memories, requiring them to reconsolidate in order to persist. Memory reactivation also occurs during slow-wave sleep (SWS) and is assumed to underlie the consolidating effect of sleep. Here, we tested whether the same principle of transient destabilization applies to memory reactivation during SWS. We reactivated memories in humans by presenting associated odor cues either during SWS or wakefulness. Reactivation was followed by an interference task to probe memory stability. As we expected, reactivation during waking destabilized memories. In contrast, reactivation during SWS immediately stabilized memories, thereby directly increasing their resistance to interference. Functional magnetic resonance imaging revealed that reactivation during SWS mainly activated hippocampal and posterior cortical regions, whereas reactivation during wakefulness primarily activated prefrontal cortical areas. Our results show that reactivation of memory serves distinct functions depending on the brain state of wakefulness or sleep.**



The concept of reconsolidation assumes that newly acquired memories are not consolidated once and forever[1]. According to this hypothesis, memories can exist in an active state where they are labile and susceptible to disturbing influences, and in an inactive or stable state during which they are resistant to amnestic treatments[2,3]. It has been proposed that stored memories can re-enter active states when they are reactivated during retrieval or by a reminder and need again to stabilize in order to persist. In animal studies, application of amnestic treatments like protein synthesis inhibitors impairs memory retrieval when applied shortly after memories are reactivated[3–5]. On the cellular level, reactivations produce a renewed instability in synaptic connections representing the memory, as well as in molecular markers of memory formation[6–8]. In humans, support for the reconsolidation hypothesis has only recently been provided[9–11]. Such reconsolidation effects are mainly observed when reactivation is followed by learning of similar but new information (that is, retroactive interference) and with indirect memory measures like the incorporation of new information into old material[12], changes in retrieval-induced forgetting[13] or forgetting of older autobiographical memories[14].

Reactivations of memory representations take place at a neuronal level also during sleep. Especially during SWS, firing patterns within hippocampal assemblies of place cells (coding the rat's position in space) express a marked similarity to firing patterns that were present during learning and exploratory behaviors before sleep[15–17]. Using neuroimaging techniques, studies in humans show that brain regions activated during learning are reactivated during subsequent SWS[18]. These neuronal reactivations of memory representations during sleep contribute to the facilitating effect of sleep on memory consolidation, presumably promoting the gradual redistribution of memory traces from hippocampal to neocortical brain regions for long-term storage[19]. Thus, cuing newly encoded memories during sleep by olfactory or auditory stimuli strengthens these memories, demonstrating a causal role of reactivations during SWS for the consolidation of memory[20,21].

In the context of reconsolidation theory, the improving effect of reactivating memories during SWS on post-sleep recall performance could be explained by a transient destabilization and subsequent reconsolidation of reactivated memories occurring sequentially during sleep[19,22]. Previous sleep studies are unclear about whether reactivations during SWS likewise transiently return memories into a labile state as during waking, mainly for two reasons. First, sleep studies commonly test memory performance after a full night of sleep or even later, that is, several hours after reactivation, whereas studies of reconsolidation during wakefulness show that memories are in a labile phase for only a short time window after reactivation and restabilize within minutes or a few hours[23,24]. Second, sleep studies typically assess the strength of a memory by a simple retrieval test, whereas the assessment of memory destabilization requires that stability is directly probed by introducing modulating influences like interference learning or amnestic treatments after reactivation[24,25]. Thus, previous sleep studies have not shown any immediate destabilizing effect of reactivations as has been observed in studies of reconsolidation during wakefulness because these studies either did not test retrieval in the appropriate time window of destabilization or lack a test of memory stability[20,21].

Transient destabilization after reactivation during sleep is a candidate mechanism to facilitate the gradual redistribution of newly encoded memory traces, for example, by gradually loosening synaptic connections in hippocampal regions in favor of direct cortico-cortical

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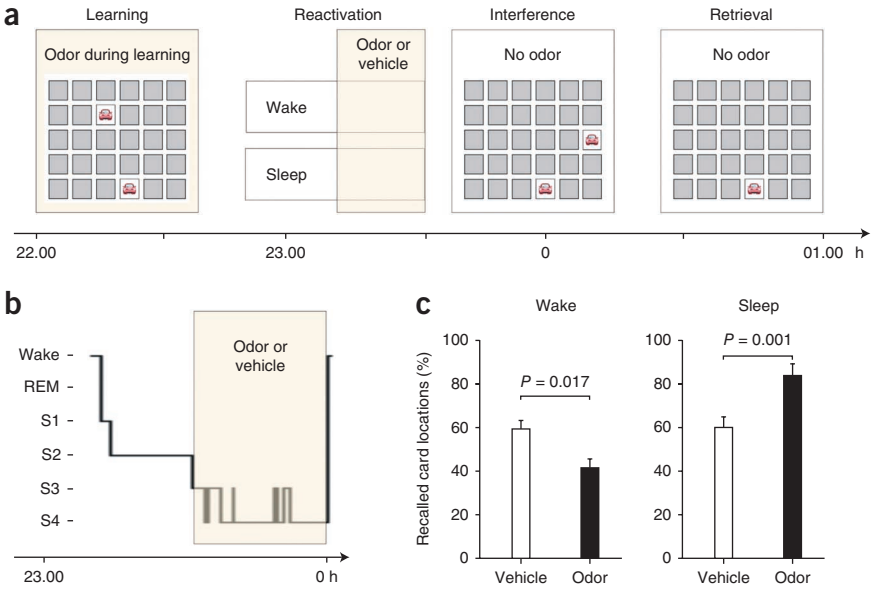


