SLEEP-DEPENDENT CONSOLIDATION IN MULTIPLE MEMORY SYSTEMS

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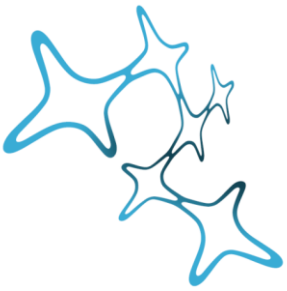
Dissertation at the Graduate School of Systemic Neurosciences
at the Ludwig-Maximilians-Universität München



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ABSTRACT

Before newly formed memories can last for the long-term, they must undergo a period of consolidation. It has been shown that sleep facilitates this process. One hypothesis about how this may occur is that learning-related neuronal activity is replayed during following sleep periods. Such a reactivation of neural activity patterns has been repeatedly shown in the hippocampal formation in animals. Hippocampally-induced reactivation can also be observed in other brain areas like the neocortex and basal ganglia. On the behavioral level, sleep has been found to benefit performance on a broad range of memory tasks that rely on different neural systems. Up to now, however, it is unclear whether the same mechanisms mediate effects of sleep on consolidation in different memory systems.

In this thesis, we investigated both the effects and the mechanisms of sleep-dependent consolidation in multiple memory systems. We find that sleep benefits performance on a broad range of procedural and declarative memory tasks (studies 1 and 2). These beneficial effects of sleep go beyond a reduction of retroactive interference as effected by quiet wakeful meditation (study 1).

In study 2, we demonstrate that the processes underlying these beneficial effects of sleep are different for different memory systems. We assessed performance on typical declarative and procedural memory tasks during one week after participants slept or were sleep deprived for one night after learning. Sleep-dependent consolidation of hippocampal and non-hippocampal memory follows different time-courses. Hippocampal memory shows a benefit of sleep only one day after learning. Performance after sleep deprivation recovers following the next night of sleep, so that no enduring effect of sleep can be observed. However, sleep deprivation before recall does not impair performance. For non-hippocampal memory, on the other hand, long-term benefits of sleep after learning can be observed even after four days. Here, delayed sleep cannot rescue performance. This indicates a dissociation between two sleep-related consolidation mechanisms, which rely on distinct neuronal processes.

We studied the neuronal processes underlying sleep effects on declarative memory in study 3, where we investigate learning-related electrophysiological activity in the sleeping brain. With the help of multivariate pattern classification algorithms, we show that brain activity during sleep contains information about the kind of visual stimuli that were learned earlier. We thus find that learned material is actively reprocessed during sleep.

In a next step, we examined whether procedural memory can also benefit from reactivation during sleep. We find that a procedural memory task that has been found to activate the hippocampus can be strengthened by externally cueing the reactivation process during sleep. Similar to study 2, this indicates that it is not the traditional distinction between declarative and procedural memory that determines how memories are consolidated during sleep. Rather, memory systems, and in particular hippocampal contribution, decide the sleep-dependent consolidation process.

In the first four studies, we examined how sleep affects memory in different memory systems. In our last study, we went one step further and investigated whether multiple memory systems can also interact during consolidation in sleep. We devised a task during which both implicit and explicit memory develop during learning. Results show that sleep not only strengthens implicit and explicit memory individually, it also integrates these formerly separate representations of the learning task. Implicit and explicit memory are negatively correlated immediately after training. Sleep renders this association positive and allows cooperation between the two memory traces. We observe this change both in behavior, using structural equation modeling, and on the level of brain activity, measured by fMRI. After sleep, the hippocampus is more strongly activated during recall of implicit memory, whereas the caudate nucleus shows stronger activity during explicit memory recall. Moreover, both regions show correlated stimulus-induced responses in a task that allows memory systems cooperation. These results provide conclusive evidence that sleep not only strengthens memory, but also reorganizes the contributing neural circuits. In this way, sleep actually changes the quality of the memory representation.

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INTRODUCTION

SLEEP'S ROLE IN MEMORY

At the end of the 19th century, Hermann Ebbinghaus studied the dynamics of forgetting. He recorded detailed retention curves for newly learned material, which illustrate the process of memory decay. Already from these curves, it becomes apparent that we forget less over periods of sleep than over periods of wakefulness (Ebbinghaus, 1885). Many early studies on the relation between sleep and memory replicate this finding, using larger samples and refined methods (Heine, 1914; Jenkins & Dallenbach, 1924; reviewed in van Ormer, 1933).

The main question in this newly emerging field of research was, why such a beneficial effect of sleep occurs. Several theories tried to explain how sleep protects memories from forgetting (reviewed in Ekstrand et al., 1977). On the one hand, forgetting was thought to result from a decay of the neurobiological traces established during learning. Because metabolism is lower during sleep, it was assumed that the decay of memory traces should occur at a slower rate during sleep than during wakefulness (Thorndike, 1913). This would explain why less forgetting occurs over sleep. On the other hand, it was found that new learning interferes with existing memories and thus causes forgetting. Since no interfering information can be learned after sleep onset, sleep was thought to shelter newly formed memory traces from these disruptive effects (McGeoch, 1932).

During the same time, Müller and Pilzecker (1900) reasoned that the gradually decreasing disruptive effect of retroactive interference on previously encoded material is evidence for an ongoing physiological process related to the learning experience, which stabilizes new memories. They termed this process memory consolidation. Consolidation is supposed to transform the memory engram into a stable state, which is enduring and makes it resistant against further interference. It has been shown that sleep following immediately after learning has a stronger effect on memory than sleep occurring after longer delays (Ekstrand, 1967; Benson & Feinberg, 1977; Gais et al., 2006; Talamini et al., 2008; Payne et al., 2012). These findings have been taken as an indication that sleep has a beneficial effect on memory consolidation. If sleep occurs long after learning, the consolidation process

may already have completed, which could explain the smaller effects of delayed sleep (Ekstrand et al., 1977).

The debate about whether sleep prevents decay, whether it provides a period of reduced interference, or whether it induces consolidation of the memory trace has long been controversial. Whereas theories about slower memory trace decay and less interfering information intake during sleep assume that sleep passively protects memories from forgetting, theories of sleep-dependent consolidation posit that sleep actively stabilizes newly formed memories. Recently, the study of the underlying mechanisms provided more insight into how sleep affects memory. In particular, it has been shown that neural activity during task acquisition is re-expressed in post-learning sleep (Wilson & McNaughton, 1994). This reactivation clearly constitutes an active process supporting memory consolidation during sleep. Many behavioral experiments also indicate that sleep holds an active role in consolidation, and contradict the notion that its beneficial effect is due to a mere passive sheltering from external interference. Findings that a period of sleep stabilizes memories and makes them more resistant against later interference learning, in particular, support the view that sleep not only allows, but also aids and accelerates memory consolidation (Ellenbogen et al., 2006; Ellenbogen et al., 2009). However, it has never been directly tested whether a reduction of interfering information after learning, which might allow a more undisturbed stabilization of memories, can at least in part explain the observed beneficial effects of sleep on declarative memory consolidation. We addressed this open question in study 1, where we manipulated the amount of interfering information during a memory retention interval and compared potential effects of reduced interference on memory consolidation to the effect of sleep.

MEMORY TYPES AND SLEEP

While the effect of sleep on memory per se is well established, there is an ongoing debate about which types of memory benefit from sleep. In its beginnings, research on sleep's function focused on declarative memory (Heine, 1914; Jenkins & Dallenbach, 1924). More recent studies show that sleep strengthens a broad range of declarative memory tasks (for a classification of the different forms of long-term memory, see Figure 1). These include wordlist and vocabulary learning, but also visual memory for pictures, and visuo-spatial tasks, such as pairs games (Plihal &

Born, 1997; Ellenbogen et al., 2006; Tucker et al., 2006; Gais et al., 2007; Gorfine et al., 2007; Rasch et al., 2007; Sterpenich et al., 2007; Wagner et al., 2007; Atienza & Cantero, 2008; Mednick et al., 2008; Tucker & Fishbein, 2008; Ellenbogen et al., 2009; Rudoy et al., 2009; Lau et al., 2010; Diekelmann et al., 2011; Wilhelm et al., 2011; Baran et al., 2012; Diekelmann et al., 2012; Payne et al., 2012; Wilson et al., 2012).

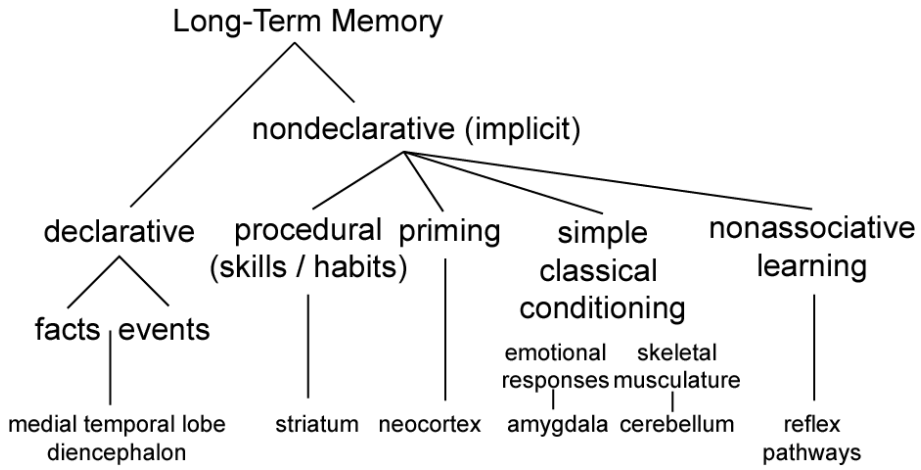

Figure 1. Classification of different forms of long-term memory. Long-term memory is traditionally divided into declarative and nondeclarative memory. Declarative memory comprises memory for both facts (semantic memory) and events (episodic memory). It is impaired after damage to the temporal lobes and especially the hippocampal formation. Nondeclarative memory comprises procedural memory for skills and habits. It is thought to depend both on the striatum und neocortical regions. Figure reprinted with permission from Squire and Zola-Morgan (1991).

Apart from declarative memory, sleep has been shown to facilitate procedural memories like finger tapping skills, visual discrimination, gross motor learning and motor adaptation (Plihal & Born, 1997; Gais et al., 2000; Fischer et al., 2002; Mednick et al., 2002; Walker et al., 2002; Mednick et al., 2003; Atienza et al., 2004; Backhaus & Junghanns, 2006; Korman et al., 2007; Doyon et al., 2009; Kempler & Richmond, 2012; Geyer et al., 2013). A characteristic of procedural memory is that it is more resistant to forgetting than declarative memory. In procedural tasks, forgetting occurs scarcely or not at all. Moreover, procedural skills are not only retained better when sleep follows learning, performance can even improve over a period of sleep. In the serial reaction time task, participants actually react faster on finger transitions in a learned sequence after consolidation during sleep than at the end of training (Fischer et al., 2002; Walker et al., 2002). Visual discrimination,

another form of procedural memory, even crucially depends on sleep for improvements to emerge and strongly requires sleep to occur shortly after learning (Stickgold et al., 2000). It does not show improvements after several nights of sleep when subjects are initially sleep deprived after learning, whereas the benefit achieved by sleep during the first night remains stable even after one week.

In summary, both declarative and procedural memory benefit from sleep (for a comprehensive review see Rasch & Born, 2013). It is, however, unclear whether all types of declarative and procedural memory are equally sleep-dependent. Furthermore, it is not known whether sleep-dependent consolidation relies on the same mechanisms for different kinds of memories. We addressed these questions in studies 1 and 2.