**Figure 1** Experimental procedures and memory performance after odor reactivation. (**a**) Participants learned an object-location task in the evening in the presence of an odor. Half of the subjects stayed awake after learning; the other half slept for 40 min. The odor was again presented for 20 min during wakefulness and (in the sleep group) during SWS, respectively (reactivation). In a second session, odorless vehicle was presented during the retention interval (no reactivation) in balanced order. Shortly after odor (or vehicle) re-exposure (after subjects in the sleep group were awakened), subjects learned an interference object-location task (without odor presentation) using the same card pairs as during learning, but with the second card of each pair presented at a different location (interference). Retrieval of the original object-location task was tested 30 min after interference learning (without odor presentation). (**b**) Typical sleep profile from a sleep subject. Odor (versus vehicle) was presented for 20 min during SWS and the subject was awakened from SWS after odor (versus vehicle) stimulation was completed. (**c**) Recall of card locations from the original object-location task was impaired after odor reactivation in the wake group, indicating an increased susceptibility to interference after reactivation, whereas recall was enhanced by odor reactivation compared to vehicle in the sleep group, indicating that reactivation during sleep makes memories resistant to interference ($P < 0.001$ for 'sleep-wake' × 'odor-vehicle' interaction). Retrieval performance, percentage of recalled locations with performance at learning set to 100%. Values are mean ± s.e.m.



connections supporting long-term storage of the memory in neocortical circuits. In addition, a transiently labilized memory trace might be more easily adapted to, and even changed with respect to, pre-existing knowledge, thereby facilitating its integration in established schemes and semantic networks[22]. After successful integration, subsequent reconsolidation, possibly involving rapid eye movement (REM) sleep, would then lead to strengthened memory traces the next day[19,22]. Because external input is considerably reduced during sleep as compared with the waking state, memories could enter a labile phase with a distinctly reduced risk of encountering interfering information. Modifications to the labile memories would be restricted to internal information originating, for example, from an interleaved reactivation of older associated knowledge and/or possibly related mentation processes occurring during SWS[26].

In this study, we aimed to test directly the potential destabilizing effect of reactivations during SWS. We examined memory performance shortly after reactivation, that is, after awakening from SWS (without intervening REM sleep), and probed memory stability by introducing interference learning after reactivation. We hypothesized that memory reactivations during SWS, similarly to reactivations during wakefulness, lead to an immediate transient destabilization of memory traces[22]. We used an olfactory stimulus previously associated with the memories during learning to cue these memories during post-learning SWS or wakefulness. Shortly after the cue-induced reactivation, participants were presented with an interference learning task to probe stability of the memory.

## RESULTS

### Memory stability after reactivation during waking and SWS

Participants learned in the evening (22:00–23:00) a visuo-spatial two-dimensional object-location task in the presence of the experimental odor (unfamiliar slightly negative smell) to establish a robust association between the odor and the learning material (**Fig. 1a**). The object-location task consisted of 15 card pairs showing pictures of animals and everyday objects. After learning, one group of subjects (wake group, $n = 12$) stayed awake for ~40 min and the odor was presented again for the last 20 min of this interval. The other group of subjects (sleep group, $n = 12$) went to sleep after learning and the odor was re-exposed for 20 min during SWS (**Fig. 1b**). The odor was always presented in an alternating mode of 30-s on, 30-s off periods to prevent habituation. Shortly after the odor presentation, and after subjects of the sleep group were awakened from SWS, subjects started learning an interference object-location task. The interference task consisted of the same card pairs as the original object-location task with the first location of each pair being always the same but the location of the second card being different from the original task. Recall of the original object-location task was tested 30 min after interference learning. Each participant was also tested in a control condition in which, instead of odor, odorless vehicle was presented during waking and SWS, respectively.

As we expected, reactivating the memories using the odor during waking rendered them susceptible to interference. In the wake group, recall of the original object-location task was distinctly reduced by interference learning when the interference was preceded by the re-exposure of odor compared with vehicle: subjects remembered 59.39 ± 4.24% of the learned card locations after vehicle (no reactivation) and only 41.43 ± 4.68% after reactivation by the odor cue ($P = 0.017$; **Fig. 1c**). Initial learning performance (**Table 1**) as well as general alertness (assessed by the Stanford Sleepiness Scale) and reaction times in a vigilance task did not differ between the odor and vehicle condition (all $P > 0.20$; see **Supplementary Table 1** for sleepiness and vigilance data). To exclude the possibility that the reactivation effect was due to sleepiness and fatigue in the wake group during the night, we replicated this effect using the same experimental setup in another group of subjects who learned in the evening between 17:00 and 18:00 ($n = 11$ and 13 in the odor and vehicle condition, respectively). In this experiment, again, recall of the original object-location task was significantly impaired by interference learning after reactivation by