LONG-TERM EFFECTS OF SLEEP ON MEMORY

Most studies examining the effect of sleep on memory consolidation test performance directly after one night of sleep or sleep deprivation, but not after longer time-spans. A persistent benefit of sleep, however, would underline the behavioral relevance of sleep effects on memory consolidation. Apart from visual discrimination learning, as described above, long-term effects have been reported only for a few tasks. Procedural motor adaptation and a number of other motor tasks show benefits of sleep when tested after a three-day interval including recovery sleep (Smith, 1995; Maquet et al., 2003). Next to these findings in the procedural domain, a small number of studies examined long-lasting effects of sleep on declarative memory. Two studies testing word pair and vocabulary memory have shown benefits of sleep not only on the first night after learning but also after a two-day interval including one night of recovery sleep for initially sleep-deprived subjects (Gais et al., 2006; Gais et al., 2007). Another study finds a lasting sleep-related increase in memory for emotional texts even after four years from encoding (Wagner et al., 2006). Overall, however, evidence for long-term effects of sleep is scarce. Therefore, we assessed both short-term and long-term effects of sleep on different types of declarative and procedural memory in study 2. We tested memory performance on the first day after learning, and again after varying delays of two, three, four, or six days. Examining this extended time-course of consolidation can also help to tease apart differential contributions of sleep to consolidation of these memory subtypes.

MEMORY TRACE REACTIVATION

Research in rodents has shown that activity patterns of hippocampal neurons observed during learning are later replayed during sleep (Wilson & McNaughton, 1994; see Figure 2). In this seminal study, rats were trained to find food in a T-maze. Hippocampal neurons that fired together during spatial learning again exhibited the same correlated firing patterns during the following sleep period. These patterns were not present in sleep before training.

This first evidence for a reactivation of waking experiences during sleep has been extended by many more recent findings (Nadasdy et al., 1999; Louie & Wilson, 2001; Lee & Wilson, 2002; Ribeiro et al., 2004; Euston et al., 2007; Peyrache et al., 2009). Replay of neuronal activity is not restricted to the hippocampus but can also be observed in other cortical areas. It has been shown that replay in the hippocampus precedes replay in the neocortex (Ji & Wilson, 2007; Peyrache et al., 2009) and subcortical areas, like the striatum (Pennartz et al., 2004; Lansink et al., 2009). Moreover, neuroimaging studies in humans find that hippocampal activity during spatial learning is re-expressed during subsequent sleep, and that the amount of this reactivation correlates with later memory performance (Peigneux et al., 2004). In study 3, we detected neuronal reprocessing of learning material in electrical brain activity during sleep. We examined which features in the electroencephalogram (EEG) reflect such reprocessing and studied how and when it preferentially occurs.

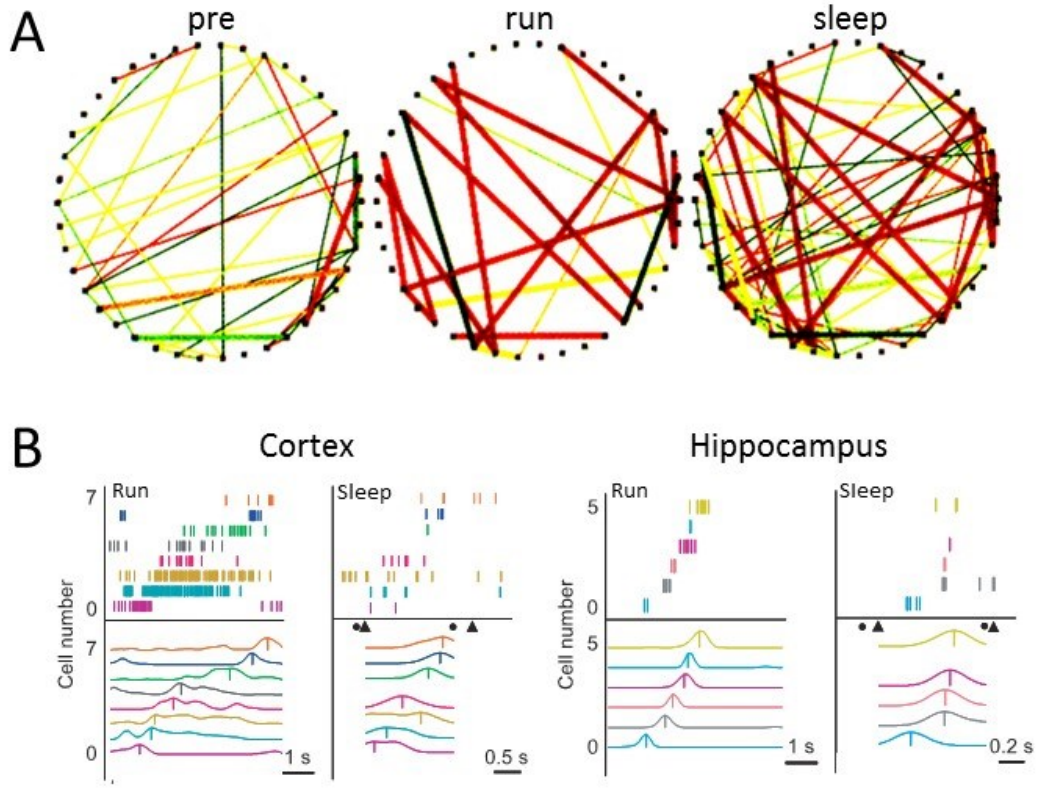

Figure 2. Evidence for memory trace reactivation in the rodent brain. (A) Neurons that exhibited correlated firing during spatial learning in a T-maze (run) showed the same firing patterns again during post-learning sleep (sleep). These patterns were not observed in a sleep recording that preceded the learning experience (pre). Figure adapted and reprinted with permission from Wilson and McNaughton (1994). (B) Replay is not restricted to the hippocampus. Also in the visual cortex, the same neuronal firing sequences as during learning can be observed during later sleep. Figure adapted and reprinted with permission from Ji and Wilson (2007).

If memory reactivation leads to enhanced consolidation, it can be speculated that triggering reactivation with external cues will increase performance. Rasch and colleagues (2007) targeted the internal reactivation process in humans by introducing such cues as a reminder of a previous learning task (see Figure 3). They presented an odor during encoding and again during the following period of sleep or wakefulness. Odor presentation during sleep, but not during wakefulness boosted memory performance on a hippocampal-dependent spatial memory task, showing that memory trace reactivation can mediate memory performance after sleep. This paradigm has been modified and replicated in several studies, which found that the effect of reactivation is not only specific to sleep, but can also be highly specific to individual items learned during the encoding session (Rudoy et al., 2009; Schreiner & Rasch, 2014). Research in animals has shown that presenting cues associated with

the learning task biases the content of memory replay during sleep (Bendor & Wilson, 2012; see Figure 3). Thus, presenting external cues during sleep does not increase the amount of reactivation per se, but it increases the number of replays for the cued parts of the memory by selecting which information is reactivated.

The behavioral effects of externally triggered reactivation have been well established for declarative memory, yet it remained unclear whether other types of memory benefit from sleep in the same way. Some findings indicate that also procedural memory may be reactivated during sleep. In a visual discrimination task, stronger activity is re-expressed during sleep in the area of primary visual cortex representing the visual quadrant that participants were trained on (Yotsumoto et al., 2009). Two other studies found similar ongoing task-related activity during later sleep in areas involved in training a serial reaction time task (Maquet et al., 2000; Peigneux et al., 2003). Moreover, it has recently been shown that targeted memory reactivation during sleep can boost memory performance in such a sequential motor task (Antony et al., 2012). Whether these processes are governed by the same mechanisms as consolidation of declarative memories, however, remained unclear. We addressed this question in study 4, where we trained participants on a finger-tapping sequence for which sounds were assigned to individual finger movements. Half of the sounds for this sequence were replayed to the subjects during subsequent periods of sleep and wakefulness. We examined effects of conscious state, sleep length, and external memory reactivation on finger-tapping performance.

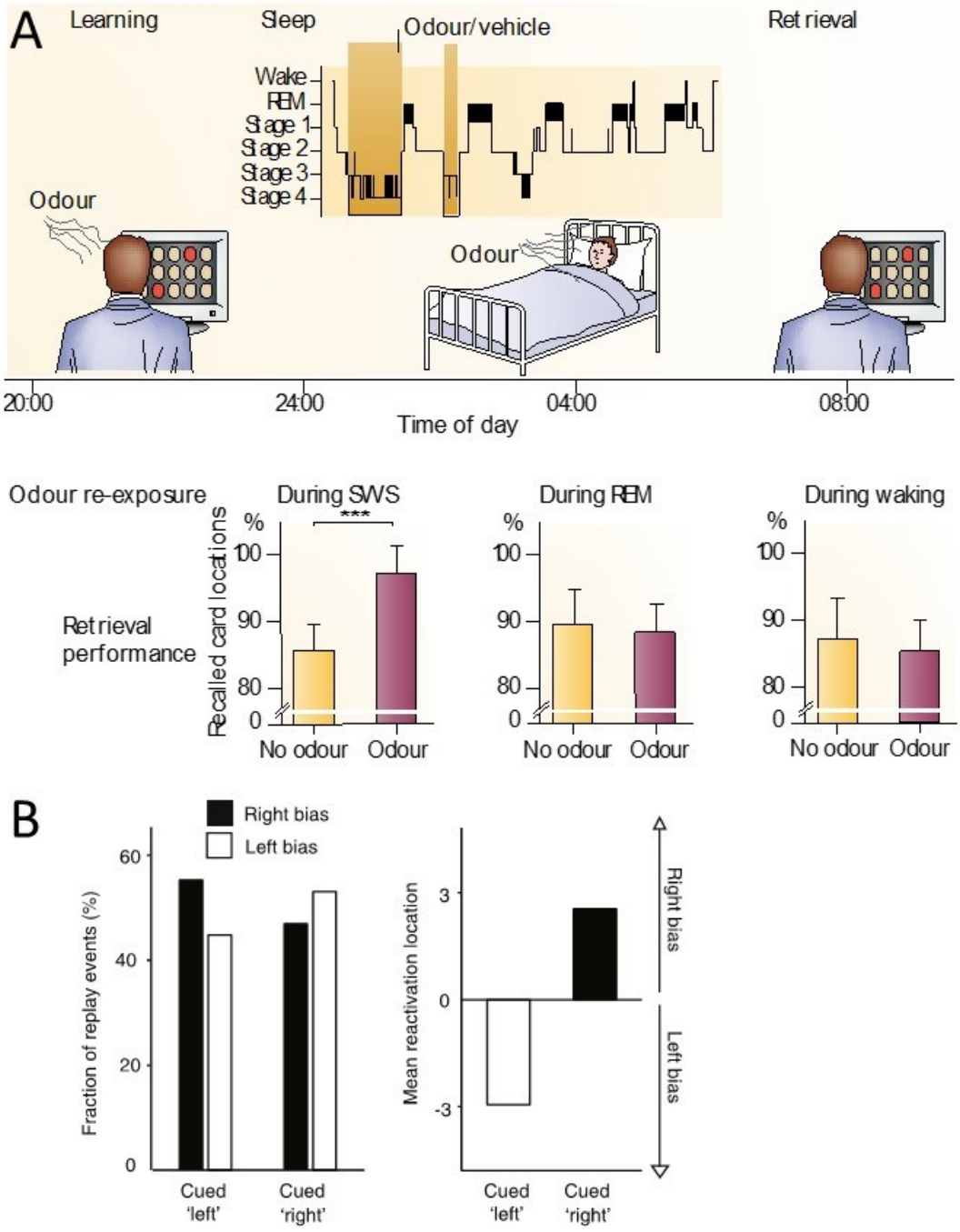

Figure 3. Targeting memory reactivation by external cues during sleep. (A) Rasch and colleagues presented the odor of a rose during encoding of a pairs game. Subjects were re-exposed to this odor during the following periods of slow wave sleep (SWS). This reactivation of the learning context improved behavioral performance. No effect of odor re-exposure was found during wakefulness or REM sleep. Figure adapted from Rasch et al. (2007). Reprinted with permission from Diekelmann and Born (2010). (B) In rats, presenting cues that have been associated with a learning experience during sleep influences the content of memory replay in the hippocampus. In a spatial task where different sounds were associated with rewards given on the left or right side of a box, playing the associated sounds led to more frequent participation of neurons in replay events that code the rats' position on the respective side of the box. Figure adapted from Bendor and Wilson (2012). Reprinted with permission from Abel et al. (2013).