**Table 1 Performance on the object-location task**

| | | No reactivation | Reactivation | *t* | *P* |
|---|---|---|---|---|---|
| Wake group | | | | | |
| Number of trials | Learning | 2.58 ± 0.55 | 2.33 ± 0.46 | 0.30 | >0.70 |
| Recalled card locations | Learning | 10.42 ± 0.41 | 10.33 ± 0.39 | 0.16 | >0.80 |
| | Retrieval | 6.17 ± 0.44 | 4.25 ± 0.41 | 2.97 | 0.013 |
| | Interference | 9.33 ± 0.84 | 8.92 ± 0.85 | 0.33 | >0.70 |
| Sleep group | | | | | |
| Number of trials | Learning | 2.25 ± 0.55 | 2.42 ± 0.46 | –0.69 | >0.50 |
| Recalled card locations | Learning | 10.67 ± 0.41 | 10.58 ± 0.39 | 0.13 | >0.80 |
| | Retrieval | 6.50 ± 0.60 | 9.00 ± 0.75 | –2.92 | 0.014 |
| | Interference | 5.75 ± 0.84 | 5.50 ± 0.85 | 0.30 | >0.70 |

The task included 15 card-pair locations. Learning trials were repeated until participants reached a learning criterion of 60% correct responses. Number of trials to reach the criterion and absolute number of card locations recalled at learning (during the criterion trial) and at retrieval are indicated. Interference refers to number of card locations recalled at learning of the interference task (after one learning trial). Values are mean ± s.e.m. Right-hand columns, *t* and *P* values for pair-wise comparisons.

the odor compared to the no-reactivation condition (58.38 ± 5.06% versus 43.90 ± 4.83%, $P = 0.05$; general alertness and vigilance performance did not differ between the two wake groups, all $P > 0.13$; see **Supplementary Table 2** for detailed results). Thus, during wakefulness memory reactivation by a contextual cue destabilized memory traces, making them susceptible to disrupting influences.

During SWS, on the contrary, and in marked contrast with our hypothesis, memory reactivation did not labilize, but stabilized memories for the original card-pair locations, making them less susceptible to interference learning. Subjects remembered 60.80 ± 4.24% of the learned card locations after vehicle (no reactivation), but 84.18 ± 4.68% after odor reactivation ($P = 0.001$; $P < 0.001$ for the interaction 'sleep-wake' × 'reactivation–no reactivation'; **Fig. 1c**). Sleep parameters during the odor and vehicle nights were comparable between conditions (all $P > 0.20$). Sleep time was on average 46.9 ± 3.4 and 47.9 ± 4.8 min for the odor and vehicle condition, respectively ($P > 0.80$; see **Supplementary Table 3** for sleep data). As in the wake group, initial learning performance (**Table 1**) as well as general alertness and reaction times in the vigilance task did not differ between the odor and vehicle conditions (all $P > 0.50$, **Supplementary Table 1**). These parameters also did not differ between the sleep and wake groups (all $P > 0.15$), excluding the possibility that the differential effect of reactivation during sleep and wakefulness was due to nonspecific differences in arousal.

Subjects in the sleep and wake group differed, however, in the degree of interference learning after reactivation, owing to an overall diminished performance at learning of the interference task in sleep subjects examined shortly after awakening (recalled card locations at interference learning: sleep group, 5.63 ± 0.65; wake group, 9.13 ± 0.65; $P < 0.01$; **Table 1**). To exclude the possibility that the different degrees of interference learning differentially affected memory performance after reactivation in the sleep and wake groups, we conducted a subgroup analysis. We compared ten sleep subjects who performed best at interference learning (including three new subjects who learned the interference task to a criterion of at least 60% correct responses) with ten subjects from the wake group who performed worst at interference learning. Notably, performance in the interference task in this case did not differ between the two subgroups (recalled card locations at interference learning: sleep group, 8.20 ± 0.72; wake group, 8.45 ± 0.72; $P > 0.80$). Analysis of these subgroups showed essentially the same results as those of the original groups, indicating destabilized memories after reactivation during wakefulness (no reactivation, 58.49 ± 3.39%; reactivation, 43.04 ± 4.59%; $P = 0.034$) and stabilized memories after reactivation during SWS (no reactivation, 61.84 ± 6.38%; reactivation, 79.29 ± 7.84%; $P = 0.026$; $P = 0.002$ for the interaction 'sleep-wake' × 'reactivation–no reactivation').