SYNAPTIC AND SYSTEMS CONSOLIDATION

As already postulated early on by Müller and Pilzecker (1900), newly acquired memories must undergo a period of consolidation before they become stable and can last for the long-term. Recent studies examine the neurophysiological processes underlying this effect and their impact on the representation of memory in the brain before and after sleep. Consolidation of newly formed memory traces happens on both the synaptic and the systems level (Dudai, 2004). Synaptic consolidation lasts only for a few minutes up to one hour after encoding and occurs in the local nodes of the memory circuit. Systems consolidation can take several days up to months or years to be accomplished. It involves a reorganization of the neural circuits that encode the memory. Sleep is suggested to benefit both forms of consolidation (Diekelmann & Born, 2010).

Several findings indicate that sleep may aid synaptic consolidation. Sleep beneficially regulates key molecular mechanisms that support memory encoding and consolidation, like gene transcription and protein synthesis (Abel et al., 2013). Transcriptional regulatory proteins, like the cAMP-response binding protein (CREB), support long-lasting synaptic plasticity and long-term cellular information storage (Bourtchuladze et al., 1994). CREB phosphorylation in the hippocampus is elevated during rapid-eye-movement (REM) sleep (Luo et al., 2013). Furthermore, the expression of immediate early genes that are critical for memory storage is upregulated during sleep after animals have been exposed to enriched environments and after induction of long-term potentiation (LTP) in the hippocampus (Ribeiro et al., 1999; Ribeiro et al., 2002). There are also indications that sleep may increase the strength of synaptic connections through similar LTP-like phenomena that underlie memory consolidation (Chauvette et al., 2012). Moreover, spiking patterns that give rise to spindle oscillations, which are typically observed in the sleep EEG, have been shown to facilitate synaptic plasticity in slice preparations (Rosanova & Ulrich, 2005). A recent study has found that sleep promotes learning-dependent synapse formation in the neocortex (Yang et al., 2014). Together, this is compelling evidence that sleep impacts memory formation on the neuronal level.

Other synaptic processes may also contribute to better memory performance after sleep. Memory encoding during wakefulness is associated with a global increase in

synaptic potentiation. The synaptic homeostasis hypothesis posits that sleep serves to downscale these elevated activity levels (Tononi & Cirelli, 2003; Tononi & Cirelli, 2006). The goal of this downscaling is a homeostatic regulation of the total synaptic weight impinging on neurons. A downscaling in synaptic strength may be necessary for keeping energy demands low. Synaptic depotentiation is thought to occur proportionally to the initial strength of the synapse. Thus, differences in strength between synapses are preserved and a neural trace of learning can be maintained over the process. Weak synapses, however, may be lost entirely because they are downscaled below a minimum strength. By increasing signal-to-noise ratios in this way, synaptic downscaling has been proposed to benefit memory performance after sleep (Tononi & Cirelli, 2003; Tononi & Cirelli, 2006). There is evidence for increases in synaptic strength during wakefulness and concurrent decreases over sleep (Liu et al., 2010). However, the findings reported above indicate that sleep can also maintain LTP in synapses. Synaptic downscaling and synaptic upscaling may occur in parallel during sleep (Born & Feld, 2012; Chauvette et al., 2012; Abel et al., 2013). How exactly this happens and in which way it supports memory performance is currently unclear.

The described synaptic processes act locally at single neurons. Furthermore, they are not selective but affect all newly formed synapses. It is therefore unlikely that synaptic consolidation can involve a reorganization of the brain circuits that encode memories. Thus, it will leave the architecture of the memory trace unchanged. The discovery of memory trace reactivation during sleep, on the other hand, has inspired theoretical models of systems consolidation (Wilson & McNaughton, 1994; see Figure 2). Systems consolidation describes changes of the neural basis of memory that relate to the level of brain systems. Networks involved in memory storage and retrieval change during this process. Thus, systems consolidation can change both the strength and the functional architecture of the memory trace (Dudai, 2004). In contrast to processes related to synaptic consolidation, memory trace reactivation does not act equally on all newly encoded memory traces, but can be highly selective to specific neural circuits. Only those synapses are reactivated that have been implicated in previous learning. Furthermore, single traces can be strengthened preferentially (Rudoy et al., 2009; Schreiner & Rasch, 2014). Reactivation in one neural network spreads to other networks associated with the memory (Ji & Wilson, 2007; Lansink et al., 2009; Peyrache et al., 2009). This process can induce

reorganization of the neural circuits that store the memory. Therefore, most of the current frameworks suppose that memory trace reactivation during sleep not only strengthens, but also changes the quality of memory representations (Diekelmann & Born, 2010; Inostroza & Born, 2013; Stickgold, 2013; Stickgold & Walker, 2013).

Systems consolidation is viewed as a solution to the plasticity-stability dilemma that is considered in two-storage models of memory (McClelland et al., 1995). Because neural systems that show rapid plasticity will also show rapid overwriting when capacity limits are reached, or similar material is encountered, important memory content must be stored in a more permanent way that is less susceptible to interference. Two-storage models of memory suggest that short-term and long-term storage are mediated by different neural systems. The hippocampus is assumed to be a highly plastic memory storage with limited capacity (McClelland et al., 1995). It has been described as an intermediate-term memory buffer, which retains information for a limited time of days, months, or even years (Rolls & Treves, 1994; Treves & Rolls, 1994; Squire & Alvarez, 1995). The neocortex, on the other hand, is thought to store long-term declarative memory (McClelland et al., 1995). It has been suggested that sleep enables a hippocampal-neocortical dialogue (Buzsáki, 1996; Hasselmo, 1999). Replay in the hippocampus can trigger replay in the neocortex (Ji & Wilson, 2007) and subcortical areas (Lansink et al., 2009). Over time, memory then becomes independent of the hippocampus (Frankland & Bontempi, 2005). Thus, sleep is supposed to aid a transfer of memory from the hippocampus to the neocortex (Buzsáki, 1996; Gais & Born, 2004; Diekelmann & Born, 2010). By this process, newly acquired memories can be integrated more tightly into existing neocortical networks, some aspects of experiences can be selectively strengthened, and memory traces can, by repetition, be made more permanent in a less plastic but more stable network (Buzsáki, 1996; Gais & Born, 2004; Diekelmann & Born, 2010).

Changes in neural substrate go along with changes in the quality of the memory trace (Dudai, 2004; Gais & Schönauer, 2013). Such shifts have been observed for both explicit declarative memory and implicit skill learning. When sleep follows declarative learning, memory systems contributions shift over time (Gais et al., 2007). Over sleep, memory recall gradually becomes independent of the hippocampus and activates neocortical areas (Takashima et al., 2006). Also in implicit statistical learning, memory representations show changes in neural

substrate after consolidation. Whereas early after learning, recognizing statistically similar tone sequences activates regions in the medial temporal lobe, sleep shifts this activation to striatal areas (Durrant et al., 2013). This shift in brain activity goes along with better behavioral performance after sleep (Durrant et al., 2011; Durrant et al., 2013). Together, these findings support that sleep aids a transfer of memories from the hippocampus to other cortical and subcortical areas.

The neocortex is thought to store generalized semantic representations, whereas the hippocampus stores detailed episodic representations (Squire & Zola-Morgan, 1991; Moscovitch et al., 2005). It has thus been suggested that sleep leads to a generalization and semanticization of memories (Stickgold & Walker, 2013). If replay originating in the hippocampus entails a concurrent activation of associated memory traces in other brain areas, it is conceivable that overlapping memory representations in the neocortex may be preferentially strengthened, because their joint nodes are frequently activated together. This mechanism has been suggested to underlie the abstraction of semantic knowledge from episodic experiences (Lewis & Durrant, 2011). Sleep is widely assumed to play a pivotal role in this generalization of knowledge from single events (Gais & Born, 2004; Marshall & Born, 2007; Diekelmann & Born, 2010; Lewis & Durrant, 2011; Inostroza & Born, 2013; Stickgold, 2013; Stickgold & Walker, 2013; Diekelmann, 2014). However, direct evidence for such qualitative changes in memory over sleep is still scarce. They have only been observed for declarative and procedural memory, separately (Diekelmann et al., 2010; Nieuwenhuis et al., 2013). In study 5, we tested whether sleep can also integrate separate aspects of memory over different memory systems.

AIMS OF THIS THESIS

Although the relation between sleep and memory is already an old topic that has been investigated by many generations of scientists (Heine, 1914; van Ormer, 1932; Ekstrand et al., 1977; Maquet, 2001), some fundamental questions are still left unanswered. Current research tries to establish the exact role of sleep in consolidation of different kinds of memories. We do not know whether sleep-related consolidation benefits all kinds of memories equally, or only specific types of memory. It is also unclear if it occurs generally, or only under specific conditions (Marshall & Born, 2007; Diekelmann & Born, 2010; Inostroza & Born, 2013). We investigated these questions in studies 1 and 2, where we examined the effect of sleep on declarative and procedural memory in a variety of memory tasks.

Effects of sleep have already been shown for different types of memory (Rasch & Born, 2013). It is, however, unclear whether the same mechanisms mediate sleep-related consolidation in different memory systems. We addressed this question in study 2. We studied short-term and long-term effects of sleep on different kinds of procedural and declarative memory tasks to identify the role of sleep in the consolidation process. Studying the extended time-course of consolidation can reveal differences in mechanisms that do not become apparent after shorter intervals.

In study 3, we examined memory reactivation, which is one mechanism that underlies memory consolidation, more closely. Reactivation-dependent memory consolidation has been extensively studied in animals (Wilson & McNaughton, 1994; Nadasdy et al., 1999; Louie & Wilson, 2001; Lee & Wilson, 2002; Pennartz et al., 2004; Ribeiro et al., 2004; Euston et al., 2007; Ji & Wilson, 2007; Lansink et al., 2009; Peyrache et al., 2009), but the available imaging methods prevent such a direct access to reactivation-related brain activity in humans. Memory trace replay during sleep is not entirely faithful and can be compressed in time (Nadasdy et al., 1999; Carr et al., 2011). Thus, it is not predictable what form reactivation of past experience will take during sleep. When studying the internal reactivation process in humans, it is therefore necessary to use methods that do not require a priori assumptions about the exact form of this activity. We employed pattern classification algorithms, which fulfill this criterion, on human sleep EEG data to

detect material-specific memory reactivation of a declarative learning task in sleep and study the dynamics of this process.

In study 4, we examined whether external memory trace reactivation is only effective for declarative memory, or whether procedural memory tasks can also benefit from reactivation during sleep. Participants were trained on a finger sequence tapping task. Sounds were associated with their finger movements during training and replayed to them during the following sleep period. By presenting such learning-related cues during sleep, we targeted the reactivation process to test whether this improves performance. Furthermore, we compared these effects of external cueing to effects of prolonged sleep time.

One of the most prominent questions to date is how sleep changes the quality of memory traces. Theoretical models assume sleep to play a pivotal role in systems consolidation and generalization of memories over individual experiences, yet data that support these hypotheses are still scarce (Stickgold & Walker, 2013). Whereas qualitative changes over sleep have been observed for different kinds of memory representations separately, it has never been examined whether sleep can also integrate individual aspects of memory over different memory systems. In study 5, we developed a behavioral paradigm that allows forming both implicit and explicit representations of the same learning task to test whether sleep also changes the quality of memory systems interactions.

STUDY 1:

EXPLORING THE EFFECT OF SLEEP AND REDUCED INTERFERENCE ON DIFFERENT FORMS OF DECLARATIVE MEMORY

Monika Schönauer, Corinna Köck, Annedore Pawlizki, & Steffen Gais

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AP and SG planned and designed the experiments. MS, CK, and AP collected the data. MS and AP analyzed the data. MS and SG wrote the manuscript.

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ABSTRACT

Study Objectives: Many studies have found that sleep benefits declarative memory consolidation. However, fundamental questions on specifics of this effect remain open. It is not clear which forms of memory are affected by sleep and whether this beneficial effect is partly mediated by passive protection against interference. Moreover, a putative correlation between the structure of sleep and its memory-enhancing effects is still being discussed.

Design: In three experiments, we tested whether sleep differentially affects various forms of declarative memory. We varied verbal content (verbal/nonverbal), item type (single/associate) and recall mode (recall/recognition, cued/free recall) to examine the impact of sleep on specific memory subtypes. We compared within-subject differences in memory consolidation between intervals including sleep, active wakefulness, or quiet meditation, which reduced external as well as internal interference and rehearsal.

Participants: 40 healthy adults aged 18-30, and 17 subjects aged 24-55 with extensive meditation experience participated in the experiments.

Results: All types of memory were enhanced by sleep if the sample size provided sufficient statistical power. Smaller sample sizes showed an effect of sleep if a combined measure of different declarative memory scales was used. In a condition with reduced external and internal interference, performance was equal to one with high interference. Here, memory consolidation was significantly lower than in a sleep condition. We found no correlation between sleep structure and memory consolidation.

Conclusions: Sleep does not preferentially consolidate a specific kind of declarative memory, but consistently promotes overall declarative memory formation. This effect is not mediated by reduced interference.

INTRODUCTION

After encoding, newly acquired memories undergo a phase of consolidation, during which the memory trace can be stabilized and strengthened. It has been shown that sleep facilitates this process[1]. Although the relation between sleep and memory has already been investigated by several generations of scientists[2-5] there are still fundamental questions left unanswered.

First, it has been suggested that only specific types of declarative tasks are affected by sleep. For example, it has been proposed that semantically unrelated word lists, but not semantically related ones benefit from sleep[6], whereas other studies showed effects using related material[7]. Similarly, some authors suggest that benefits from sleep are greater for weaker compared to stronger memory traces[8], while other studies – using similar verbal material – report that only subjects who show a strong initial encoding benefit from subsequent sleep[9]. Furthermore, recent studies have found that future relevance[10] might mediate the effect sleep has on memory consolidation, but that future reward does not[11]. Sometimes, sleep-related changes can only be found in brain activity, but not in overt changes in behavioral performance[12]. Finally, it has been suggested that mainly the types of memory which rely on the hippocampus are sleep-dependent[5,13,14]. Therefore, it has been put forward that the mode of retrieval could also mediate whether memory performance is influenced by sleep, as recollection-based memory is thought to depend more on the hippocampus than familiarity-based memory[15,16]. Currently, it is unclear whether any of these types of memory tasks is actually not sleep dependent.

Second, it is not yet clear whether the positive effect of sleep on declarative memory formation stems from an active role of sleep in consolidation or whether it is passively emerging from reduced interference during an interval spent asleep as compared to one spent awake. In the procedural domain, this matter has already been addressed. To reduce interference generated by motor activation, Walker et al.[17] stabilized the participants' arms during consolidation of a motor skill learning task, so that there were no differences in muscle activity between wake and sleep groups. Similarly, Mednick et al.[18] tested whether the benefit of sleep for a visual texture discrimination task resulted from absence of visual input in the sleep group

by adding a control group that was blindfolded while awake. Both studies show that sleep, but not interference-free wakefulness, improved procedural memory consolidation.

To establish an interference-free control for a declarative memory task is not as straightforward as blindfolding subjects and letting them rest comfortably. Deliberate rehearsal can occur even in the absence of any external stimulation and leads to improved memory performance. Random cogitation, on the other hand, can constitute interference. An interference-free wake control for a declarative task must therefore involve not only suppression of all external sensory stimuli but also of internal stimuli. To control external stimulation, subjects can be seated in a dark and quiet room while their state of consciousness is monitored by electroencephalography (EEG). An efficient way to control cognition is by focusing attention on one single thought, limiting self-generated information processing to a minimum. This kind of concentration is strongly developed in people who practice meditation. Meditators have learned to focus their thoughts for a very long time[19], thus reducing cognitive interference from internal stimuli most effectively and constituting a suitable low-interference control. A third topic of ongoing discussion is the question whether the strength of sleep-dependent memory consolidation is mediated by the amount of time spent in a specific sleep stage, i.e. by the "macrostructure" of sleep[9,20-22]. For example, declarative memory has been proposed to benefit mainly from slow-wave sleep (SWS)[1]. On the other hand, the amount of time spent in sleep stage 2 has been found to predict declarative memory consolidation[23]. Other studies focus on sleep "microstructure," i.e. sleep-related mechanisms that could mediate the effect on declarative memory consolidation. Sleep spindles, brief bursts of synchronous neuronal firing in the frequency range of 12-16 Hz, have been shown to favor synaptic plasticity[24]. It has also been shown that previous learning experience modulates spindle activity during sleep[25] and that sleep spindle activity correlates with the strengths of the observed benefits on memory consolidation or memory performance[26,27,23,10,28]. Recently, sleep spindles have also been found to be a pacemaker for memory reactivation[29]. Furthermore, several studies show the functional importance of slow wave activity for sleep-dependent declarative memory consolidation[30,31]. Even though several models regarding effects of different sleep stages on memory consolidation have been proposed[7,32], we agree with a recent paper concluding that "no model for the sleep-

stage dependencies of memory processing has been found to adequately explain the existing data", and that results of correlational studies currently remain inconclusive[33].

We tried to systematically address these open questions regarding the nature of the declarative sleep effect in a series of experiments. We tested whether differences in learning material can explain previous inconsistent findings. First, we tested a broad range of declarative memory tasks, varying in type of material and recall mode. Second, we investigated whether sleep holds an active role in declarative memory consolidation by comparing an active wake condition, a reduced-interference wake condition and a sleep condition. Third, we studied the influence of sleep macrostructure and microstructure by comparing sleep with varying amounts of time spent in different sleep stages and analyzing the relationships between sleep parameters and memory consolidation.

MATERIAL AND METHODS

TEST MATERIAL

A standard diagnostic test battery was used to test effects of sleep on different types of declarative memory ("Lern- und Gedächtnistest", LGT-3[34]). The LGT-3 is a speeded memory test commonly applied in German performance diagnostics. The LGT-3 measures long-term memory performance over a broad range of declarative material and is constructed as a parallel test with two equally difficult versions (A and B), allowing within-subject testing of performance after the retention interval in sleep as compared with wakefulness. For Experiment 3, which tested three conditions within each subject, a third parallel version C was created. Versions were counterbalanced between experimental conditions. Equal level of difficulty was confirmed in a pre-test sample of ten subjects. Each parallel version consists of six subtests: "city map," "vocabulary," "objects," "phone numbers," "story details" and "signs." These subtests are always presented in a fixed, standardized order during learning and recall. *The city map:* remembering a path between two points on a map of a complex maze within one minute. For retrieval, the path has to be re-drawn on the map from memory within two minutes. *Vocabulary:* learning 20 Turkish-German word pairs within one minute. For retrieval, the German words, along with five Turkish words to choose from, are presented within four minutes. *Remembering*

objects: remembering 20 simple objects within one minute, free recall in two minutes. *Phone numbers:* association of 13 3-digit numbers with names within two minutes, recall of the numbers cued with the names within two minutes. *Details of a story:* reading a story within one minute, answer 14 questions concerning numbers and names in the text within four minutes. *Signs:* learning abstract and concrete drawings inside different frames within one minute. For retrieval, the drawings and four frames to choose from are given within four minutes. We followed all procedures as described in the manual of the LGT-3, except for the length of the retention interval, which was adapted to fit our sleep and wake intervals. Performance in the LGT-3 is measured as number of correct items in all subtests, as defined in the test manual. Raw scores for all subtests is transformed to T-scores (mean: 50, standard deviation: 10) according to standard norms.

The subtests were subsumed into mutually exclusive scales, classified as verbal (vocabulary, story details) and nonverbal (city map, signs), single item learning (objects) and item association learning (vocabulary, phone numbers, signs), recall (city map, objects, phone numbers, story details) and recognition (vocabulary, signs), as well as cued recall (phone numbers, city map) and free recall (objects, story details). Additionally, a total score is computed according to the LGT-3 test manual[34], consisting of the sum of T-transformed scores of all six subtests. This score serves as a general measure of declarative memory performance. Classification into mutually exclusive scales followed description of demands on memory operations given in the test manual. Only subtests that belonged unambiguously to one category were included in a scale.

GENERAL DESIGN AND PROCEDURE

Sixty-one paid volunteers recruited from the Ludwig-Maximilians-Universität and different Buddhism centers in Munich participated in three experiments. They were healthy, 18-55 years old, non-smokers and had no Turkish skills. They did not ingest any medication, alcohol or caffeine on the days of the experiments. All participants reported sleeping between 6 and 10 hours per night, had a regular circadian rhythm, and were not extreme morning or evening types, as confirmed by the Munich Chronotype Questionnaire (MCTQ)[35]. They had no shift work or long-distance flights within the last six weeks before the experiment, and experienced no sleep-related pathology.

In all experiments, subjects learned a battery of declarative memory tasks (LGT-3)[34] before a period of sleep or wakefulness. After this period, the learned material was tested. No immediate test was given after learning, because the LGT-3 does not permit more than one testing per parallel test version. Experiment 1 compared afternoon periods of sleep (with a higher proportion of SWS) and wakefulness. Experiment 2 compared morning periods of sleep (with a higher proportion of REM sleep) and wakefulness. In Experiment 3, highly trained meditators participated in a sleep condition, an active wake condition during which they were involved in discussion with the experimenter, and an interference-reduced quiet meditation condition. Sleep opportunity in all experiments was two hours. Subjects showing less than 20 min of sleep or not reaching S2 were excluded from analysis. All experiments used within subject comparisons. To facilitate daytime sleep, participants were asked to rise one hour earlier than usual the two mornings before all conditions. Sleep logs were used to confirm that participants kept to the administered sleep schedule and to control the sleep history. Subjects had the same amount of sleep in the nights preceding the different experimental conditions (sleep 6 h 23 min ± 1 h 7 min [mean ± SD], wake 6 h 34 min ± 1 h 1 min, meditation 6 h 47 min ± 1 h 2 min; all $p > 0.31$ for pairwise comparisons within experiments and in combined data), and there were no differences in average sleep times during the week preceding the experimental sessions (sleep 7 h 07 min ± 54 min, wake 7 h 11 min ± 46 min, meditation 6 h 54 min ± 1 h 11 min, all $p > 0.22$ for pairwise comparisons within experiments and in combined data). Our experimental design employed short daytime sleep windows as an additional sleep opportunity to exclude confounding effects of sleep loss on memory recall performance. Daytime naps have been found to have no quantitative effect on levels of fatigue and alertness, as measured by the psychomotor vigilance task (PVT)[36,37]. This makes daytime sleep an ideal condition to compare consolidation effects on memory. In our experiments, we controlled alertness using the standard 10-min version of the PVT[36]. The PVT was administered after the memory test session, following sleep, wakefulness, and meditation. There were no significant differences between conditions in the most sensitive measure number of lapses (reaction times > 500 msec)[38] nor in median response times (all $p > 0.33$). The minimum time between two experimental conditions was one week in order to avoid interference between sessions. The order of conditions and the order of parallel LGT-3 test versions were counterbalanced in all three experiments. In addition, we confirmed

that overall test performance did not differ between the first and second test session in all three groups (all $p > 0.35$ for total score differences; see Supplementary Table 1 for more detailed information regarding all scales).