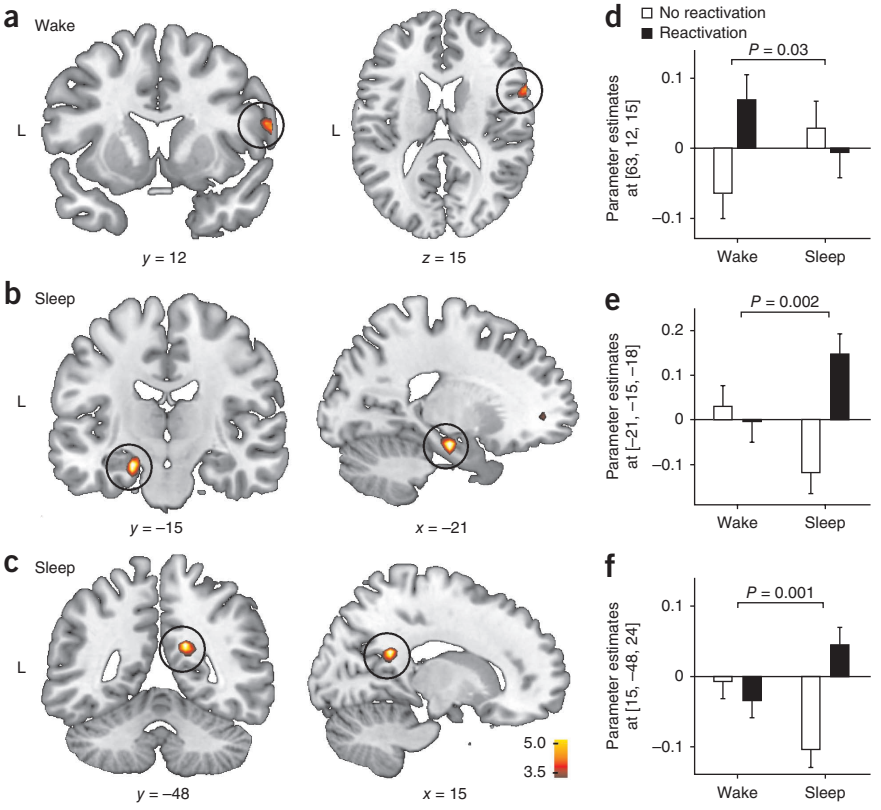


**Figure 2** Brain activity associated with odor-induced memory reactivation during wakefulness and sleep. (**a**) During wakefulness, brain activity during 30-s odor-on periods in the reactivation condition (that is, odor during learning and during the wake retention interval) was increased in the lateral prefrontal cortex (PFC) as compared with the no-reactivation condition (odor only during the retention interval). (**b**) During SWS, odor presentation strongly activated the left anterior hippocampus only when it was previously paired with the learning material. (**c**) During SWS, the retrosplenial cortex also showed stronger activation in the reactivation than in the no-reactivation condition. Coronal and sagittal sections are shown, thresholded at $P < 0.001$ uncorrected, superimposed on a $T_1$-template image. (**d**–**f**) Parameter estimates of the peak voxel in the lateral PFC, left hippocampus and retrosplenial cortex for all four experimental conditions. Values are mean ± s.e.m. *P* values, significant interactions for 'sleep-wake' × 'reactivation–no reactivation'.

**Table 2 Results summary of the fMRI experiment**

| | MNI coordinates (mm) | | | | |
|---|---|---|---|---|---|
| | *x* | *y* | *z* | Peak *Z* | *P* |
| Wake group | | | | | |
| Reactivation > no reactivation | | | | | |
| Right lateral prefrontal cortex (44) | 63 | 12 | 15 | 3.28 | 0.001 |
| No reactivation > reactivation | | | | | |
| Right inferior frontal gyrus (47) | 18 | 27 | −12 | 3.92 | 0.0001 |
| Anterior cingulate (32) | 9 | 45 | 6 | 3.28 | 0.001 |
| Sleep group | | | | | |
| Reactivation > no reactivation | | | | | |
| Left anterior hippocampus | −21 | −15 | −18 | 4.21 | 0.00003 |
| Retrosplenial cortex (31) | 15 | −48 | 24 | 4.14 | 0.00003 |
| Left middle temporal lobe (21) | −42 | 0 | −30 | 3.47 | 0.0005 |
| Medial frontal gyrus (8) | 3 | 51 | 48 | 3.37 | 0.0007 |
| Right middle frontal gyrus (11) | 27 | 42 | −3 | 3.87 | 0.0009 |
| Right inferior parietal lobule (40) | 51 | −36 | 30 | 3.86 | 0.0009 |
| Left superior frontal gyrus (10) | −21 | 48 | 0 | 3.76 | 0.001 |
| Left superior frontal gyrus (10) | −33 | 54 | 18 | 3.72 | 0.001 |
| No reactivation > reactivation | | | | | |
| No suprathreshold voxel | | | | | |

Brain regions showing significantly higher activity during odor-on periods in the reactivation condition (odor during learning and retention interval) as compared with the no-reactivation condition (odor during retention interval only) are indicated (thresholded at $P < 0.001$; minimal voxel size $k = 3$). Numbers in parentheses refer to respective Brodmann areas. MNI, Montreal Neurological Institute.

Thus, our findings indicate that the reactivations of memory traces have opposite effects on memory depending on whether they occur during SWS or wakefulness. Whereas reactivation during waking destabilized memory traces, returning them to a labile state, the same odor-cued reactivation stabilized memory traces when induced during SWS.