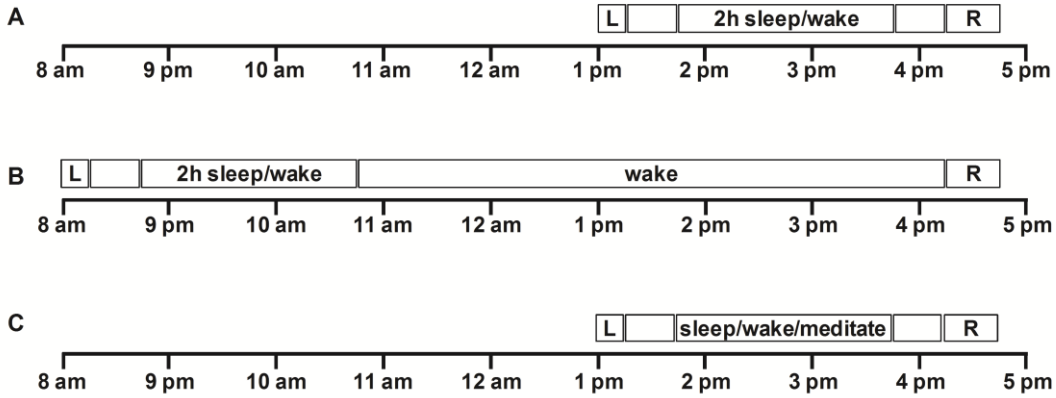

Figure 1. Experimental design. We compared the effect of sleep on memory consolidation in three different experiments. The first (A) and second (B) experiment tested sleep at different times of day and with different delay between learning and testing. Subjects slept either in the afternoon (A, C) or in the morning (B). In the first and third (C) experiment, subjects were tested after 3 hours, whereas there was an 8-hour retention interval in the second experiment (B). The third experiment (C) compared an additional interference-reduced wake control with sleep and active wakefulness, where participants had to meditate for two hours, directing their focus of attention on a single object, such as their breath or a mantra. L: Learning, R: Recall. Figure reprinted with permission.

Experiment 1. A sample of 20 students (10 male), aged between 18 and 30 years (21 ± 3 years [mean ± SD]), participated in the experiment. One participant was excluded because she did not fall asleep during the sleep condition. For one participant, not all memory scores could be calculated because of a missing subscale. EEG data was missing for two participants, because of technical problems during the measurements. Participants arrived at the sleep lab at 1 p.m. After learning the LGT-3 memory test battery (15 min) and application of EEG electrodes (30 min), subjects either had the opportunity to sleep for two hours or had to stay awake until testing. At 4:15 p.m., memory retention was tested (30 min; see Figure 1A). In the wake condition, participants played easy, non-verbal board games with the experimenter.

Experiment 2. A sample of 20 students (7 male), aged between 18 and 30 years (24 ± 4 years), participated in the experiment. Two participants were excluded, because they did not fall asleep during the sleep condition. Participants arrived at the sleep lab at 8 a.m. After learning the LGT-3 memory test battery (15 min) and application

of EEG electrodes (30 min), subjects either had the opportunity to sleep for two hours or had to stay awake until testing. Subjects slept in the laboratory, but were allowed to leave for the remaining time until memory retention was tested at 4:15 p.m. (see Figure 1B). During the time spent outside the sleep lab, activity was monitored by actimetry.

Experiment 3. 17 highly trained meditators (14 male), aged between 24 and 55 years (40 ± 10 years), with an average meditation experience between 2 and 28 years (10 ± 8 years), participated in the experiment. Participants were required to have at least two years of meditation experience with daily meditation practice to be included in the study. Two subjects were excluded because they slept less than 20 min in the sleep condition, and one subject was excluded, because he fell asleep during meditation. In one subject not all memory scores could be calculated because of a missing subscale, and one subject had to be excluded because of exceptionally low learning performance in all conditions, leaving a total of 12 subjects.

Participants arrived at the sleep lab in the afternoon between 12 noon and 5 p.m., at the same time for all three study conditions. After learning the LGT-3 memory test battery (15 min) and application of EEG electrodes (30 min), subjects were assigned to one of three conditions. To keep procedures in all three conditions identical, EEG electrodes were applied also in the active wake condition, where they were not required. Afterwards, in the sleep condition, subjects had the opportunity to sleep for two hours. In the active wake condition, they discussed various topics with the experimenter while sitting in a busy street café. In the interference-reduced wake condition, they meditated for two hours in a quiet room. During meditation, participants directed their focus of attention on a single object such as their breath or a mantra. Four hours after learning, memory retention was tested (see Figure 1C). Meditation was monitored with EEG.

STATISTICAL ANALYSIS

Because retrieval performance differed to some extent between the three experiments due to different length of the retention interval and different age groups, all LGT-3 scales were standardized to z-scores (mean: 0, standard deviation: 1) separately for the experimental groups. Thus, effects of conditions and scales are made comparable between experiments. In addition, this transformation allows

results of all three experiments to be pooled and analyzed as one large sample. Note that all experiments employed within-subject comparisons. Thus, differences between experimental conditions (sleep, wake, meditation) are preserved by this transformation. T-tests for dependent measures and repeated-measures ANOVAs with a two-sided significance threshold of 0.05 were used to compare performance between conditions. Results are given as mean ± standard error of mean (SEM). Correction for multiple testing was done using the Holm-Bonferroni method, which adjusts α according to a stepwise procedure that no longer corrects for the tests that survived a stricter Bonferroni correction in previous steps. Holm-Bonferroni adjusts the corrected significance threshold α to n − (k − 1), where n is the total number of tests performed and k is the rank of the test among all tests ordered from lowest to highest p-value. Tests are considered significant according to the chosen threshold α as long as $p < \alpha / (n - (k - 1))$.

EEG RECORDING AND ANALYSIS

In all experiments, sleep was recorded polysomnographically in the sleep lab. EEG (C3 and C4, according to the international 10-20 system) against a nose reference, and bipolar EOG and EMG were recorded. Data was recorded and scored offline by two independent raters according to the Rechtschaffen and Kales standard criteria[39]. Discrepancies in scorings were decided by a third rater. Meditation was monitored with the same setup to ascertain that no sleep occurred in this condition.

For spectral analysis, EEG data was segmented into one-second epochs and artifacts were rejected using a semi-automatic process that detects muscle activity, signal jumps and bad channels. Only artifact-free data was entered into analysis. Log transformed band power was calculated in the frequency range of slow oscillations (0-1Hz), delta waves (1-4 Hz) and sleep spindles (12-16 Hz). Sleep spindles were determined and counted by a previously published algorithm[25]. The individual peak frequency was detected in the spindle frequency range (12-16 Hz) and EEG data was bandpass-filtered in the individual spindle range for each subject (peak frequency ± 1.5 Hz). Spindles were defined as a root-mean-square signal that stayed above an individual, SD-based threshold (1.5 SDs above average power) for 0.5 to 3 seconds, with at least 0.5 seconds between events. Both number of spindles and spindle activity [duration×amplitude] were calculated.

RESULTS

BEHAVIORAL DATA

In all three experiments, we tested whether sleep benefits the consolidation of different kinds of declarative memory equally. In Experiment 1, subjects learned in the afternoon and were tested after a 3-hour retention interval during which they took a 2-hour nap in the sleep condition. They performed significantly better after sleep than after wakefulness on a number of scales (for inferential statistics, see Table 1A).

Table 1A. Mean z-differences (sleep – wake) for scales and single subtests

	Experiment 1			*Experiment 2*			*All three experiments*		
Scale	Mean ± SEM	t_{17}	p	Mean ± SEM	t_{17}	p	Mean ± SEM	t_{47}	p
Verbal	0.23 ± 0.18	1.58	0.23	0.33 ± 0.15	2.15	0.05	0.38 ± 0.11	3.62	0.001
Nonverbal	0.46 ± 0.20	2.24	0.04	-0.09 ± 0.21	-0.42	0.68	0.20 ± 0.13	1.57	0.12
Single item	0.15 ± 0.23	0.65	0.53	0.64 ± 0.21	3.06	0.006	0.48 ± 0.13	3.30	0.002
Associated items	0.43 ± 0.14	3.06	0.007	0.25 ± 0.16	1.55	0.14	0.35 ± 0.09	3.47	0.001
Recall	0.22 ± 0.14	1.57	0.14	0.37 ± 0.13	2.80	0.01	0.32 ± 0.08	3.84	< 0.001
Recognition	0.48 ± 0.17	2.75	0.01	0.10 ± 0.14	0.70	0.49	0.33 ± 0.11	2.99	0.004
Cued recall	0.31 ± 0.18	1.71	0.11	0.25 ± 0.24	1.04	0.31	0.38 ± 0.12	3.30	0.002
Free recall	0.13 ± 0.21	0.62	0.55	0.49 ± 0.17	2.89	0.01	0.26 ± 0.12	2.00	0.05
Test									
City map	0.30 ± 0.31	0.96	0.35	-0.05 ± 0.27	-0.20	0.85	0.18 ± 0.18	1.03	0.31
Vocabulary	0.34 ± 0.20	1.75	0.10	0.32 ± 0.15	2.20	0.04	0.43 ± 0.12	3.58	0.001
Objects	0.15 ± 0.23	0.65	0.53	0.64 ± 0.21	3.06	0.007	0.43 ± 0.13	3.30	0.002
Phone numbers	0.33 ± 0.22	1.46	0.16	0.55 ± 0.32	1.73	0.10	0.33 ± 0.16	2.00	0.05
Story details	0.12 ± 0.27	0.42	0.68	034 ± 0.22	1.53	0.15	0.33 ± 0.15	2.26	0.03
Signs	0.62 ± 0.26	2.39	0.03	-0.13 ± 0.5	-0.52	0.61	0.22 ± 0.16	1.36	0.18
Total score	0.47 ± 0.18	2.58	0.02	0.41 ± 0.17	2.37	0.03	0.49 ± 0.11	4.29	< 0.001

Significance threshold according to Holm–Bonferroni method correcting for all tests in display at α_{corr} = 0.01.

The benefit from sleep as measured by sleep-wake differences was significant for nonverbal memory, for memory for associated items, and when recognition was tested. Sleep did not yield a significant benefit for verbal memory, for memory for single items, or when recall was tested, regardless of whether cued recall or free recall was tested. In addition, overall performance as measured by the sum score of the test battery was significantly better in the sleep than in the wake condition (see Fig. 2A). However, it is important to note that our data does not show that sleep preferentially affects specific types of declarative memory. Interaction analyses tested whether in pairs of mutually exclusive task dimensions (verbal vs. nonverbal [ve–nv], single item vs. associated items [si–as], recall vs. recognition [rc–rg], cued

recall vs. free recall [cr–fr]) one type of memory benefitted more from sleep than the other type. All interactions remained non-significant (ve–nv × s–w: $F_{1,17}$ = 0.73, p = 0.41; si–as × s–w: $F_{1,17}$ = 0.86, p = 0.37; rc–rg × s–w: $F_{1,17}$ = 1.24, p = 0.28; cr–fr × s–w: $F_{1,17}$ =0.42, p = 0.53; Table 1A). In the above analyses, the subtests of the LGT-3 were subsumed into scales according to type of memory. When looking at single subtests, subjects performed significantly better in the sleep than in the wake condition in the signs test (see Table 1A).

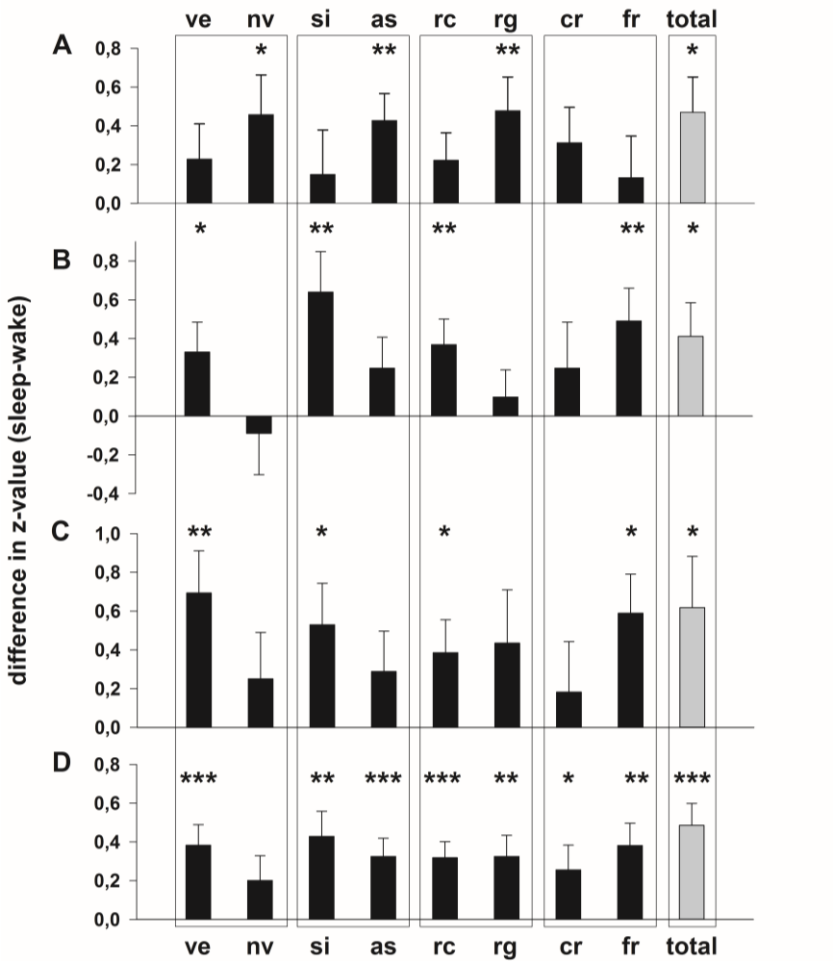

Figure 2. Differences in memory scores between the sleep and wake condition for the verbal (ve), the nonverbal (nv), the single item (si), the associate (as), for recall (rc), recognition (rg), cued recall (cr) and free recall scale (fr) for the sleep and wake condition for (A) the first experiment (n = 18), (B) the second experiment (n = 18), (C) and the third experiment (n = 12) separately, (D) and for all data combined (n = 48). *: p = 0.05, **: p = 0.01, ***: p = 0.001. Figure reprinted with permission.

In Experiment 2, subjects learned in the morning, then took a 2-hour morning nap in the sleep condition and stayed awake until they were tested eight hours after encoding. They again performed significantly better after sleep than after wakefulness on a number of scales (see Table 1A). Significant sleep-wake differences were found for verbal memory, for memory for single items, and when recall was tested, in particular when a free recall procedure was used. Sleep did not yield a significant benefit for nonverbal memory, for memory for associated items, when recognition was tested, or when cued recall was tested. In addition, overall performance as measured by the sum score of the test battery was significantly better in the sleep condition (see Fig. 2B). Again, it is important to note that we find no sign that sleep affects one form of memory more than another. For all mutually exclusive dimensions, interactions with state (sleep/wake) remained non-significant (ve–nv × s–w: $F_{1,17} = 2.00$, $p = 0.17$; si–as × s–w: $F_{1,17} = 2.22$, $p = 0.16$; rc–rg × s–w: $F_{1,17} = 2.51$, $p = 0.13$; cr–fr × s–w: $F_{1,17} = 0.58$, $p = 0.46$; Table 1A). Again, in the above analyses, the subtests of the LGT-3 were subsumed into scales. Looking at single subtests, subjects performed significantly better in the sleep than in the wake condition in the vocabulary and objects tests (see Table 1A).

In Experiment 3, three conditions (sleep/active wake/meditation) were compared. Procedures corresponded to those of experiment 1. Subjects learned in the afternoon and slept or meditated for two hours in the respective conditions during the 3-hour retention interval. Conditions differed on a number of scales; and post-hoc tests confirmed that subjects were significantly better in the sleep condition than in the two wake conditions (see Table 1B). We found a significant main effect of condition for verbal memory, for memory for single items, and when free recall was tested. Post-hoc t-tests confirmed that subjects performed significantly better in the sleep (s) condition than in the wake (w) or meditation (m) conditions for verbal memory (s–w: $t_{11} = 3.17$, $p = 0.01$, s–m: $t_{11} = 2.79$, $p = 0.02$), for memory for single items (s–w: $t_{11} = 2.48$, $p = 0.03$, s–m: $t_{11} = 2.35$, $p = 0.04$), and when free recall was tested (s–w: $t_{11} = 2.93$, $p = 0.01$, s–m: $t_{11} = 2.89$, $p = 0.02$). There were no significant main effects for nonverbal memory, for memory for associated items, when recognition was tested, when recall in general was tested, or when a cued recall procedure was used. In addition, overall performance as measured by the sum score of the test battery showed a significant main effect. Post-hoc t-tests confirmed that subjects performed significantly better in the sleep condition than in the wake or

meditation conditions (s–w: $t_{11} = 2.34$, $p = 0.04$, s–m: $t_{11} = 3.45$, $p = 0.01$; see Figs. 2C and 3).

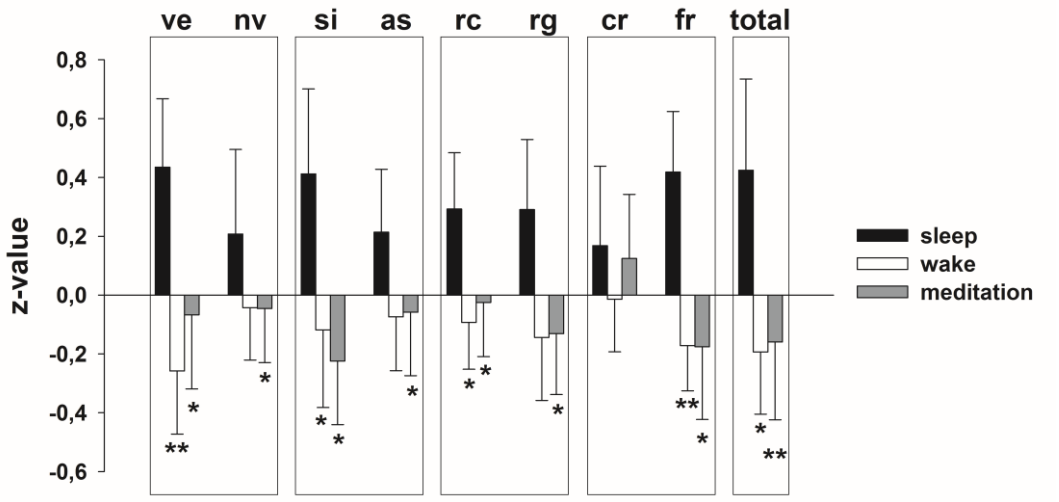

Figure 3. Memory scores for the sleep and wake and meditation condition in the third experiment for the verbal (ve), the nonverbal (nv), the single item (si), the associate (as), for recall (rc), recognition (rg), cued recall (cr) and free recall scale (fr). N = 12. Asterisks mark significant difference to the sleep condition. *: p = 0.05, **: p = 0.01. Figure reprinted with permission.

As in Experiments 1 and 2, interactions between state (sleep/wake) and test dimension showed no sign that sleep differentially affects different forms of memory (ve–nv × s–w–m: $F_{2,22} = 1.05$, $p = 0.37$; si–as × s–w–m: $F_{2,22} = 0.80$, $p = 0.46$; rc–rg × s–w–m: $F_{2,22} = 0.05$, $p = 0.95$; cr–fr × s–w–m: $F_{2,22} = 1.76$, $p = 0.20$). To test whether interference-reduced wakefulness differed from active wakefulness, post-hoc tests were calculated also between the meditation and wake conditions. These remained non-significant for all dimensions (ve: $t_{11} = 0.70$, $p = 0.50$, nv: $t_{11} = 0.01$, $p = 0.99$, si: $t_{11} = 0.37$, $p = 0.72$, as: $t_{11} = 0.08$, $p = 0.94$, rc: $t_{11} = 0.31$, $p = 0.76$, rg: $t_{11} = 0.05$, $p = 0.96$, cr: $t_{11} = 0.53$, $p = 0.61$, fr: $t_{11} = 0.02$, $p = 0.99$; Table 1B). There was also no difference in performance on the total score between the wake and meditation condition ($t_{11} = 0.13$, $p = 0.90$). Again, in the above analyses, the subtests of the LGT-3 were subsumed into scales. Looking at single subtests, subjects performed significantly better in the sleep than in the wake condition in the vocabulary and objects tests (see Table 1B).

Table 1B. Mean z-values for scales and single subtests in experiment 3

Scale	*Sleep* (mean ± SEM)	*Wake* (mean ± SEM)	*Meditation* (mean ± SEM)	$F_{2,22}$	p
Verbal	0.44 ± 0.23	-0.21 ± 0.21	-0.07 ± 0.25	4.98	0.02
Nonverbal	0.21 ± 0.29	-0.04 ± 0.18	-0.05 ± 0.18	0.77	0.47
Single item	0.41 ± 0.29	-0.12 ± 0.26	-0.22 ± 0.22	3.49	0.05
Associated items	0.21 ± 0.21	-0.07 ± 0.18	-0.06 ± 0.22	1.56	0.23
Recall	0.29 ± 0.19	-0.09 ± 0.16	-0.03 ± 0.18	2.72	0.09
Recognition	0.29 ± 0.24	-0.14 ± 0.21	-0.13 ± 0.21	1.92	0.17
Cued recall	0.17 ± 0.27	0.01 ± 0.18	0.13 ± 0.22	0.33	0.28
Free recall	0.42 ± 0.21	-0.17 ± 0.15	-0.18 ± 0.25	4.35	0.03
Test					
City map	0.27 ± 0.29	-0.09 ± 0.29	0.16 ± 0.30	0.62	0.55
Vocabulary	0.06 ± 0.32	0.06 ± 0.27	0.09 ± 0.34	3.52	0.05
Objects	0.41 ± 0.29	-0.12 ± 0.26	-0.22 ± 0.22	3.49	0.05
Phone numbers	0.06 ± 0.32	0.06 ± 0.27	0.09 ± 0.34	< 0.01	> 0.99
Story details	0.43 ± 0.26	-0.22 ± 0.26	-0.13 ± 0.34	2.75	0.09
Signs	0.14 ± 0.34	0.00 ± 0.28	-0.25 ± 0.30	0.58	0.57
Total score	0.43 ± 0.31	-0.19 ± 0.21	-0.16 ± 0.26	4.40	0.03

Finally, we pooled data from all 48 subjects in a combined analysis of all three experiments. A multivariate ANOVA over all scales revealed no interaction between experiment and condition ($F_{16,78} = 0.77$, $p = 0.71$, all univariate comparisons $p > 0.17$), whereas there was a highly significant main effect of condition (sleep vs wake) over all scales ($F_{8,38} = 3.7$, $p = 0.003$). This confirms that differences in experimental procedures (time of day, subjects' age etc.) did not significantly influence the effect of sleep. Furthermore, individual ANOVAs confirmed that sleep benefited memory performance significantly for all but one subscale (see Table 1A).