### Neuronal correlates of reactivation during waking and SWS

In another experiment, we sought to specify the particular brain circuitry implicated in reactivation of memories during waking and SWS by applying functional magnetic resonance imaging (fMRI). Participants in the evening learned the same object-location task as in the main experiments. After learning, they were positioned in the fMRI scanner, and the wake group stayed awake ($n = 24$), whereas the sleep group was allowed to sleep for ~40 min ($n = 23$). Participants of the wake group were instructed to lie quietly in the scanner and press a button every ~1 min (to avoid falling asleep). All participants received odor stimulation while brain activity was recorded. One group of the sleep and wake subjects, respectively, received the odor during learning and during the post-retention interval (reactivation condition), whereas the others were not presented with odor during learning (no reactivation). Thus, in the latter no-reactivation condition, the odor presented during the retention interval was not associated with the learning material and therefore not capable of reactivating memory presentations of the object-location task.

Re-exposure of the associated odor during waking, compared with the no-reactivation wake group, activated mainly the right lateral prefrontal cortex (lateral PFC; [63,12,15]; $Z = 3.28$, $P = 0.001$; **Fig. 2** and **Table 2**). Some evidence suggests that this brain region is implicated in the control of memory retrieval[27,28]. We also observed left hippocampal activation, but only at a much more lenient threshold ([−33,−12,−18]; $Z = 1.79$, $P = 0.04$). It is very improbable that these activations were due to either retrieval effort or fatigue because brain activation was assessed only during the reactivation period and not during retrieval testing and fatigue should have similarly affected both the reactivation and no-reactivation condition and, thus, cannot explain differential activations in these conditions. In contrast to wake reactivations, odor-induced reactivation during SWS led to a markedly different activation pattern. Compared with the no-reactivation sleep group, odor re-exposure during SWS strongly and reliably activated the left hippocampus ([−21,−15,−18]; $Z = 4.21$, $P = 0.00003$; **Fig. 2b**). In addition, posterior neocortical regions implicated in memory, like the retrosplenial cortex (**Fig. 2c**) and temporal cortex, were activated (**Table 2** and **Fig. 2d–f**).

An interaction analysis confirmed that odor-induced reactivation during sleep activated the left hippocampus and several neocortical areas (including the retrosplenial cortex, the temporal cortex and additional medial frontal areas) to a significantly larger extent than with odor-induced reactivation during wakefulness (all $P < 0.001$; see **Supplementary Table 4**). These differences in activation patterns support the notion that different processes and mechanisms are implicated in reactivations during wakefulness and sleep.

## DISCUSSION

There is now evidence that reactivation is a key mechanism of memory formation, which is a principally dynamic process[1,19,29]. Our findings demonstrate that reactivating a memory has distinct effects on memory stability and reactivation-related activity that critically depend on the brain state of sleep or wakefulness. Whereas in the wake state reactivation returned memories into an active and labile state susceptible to interference, reactivation during sleep immediately stabilized memories, making them resistant to interference learning. The stabilizing effect occurred in the absence of subsequent REM sleep, suggesting that REM sleep might not be necessary for this process. The interplay over time between wake and sleep 'modes' of memory reactivation suggests a superordinate process of adaptive memory formation in which wake and sleep reactivations support different but complementary functions.

Our finding that, during waking, reactivation returned memories into a labile state is in agreement with many studies in animals showing similar labilization in different memory tasks, with different reminders and different types of disrupting agents[1,3,30]. In humans, reconsolidation in the waking state has only recently been demonstrated[9–11], mostly through indirect memory measures like the incorporation of new information into old material[12], changes in retrieval-induced forgetting[13] or forgetting of older autobiographical memories[14]. Here, we show for the first time, to the best of our knowledge, in humans a direct impairment of previously learned declarative memories by interference learning after reactivation by a contextual reminder cue. Reconsolidation in our study was expressed as an impairment of original memories rather than a complete blockade of memory recall. This differs from animal studies of reconsolidation, in which reactivation is followed by an injection of protein synthesis inhibitors or other strong amnestic treatments that often completely block subsequent memory. However, interference learning used to challenge memory stability in humans probably modulates memory less strongly, diminishing the previously learnt declarative memories rather than inducing complete forgetting[12,13]. Additionally, reactivation in our study occurred relatively shortly (~30 min) after learning, whereas memories are typically reactivated after 24 h or longer in most animal studies examining reconsolidation. Our findings show that reactivation during wakefulness destabilizes newly encoded memories, but these findings should be regarded as preliminary with respect to older, fully consolidated memories. It remains to be elucidated whether similar effects would be found when reactivating already consolidated memories after retention intervals of 24 h or longer. Further studies must also clarify whether memories impaired by interference learning after reactivation are permanently lost or recover over time.

Reconsolidation is increasingly recognized as an important process in memory formation[31]. Notably though, reconsolidation does not occur under all conditions but presumably depends on certain boundary conditions such as the age of the memory and the type and duration of the reminder[32–34]. Thus, specific conditions seem necessary to enable labilization of memory traces. Under some conditions reactivation does not labilize memories and in other conditions impaired memories recover over time. It has recently been suggested that reconsolidation specifically occurs under conditions in which an updating of the reactivated memory with new information is probable[31]. Although reconsolidation presumably serves multiple functions, the dynamic updating of stored information through reconsolidation during wakefulness might be an important adaptive function to maintain the relevance of these memories for the long term[22]. In everyday life, older memories are reactivated by retrieval or a reminder when these memories are used to deal with new situations. If these memories are successfully applied to manage the new situation, they will be restrengthened, that is, reconsolidated, and concurrently updated by incorporating new experiences into the reactivated older representations. In this way, 'successful' memories are optimized, keeping their potential relevance for future situations, whereas other 'unsuccessful' memories become overwritten by new and more useful information. The results of our fMRI study indicate that during reactivation in the wake state, mainly the lateral PFC is activated. This brain region is implicated in the control of memory retrieval with respect to the actual context, in upholding retrieved information in working memory and in the evaluation of stored representations[27,28,35,36]. This evaluative processing of reactivated memories during wakefulness might help return these memories to a labile state, allowing for updating of the memory representations with information concurrently encoded in the new context. However, although it is generally adaptive, this process of updating could in some cases also produce faulty or distorted memories[37].

We originally hypothesized that reactivations during SWS would likewise return memories to an active and changeable form to allow for further processing of the underlying neuronal representations. However, contrary to this hypothesis, reactivation during sleep did not labilize but directly stabilized memory representations. Although such rapid stabilization could occur solely on the hippocampal level (synaptic consolidation), a common model of sleep-dependent memory consolidation assumes that reactivations during SWS facilitate the transfer of new memory representations from the hippocampus to neocortical sites for long-term storage (system consolidation)[19,38–41]. We speculate that reactivations occurring during SWS after learning initiate this process, thereby protecting these memories against interference from information subsequently encoded in the hippocampus[11]. Signs of a redistribution from hippocampal to neocortical brain areas were revealed in several foregoing studies[38,39]. In line with this view, we observed activations of hippocampal and neocortical regions in our fMRI study during reactivations in SWS. Direct evidence for an involvement of these structures in system consolidation requires recording of brain activation also during learning and retrieval, which was not done here. Furthermore, the transfer of a memory takes days or even years, and this process is probably not completed within a single period of SWS[42–44]. However, coordinated reactivations of memory traces in hippocampal and neocortical structures during sleep have been observed shortly after learning[16], thus constituting the initial phase of redistribution. The importance of the first SWS period after learning is also supported by findings of local increases in slow-wave activity (SWA) after learning that are most pronounced during the first 30 min of non-REM sleep and are predictive of performance improvements after sleep[45].

Alternatively, reactivations during sleep might lead to a transient destabilization of memory traces, but with restabilization occurring at a much faster rate during sleep, leading to a straightforward reconsolidation of the reactivated representations. Fast processes of synaptic consolidation during SWS might target ultra-short periods of memory instability during which neuronal connections could be instantly strengthened, thus becoming rapidly resistant to interference. Future studies should clarify whether reactivation during SWS induces such rapid stabilization of memory and whether this effect relies on processes of synaptic consolidation, system consolidation or both.

Whatever the underlying mechanisms, our findings demonstrate that the effects of memory reactivation on memory stability differ fundamentally depending on the brain state of sleep or wakefulness. The factors responsible for this brain state–dependent difference are currently unknown and could involve any of the many differences between sleep and wakefulness. For example, during wakefulness as opposed to sleep, subjects might have become aware of the reactivation, stimulating conscious or unconscious retrieval of associated memory contents. Wake subjects are also necessarily engaged in other concurrent activities and might have formed new associations during the reactivation phase, potentially impacting and interfering with the reactivated memories. In fact, differences in awareness levels and encoding ability may be crucial components in determining the state-dependent function of memory reactivations. We propose that memory reactivation is not a unitary phenomenon but activates distinct processes depending on whether reactivation occurs during an 'encoding' mode or a 'consolidation' mode of the brain[25], as established during waking and sleep, respectively. Both modes of reactivation might dynamically interact over time to serve complementary functions in updating (during wakefulness) and strengthening memories (during sleep), thereby optimizing adaptive memory formation for the long term.

Finally, our findings also have clinical implications. Our results strongly suggest that contextual cues presented during wakefulness might be capable of reactivating and destabilizing unwanted and maladaptive memories in a psychotherapeutic setting, for example, in patients with panic disorder or post-traumatic stress disorder. Thus, reactivation before a psychotherapeutic intervention might facilitate reprocessing and unlearning of unwanted memories, thereby making therapy more effective[46]. Subsequent reactivation of the newly learned concepts during ensuing SWS could then help to consolidate the desired therapeutic effects for the long term.

## METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/natureneuroscience/.

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

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**AUTHOR CONTRIBUTIONS**

S.D., J.B. and B.R. designed the experiments and wrote the paper. S.D. and B.R. carried out the experiments and analyzed the data. C.B. provided analytic tools.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

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## ONLINE METHODS

**Participants.** Twenty-eight healthy nonsmoking adults participated in the study. All reported having regular sleep-wake cycles, no history of any neurological, psychiatric or endocrine disorder, and no shift work for at least six weeks before the experiments. They did not take any medication and had no nasal infections at the time of the experiments. Ingestion of caffeine and alcohol was not allowed on experimental days. Before the experiments, subjects in the sleep group spent one adaptation night in the sleep laboratory under the conditions of the experiment (including placement of electrodes and the nasal mask). Data from four subjects were excluded owing to difficulties with sleep (long sleep latency and/or long wake times after sleep onset). Mean age ± s.d. in the remaining 24 subjects was 22.3 ± 2.5 years (range: 18–27 years, 5 females). All participants gave written informed consent and were paid for participation. The study was approved by the local ethics committee.

**Design and procedure.** Participants in the wake group reported to the laboratory at 21:30 and, after filling in standard questionnaires, were attached to a nasal mask. Subjects then performed an odor detection test to assure normal olfactory sensitivity (see **Supplementary Results**). The learning phase started at 22:00 with the visuo-spatial object-location task. During performance of this task, the experimental odor was presented at the same time as the presentation of each stimulus to be learned via the nasal mask. After learning, the odor detection test was repeated. Thereafter, subjects had a short break (~30 min) to account for the slight delay between learning and sleep onset in the sleep group, and then performed a nondisturbing motor task without any learning component for on average 48.7 ± 1.6 min (48.3 ± 1.5 and 49.1 ± 1.9 min in the odor and vehicle conditions, respectively, $P > 0.60$), which was comparable to the sleep time of subjects of the sleep group. Re-exposure of the odor stimulus started ~20 min after the beginning of the task, which was roughly comparable to the time between sleep onset and odor presentation during slow-wave sleep (SWS) in the sleep group, and continued for ~20 min. Odor stimulation followed an alternating pattern of 30-s on, 30-s off phases. Although habituation was not measured here, a similar odor on–odor off procedure effectively reduces habituation[47]. Odor presentation continued for ~20 min. The long presentation duration was chosen to ensure that the odor cue would reliably induce reactivations. Immediately after the last odor-on trial, the motor task was stopped and the nasal mask removed. Then, subjects started learning the interference object-location task (without the odor). After a 30-min break, recall of the original object-location task was tested.

For the sleep group, sessions started at 20:30 to prepare the subject, after completion of the questionnaires, for standard polysomnographic recordings and odor delivery with the nasal mask. The participants then performed the odor detection test (see **Supplementary Results**). The learning phase (starting at 22:00) was identical to that in the wake group. Participants went to bed at 23:00 to enable a 40-min period of sleep. Odor was presented for ~20 min during periods of SWS. Presentation of the odor started as soon as online polysomnographic recordings indicated the presence of SWS, that is, >20% delta waves during a 30-s period. The stimulation was interrupted whenever polysomnographic signs of arousal, awakening or changes in sleep stage appeared. Although a 20-min period of stimulation was envisaged in each case, the duration of stimulation could be extended (to a maximum of 30 min) or reduced (to a minimum of 15 min) depending on the experimenter's judgment of the stability of ongoing SWS (actual mean duration of odor stimulation: 24.83 ± 2.62 min in the sleep group, 22.00 ± 1.76 min in the wake group). The experimenter was unaware whether odor or vehicle was applied on a given night. On each night, the olfactometer contained both odor and vehicle, and the selection was carried out automatically by a preprogrammed algorithm unknown to the experimenter. After odor stimulation, subjects were awakened from SWS (upon completion of a 30-s odor-on phase) and the nasal mask was removed. Subjects then, as in the wake group, started learning the interference object-location task (without odor) and 30 min later, recall was tested on the original object-location task (again without odor).

**Object-location task.** The two-dimensional object-location memory task resembles the game "concentration" and has been described in detail[20]. In brief, the task requires learning the location of 15 card pairs showing colored pictures of different animals and everyday objects presented on a computer screen. During learning, each card pair is presented by first showing one card alone, followed by presentation of both cards. The whole set of card pairs is presented twice in different orders. Immediately after these two runs, recall of the spatial locations is tested using a cued recall procedure, that is, the first card of each pair is presented and the subject has to indicate the location of the second card with a computer mouse. The cued recall procedure is repeated until the subject reaches a criterion of 60% correct responses. In this study, the odor was delivered in a stimulus-locked way during learning, starting with the onset of the presentation of the first card of a pair and stopping when presentation of both cards ended. At retrieval testing after the retention interval, the same cued recall procedure was used as during the learning phase, but without odor presentation.

For interference learning, the same object-location task with the same 15 card pairs as during original learning was used, with the only difference that the second card of each pair was presented at a different location (resembling an A-B, A-C interference learning paradigm with A, B and C representing the locations). The interference learning procedure was identical to the original procedure described above, except that no odor was presented during interference learning and that there was only one cued recall trial for all subjects (that is, no learning criterion to ensure comparable sensory interference). For the two experimental conditions, two versions of the interference learning task were designed corresponding to the two parallel versions of the original object-location task. Only subjects who remembered at least two card locations (that is, performed above chance level) in the immediate cued recall were included in the final analyses (see **Supplementary Results** and **Supplementary Table 5** for detailed results of interference learning).