Significant sleep-wake differences were found for verbal memory, for memory for single items, for memory for associated items, when memory recall was tested, when either a cued recall procedure, or a free recall procedure was used, and also when memory recognition was tested. Sleep did not yield a significant benefit for nonverbal memory tests, although a numerically higher value was found in the sleep condition. Finally, overall performance as measured by the sum score of the test battery was significantly better in the sleep condition (see Fig. 2D). Again, also in this larger sample with higher statistical power, we found no indication that sleep affects some forms of memory more than others. There was no significant interaction between state (sleep/wake) and any of the mutually exclusive dimensions tested (ve–nv × s–w: $F_{1,47} = 1.17$, $p = 0.29$; si–as × s–w: $F_{1,47} = 0.38$, $p = 0.54$; rc–rg × s–w: $F_{1,47} < 0.01$, $p = 96$; cr–fr × s–w: $F_{1,47} < 0.01$, $p = 0.94$; Table 1A). Effect sizes (Cohen's d) for the scales range between 0.3 and 0.5, translating to small

or medium sized effects (ve: d = 0.47, nv: d = 0.26, si: d = 0.44, as: d = 0.46, rc: d = 0.52, rg: d = 0.42, cr: d = 0.34, fr: d = 0.48, total score: d = 0.51).

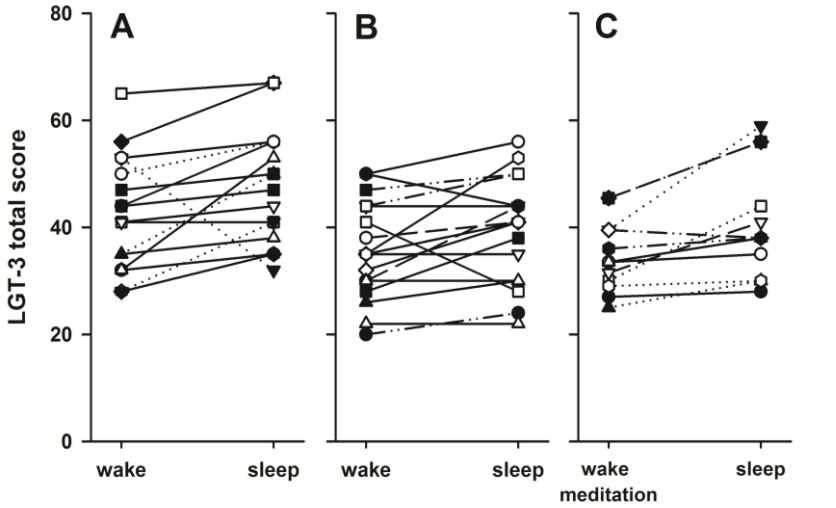

Figure 4. Performance of individual subjects on the LGT-3 total score in all three experiments. Note that this figure shows raw scores instead of z-transformed scores for individual subjects. Almost all subjects show better performance in the sleep compared with the wake condition (A and B) or the average of active wake and meditation conditions (C). (A) In experiment one, only one of 18 subjects was better after an interval spent waking. (B) In the second experiment two out of 18 subjects performed slightly worse in the sleep condition. (C) In experiment three, again only one subject in 12 does not show a beneficial effect of sleep. Subjects performed better on the test when there were only 3 hours between learning and testing (A) than when encoding and testing were spaced 8 hours apart (B). The older subject pool (C), on average, performs not as good as the younger subjects who had the same delay between learning and testing (A). Still, we observe a significant benefit of sleep in all three populations, indicating that initial performance does not have an impact on whether sleep boosts memory consolidation. Dashed lines: subjects who did not show SWS, dotted lines: subjects who did not have REM sleep during the nap, dash-dotted lines: subjects who neither had SWS nor REM sleep, solid lines: subjects who had both SWS and REM sleep during the sleep interval. Figure reprinted with permission.

Looking at single subtests, without subsuming them into scales, subjects performed significantly better in the sleep than in the wake condition in the tests vocabulary, objects, phone numbers, and story details. Sleep did not yield a significant benefit for the city map and signs tests (see Table 1A). Although significant differences do not emerge for the same memory scales and individual subtests in the three experiments, the overall sum test score was significant for all three experiments. To further demonstrate the stability of this effect, Figure 4 shows that, on an individual level, most subjects show equal or better performance in the sleep than the wake conditions.

EEG DATA

As intended in the experimental design, the amount of time spent in different sleep stages differed significantly between Experiments 1, 2 and 3. Whereas afternoon sleep in Experiment 1 had a higher proportion of SWS, morning sleep in Experiment 2 showed more REM sleep. Subjects in Experiment 3 had generally less deep sleep and fewer sleep spindles, as can be expected from the older subject population (Table 2). Because the difference between sleep and wakefulness was most robust for the sum scores of the test battery, we correlated this measure with the amount of time spent in different sleep stages.

Table 2. Sleep parameters in the different experiments

	Experiment 1 *Afternoon nap N=16*	***Experiment 2*** *Morning nap N=18*	***Experiment 3*** *Afternoon nap N=12*	***Experiment 1 vs. experiment 2***		***Experiment 1 vs. experiment 3***		***Experiment 2 vs. experiment 3***	
	mean ± SEM	mean ± SEM	mean ± SEM	t_{32}	p	t_{26}	p	t_{28}	p
S1	11.0 ± 2.4 min	15.0 ± 1.7 min	13.0 ± 2.9 min	-1.36	0.18	-0.53	0.60	0.64	0.53
S2	50.0 ± 4.3 min	53.7 ± 4.0 min	43.1 ± 6.5 min	-0.63	0.54	0.92	0.36	1.48	0.15
SWS	26.3 ± 3.7 min	9.5 ± 2.7 min	10.5 ± 2.9 min	3.69	0.001	3.15	0.004	-0.25	0.80
REM	6.3 ± 2.1 min	11.4 ± 1.9 min	4.5 ± 3.0 min	-1.74	0.09	0.51	0.61	2.01	0.05
Total sleep time	93.7 ± 4.6 min	89.6 ± 6.1 min	71.1 ± 9.7 min	0.53	0.60	2.29	0.05[1]	1.69	0.10
NREM spindle count	333 ± 21	292 ± 32	197 ± 32	1.05	0.30	3.74	0.001	2.01	0.05
Percentage of subjects without SWS	0%	33%	33%	U=96 z=-2.5	0.01[2]	U=64 z=-2.4	0.01[2]	U=108 z= 0.0	1.00[2]
Percentage of subjects without REM sleep	38%	17%	67%	U=114 z=-1.4	0.18[2]	U=76 z=-1.1	0.28[2]	U=63 z=-2.3	0.02[2]

[1] value for comparing groups with unequal variances, [2] Mann-Whitney-U Test

No significant correlations between sleep macrostructure (time spent in sleep stages 1, 2, SWS and REM) and the sum score of the test battery were found (see Table 3). The amount of time spent in specific sleep stages did not correlate with any measure of memory performance (for 144 correlations of eight sleep parameters and nine scales, both with the difference between sleep and wake and with performance in the sleep condition alone, all $p > 0.08$). In addition, we also investigated whether subjects who did not have SWS or REM sleep still showed sleep-related improvements. The individual traces in Figure 4 show that the effect of sleep is not limited to those subjects that show all sleep stages. In fact, Chi-squared tests confirm that the distribution of subjects showing sleep-related improvement is equal among subjects with and without SWS/REM sleep (SWS: $X^2 = 0.55$, $p = 0.46$; REM sleep: $X^2 = 0.70$, $p = 0.40$).

Table 3. Correlations between sleep parameters and performance on the LGT total score

		LGT sleep		*LGT wake*		*Benefit of sleep*	
		r_{46}	p	r_{46}	p	r_{46}	p
Macrostructure[1,3]	S1	-0.13	0.37	-0.32	0.03	0.18	0.22
	S2	0.03	0.87	0.02	0.92	0.02	0.92
	SWS	0.01	0.95	0.01	0.96	0.003	0.98
	REM sleep	-0.12	0.43	0.02	0.91	-0.17	0.25
	Total sleep time	-0.06	0.68	-0.09	0.57	0.01	0.92
Microstructure[2,3]	NREM spindle activity (12-16Hz)	0.11	0.46	0.38	0.009	-0.28	0.06
	NREM slow oscillations (<1Hz)	0.01	0.93	-0.03	0.86	0.05	0.75
	NREM delta power (1-4Hz)	-0.06	0.72	-0.03	0.85	-0.04	0.80

[1] *Significance threshold according to Holm–Bonferroni method for groups of independent tests at* $\alpha_{corr} = 0.006$
[2] *Significance threshold according to Holm–Bonferroni method for groups of independent tests at* $\alpha_{corr} = 0.01$
[3] *Significance threshold according to Holm–Bonferroni method for all tests in table at* $\alpha_{corr} = 0.002$

To investigate the influence of sleep microstructure on memory consolidation, we correlated spindle activity and EEG band power in the slow oscillation and delta wave range with the overall sum score of our test battery. We found a correlation between spindle activity in stage 2 and the total score of the LGT-3. This correlation, however, was higher and only reached significance with performance in the wake condition (sleep: $r_{46} = 0.11$, $p = 0.46$, wake: $r_{46} = 0.38$, $p = 0.009$; see Figure 5 and Table 3). Sleep-related improvement (difference sleep-wake), on the other hand, showed a marginally significant negative correlation with spindle activity ($r_{46} = -0.28$, $p = 0.06$). Thus, higher spindle power during sleep seems to be associated with general performance in the LGT-3 (trait), but not the memory benefit related to sleep. Band power in the range of slow oscillations and in the delta frequency range did not predict performance after sleep, wakefulness, or the benefit achieved by consolidation in sleep (all $p > 0.71$, see Table 3).