**Odor delivery and substance.** The experimental odor was delivered via a computer-controlled olfactometer as described[20]. The olfactometer was placed in a separate room and was connected to the subject's mask via Teflon tubes, which allowed odor stimulation to be regulated without disturbing the subject. The subject received the odor via a small nasal mask that assured constant stimulation but permitted normal breathing. The experimental odor was isobutyraldehyde (≥99%) diluted in 1,2-propanediol at a concentration of 1:200. Odorless 1,2-propanediol alone served as vehicle stimulus.

**Vigilance performance and subjective sleepiness.** Before learning and after retrieval testing subjects performed a vigilance task to test general alertness. A red dot randomly appeared at the left or right side of a computer screen every 2–10 s and participants had to respond as quickly as possible by pressing the corresponding left or right button. Subjects additionally rated their subjective sleepiness on the Stanford Sleepiness Scale ranging from 1 ("feeling active, vital, alert or wide awake") to 7 ("no longer fighting sleep, sleep onset soon; having dream-like thoughts").

**Sleep recordings.** Sleep was recorded by standard polysomnography including electroencephalographic (EEG), electromyographic and electrooculographic recordings. EEG was recorded from six scalp electrodes (F3, F4, C3, C4, P3 and P4 according to the International 10–20 System) and a nose reference. EEG signals were filtered at 0.15–35 Hz and sampled at 200 Hz. In addition to the online identification of sleep stages, polysomnographic recordings were scored offline according to standard criteria as wake, sleep stages 1–4 and REM sleep, with sleep stages 3 and 4 defining SWS[48].

**Statistical analyses.** Data were analyzed using multifactorial analyses of variance (ANOVA). Where appropriate, post-hoc tests were conducted using univariate ANOVAs and *t*-tests. The level of significance was set to $P = 0.05$.

**fMRI experiments.** 59 healthy young subjects participated in the fMRI experiment. Twenty-four subjects took part in the wake group (25.9 ± 0.7 years; range 20–32 years, 11 men). In the sleep group, 12 participants had to be excluded because they did not enter SWS while sleeping in the scanner. The remaining 23 participants (26.7 ± 0.8 years; range 22–36 years; 12 men) had at least one period of SWS (average time: 14.5 ± 2.4 min; the sleep group partly overlapped with a subject sample whose data have been published[20]). To increase sleep propensity, participants in the sleep group were asked to sleep no longer than 3 h on the nights before the sleep session and not to take any naps throughout the day. Although we cannot exclude the possibility that the partial sleep restriction slightly changed sleep patterns, sleep architecture during the single cycle of non-REM sleep (without subsequent REM sleep) was comparable between the behavioral and fMRI experiments. Mean length of stable SWS was still shorter

(14.5 min) as compared with the behavioral experiments (26 min) in spite of earlier partial sleep deprivation. Sessions for both sleep and wake groups took place between 20:00 and 02:00.

In the learning phase subjects performed the object-location task. Participants in both the sleep and wake groups were randomly assigned to one of two experimental conditions. In the reactivation condition, the experimental odor was presented during learning of the object-location task. In the no-reactivation condition, odor presentation during learning was omitted. After learning, all subjects were carefully positioned in the scanner, where they wore earplugs and headphones to reduce noise. Functional image acquisition and concurrent EEG recording started immediately after acquisition of an anatomical T1-weighted image. Scanning was stopped after ~1.5 h. Odor presentation during scanning followed the same procedures used in the main experiment, encompassing an alternating pattern of 30-s on, 30-s off, which in the sleep group was presented contingent on the occurrence of SWS. In the wake group, stimulation included 15 on-off periods (15 min) starting 45 min after learning. This delay was roughly equivalent to the delay of odor stimulation in the sleep group. To assure wakefulness, in the wake group subjects were asked to press a hand-held button every ~1 min, and they were reminded of this task (via headphones) whenever they failed to do so for >2 min.

Functional imaging was carried out on a 3 T Siemens Trio scanner using the following parameters: 38 axial slices, 1 mm; gap acquisition time, 2.61 s, 3 s gap; echo time, 25 ms; flip angle, 90°; field of view, 192 $mm^2$; matrix, 64 × 64. fMRI data was analyzed using Statistical Parametric Mapping (preprocessing, SPM2; data analysis, SPM5, Wellcome Department of Cognitive Neurology, London, UK). For the sleep group, only scans in SWS were evaluated. The images were realigned, normalized and smoothed (10 mm full width at half maximum). The 30-s periods of odor presentation were modeled using a boxcar function convolved with the hemodynamic response function in the context of a linear regression analysis (high-pass filter, 128 s). A statistical threshold of $P < 0.001$ in three adjacent voxels was used.

During scanning, standard polysomnographic recordings (EEG at C3 and C4, electrooculogram, electromyogram and electrocardiogram) were obtained to monitor sleep using a BrainAMP MR amplifier. Sampling rate was set to 5 kHz (filter settings, 0.03–250 Hz). Preprocessing of EEG data included correction of scanner artifacts and cardioballistic artifacts as described[20]. Resulting data were used to score sleep stages according to standard criteria[48].

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