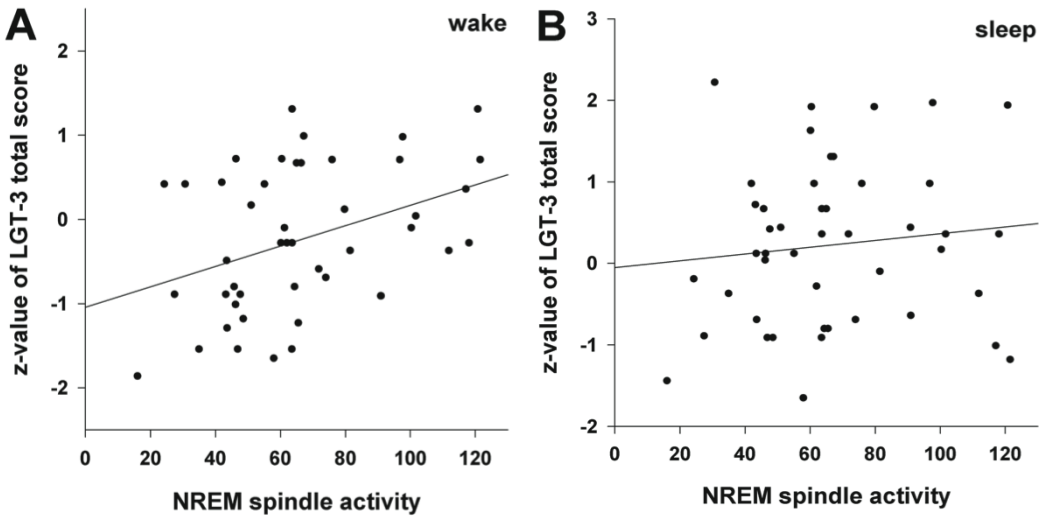

Figure 5. Correlation of EEG sleep spindle activity with LGT-3 performance. (A) Spindle activity measured in the sleep condition shows a significant positive correlation with performance on the LGT-3 in the wake condition (r_{46} = 0.38, p = 0.009). (B) Sleep spindle activity shows a weaker association with performance after sleep, with this correlation remaining non-significant (r_{46} = 0.11, p = 0.46). Thus, present data indicate that spindle activity is rather a trait than a state marker of memory performance. Figure reprinted with permission.

DISCUSSION

In three different experiments, we tried to address some of the unanswered questions regarding the effect of sleep on declarative memory. Although the first experiments on this topic were performed 100 years ago[2], it is still not known whether the effect seen occurs for all types of declarative memory tasks. It has also been debated for a long time whether sleep actively contributes to consolidation or whether it simply protects memory from interference. Finally, it has been proposed that specific sleep stages ("macrostructure") or features ("microstructure") are of importance for memory consolidation, yet, no definite relations have emerged thus far. We used a comprehensive test battery to test whether sleep has a differential effect on various kinds of declarative memory. The test battery contained scales with verbal and nonverbal material, associative and non-associative items, and different types of recall. We found that sleep consistently promotes declarative memory consolidation over the whole range of tasks, if an adequate sample size is used. A combined measure of different declarative memory scales showed consistent sleep effects also in smaller samples. This enhancing effect is restricted to sleep, and does not occur in quiet wakefulness. There is no difference between an

active wakefulness condition, in which subjects were in a busy environment and actively discussed different topics with an experimenter, and a condition in which subjects reduced external and internal interference by focusing their attention on a single thought in meditation. Thus, it is reasonable to assume that the underlying process is sleep-specific, active in nature, and not passively mediated by reduced interference. This beneficial effect of sleep seems not to be related to the time spent in a certain sleep stage, nor by sleep microstructure.

DIFFERENT TYPES OF MEMORY AND SLEEP

We find a positive effect of sleep on declarative memory for a variety of different memory tasks, independent of the material used and by which method it was retrieved. Although not all scales were significant in all three samples, pooling all 48 subjects reveals significant effects in nearly all tests. We conclude from this observation that the extent and variability of the effect of sleep is not large enough to be consistently detected in sample sizes of less than 20 subjects, which are habitually used in sleep research. In particular, experiments showing conditions with no effect must be interpreted with great caution, especially if no significant sleep × condition interaction is present. Our results show that the standardized effect size is between 0.3 and 0.5, which translates to small to medium effects according to Cohen[40]. To detect an effect of this size with a statistical power of at least 0.5, (i.e. with the ability to detect a significant effect at least in every second experiment,) a sample size between 20 and 50 per condition is required. This estimate is considerably smaller than that of other studies, which rely on smaller sample sizes. It might be partly accounted for by our experimental design. For example, we use naps, which probably produce smaller effects than full nights of sleep, although some nap studies also report larger effect sizes[41]. However, a systematic investigation of the size of the sleep effect on memory has not been done, and any estimate of effect size, which does not consider the publication bias against null results, will result in overestimation of effects. If we want to systematically investigate the relation between sleep and memory, and not only focus on the odd and rare findings with exceptionally large effects, sample sizes have to increase considerably.

Our results also suggest that it can be of advantage to combine a number of different memory scales when trying to measure sleep-related consolidation. No subscale was

significant in all three experiments. However, the total test score always reached significance. It should therefore be considered to use a broader spectrum of tests particularly when the effects of sleep in more realistic settings are to be studied and a precise differentiation between memory tasks is of lesser importance. Effects of interference among multiple tests during encoding and their consequences for subsequent memory consolidation, however, are not well characterized and should be addressed in future studies.

We tested whether material specific differences in sleep-related memory consolidation exist, and found none. This means that the processes in sleep occur fairly generally for declarative memory. There might still be differences between specific types of memory, even though we did not find them in our experiments. We find similar effects on verbal as well as on nonverbal memory, and on single item memory as well as on associated items. In the literature, effects have been reported on emotional as well as on neutral material[42-46]. Wordlist learning of both related[47,7,10,48,49] and unrelated word pairs[50,9,51,6] has been shown to benefit from sleep. Yet, there are some indications that only specific material benefits from sleep[6,23]. When combining data across experiments, all tested scales reached significance except for non-verbal memory. The interaction effect between verbal and non-verbal memory, however, was not statistically significant. In the literature, reports of significant effects of sleep on non-verbal memory are rare, maybe because it is less frequently tested, maybe because effect sizes are smaller. Many authors find effects of sleep only on consolidation of emotional pictures[43,52], but only few find the same effect for emotionally neutral pictures[44]. In view of the above considerations on statistical power, and taking into account the compiled literature, it is probably too early for final conclusions on how specific types of declarative memory are affected by sleep.

The mode of memory retrieval was, in the present experiments, not relevant for whether sleep had an effect on memory performance. This is in line with a number of studies showing sleep effects with free recall[41,53], cued recall[54,9,51], or recognition[20,55,56]. However, there are several studies indicating that only the recollection and not the familiarity aspect of recognition memory is enhanced by sleep[45,15,57]. This would speak for a particular influence of sleep on hippocampal memory because only recollection, but not familiarity, is supposed to depend on the

hippocampus proper[58]. Our finding is not in conflict with these studies because our test battery was not designed to separate familiarity and recollection.

Because the LGT-3 tests various types of declarative memory, we cannot exclude interference between tasks. However, although subtests were always presented in the same order during learning, we did not observe preferential consolidation of any of the studied subtests. This is consistent with the finding that sleep after learning removes effects of retroactive interference[8]. Additionally, because interference between tasks was identical over conditions, our reported findings cannot be attributed to effects of task interference.

AN ACTIVE OR PASSIVE ROLE OF SLEEP

Interference can disturb previously learned memories. Because of thalamic gating, interference during sleep is reduced to a minimum. Thus, the question arises whether sleep produces its beneficial effect passively by preventing interference or actively by strengthening new memory traces[59,60]. Early work by Ekstrand and colleagues examined the effect of interference on memory, but did not come to a final decision whether interference or other processes mediate the sleep effect[4]. Still today, active and passive views of sleep are discussed[61,62,1], although evidence from many directions points to an active role of sleep. Animal and human studies show reactivation of neural activity patterns in the hippocampus and neocortex during sleep that can be related to previous learning[63-66]. External reactivation by presenting memory cues during sleep can selectively enhance the consolidation of individual memories[67] and also accelerates the consolidation process[68]. Other studies tested whether interference before or after the consolidation interval interacted with the effect of sleep. Ellenbogen et al. tested whether interference leads to similar memory deterioration after a period of sleep and after a period of wakefulness[50]. Their results show that sleep makes the memory trace less susceptible to interference. This can be taken as evidence of an active consolidation process during sleep. Drosopoulos et al. showed that sleep also rescues memory from retroactive interference that occurred directly after learning[8]. This, again, speaks for an active role of sleep.

The present study concerned itself with the question whether reduced interference during the retention interval contributes to the beneficial effects of sleep on

declarative memory. Reduced interference during wakefulness did not improve memory consolidation. Potentially, interference may still have occurred during the 30-min electrode application period following learning. However, this experimental procedure was the same for all the conditions, including sleep and active wakefulness. Hence, any interference during this period cannot account for our findings.

Reducing interference during the retention period has been shown not to interact with the effect of sleep on procedural memory[17,18]. The current experiments did not find a difference between an active and a quiet wake condition for declarative memory either. This is also remarkable because subjects, being expert meditators, were admittedly motivated to show maximal performance in the meditation condition. If reduced interference, increased motivation or potential rehearsal had any influence on performance, the meditation condition should show improvement over the active wake condition, which was not the case. Therefore, in addition to providing an interference-free period, sleep must have additional properties that support memory consolidation. These could be related to the switching between neuromodulatory states, which happens when the organism falls asleep, or they could be linked to electrophysiological characteristics of sleep, like sleep spindles and delta waves. Although we could not find any correlations, such effects have been reported in other studies[56,69-71,33]. The exclusivity of some consolidation processes to sleep is also supported by the fact that external reactivation only benefits memory consolidation when subjects sleep, not when they were awake[72,73]. Sleep thus seems to hold a special and active role in the consolidation of declarative memory, which may not be explained by lack of interference during the consolidation interval.

MACRO VS. MICROSTRUCTURE OF SLEEP

A third point of debate is whether specific sleep stages (sleep macrostructure) contribute particularly to memory consolidation, or whether specifics in sleep physiology (sleep microstructure) are at the base of consolidation[74]. It has often been suggested that the amount of time spent in a particular sleep stage mediates the positive effect of sleep on memory formation[75]. However, no consensus on this point has been reached, yet[33]. In the present study, although drawing on a large sample with 46 subjects and sufficient variance in sleep and in memory performance, no correlation between sleep structure and sleep-related memory

improvement has been found. Apart from the missing correlation, another argument against an influence of sleep macrostructure on memory consolidation is that the amount of time spent in different sleep stages in our three experiments varied because morning and afternoon naps were investigated, which showed higher amounts of REM sleep and SWS, respectively. However, no consistent pattern emerged that could relate time spent in different sleep stages with specific types of memory, and even subjects without SWS or REM sleep show sleep-related improvements.

Sleep spindles and slow-waves, which are the neural mechanisms associated most often with memory consolidation, also show no correlation with sleep-related memory benefits in our data. Literature suggests that sleep spindles can be markers of trait-like and state-like performance indices[74]. We therefore correlated spindle activity with performance in the wake condition (trait) and with performance increase in the sleep condition (state). The only significant relation between sleep microstructure and performance is a positive correlation between spindle activity and memory performance in the wake condition. Thus, spindle activity during sleep seems to be a marker of a trait-like feature. The correlation we report does not survive a very conservative Bonferroni correction, which accounts for all tests performed on all correlations, and should thus be treated with caution. Yet, it remains significant when using the more common correction only for the independent comparisons of each group of tests and is the strongest association of sleep parameters and performance in this large dataset. Our finding is in line with a number of studies that show an association between spindle activity and general cognitive abilities[76-79]. It must, however, be noted that our experiments do not exclude causal relationships between sleep microstructure and memory processing that extend beyond simple quantitative association[25]. In fact, we believe that consolidation processes are rather related to changes in neurotransmitter activity and memory trace-specific reactivation, both of which are probably not directly reflected in global measures of sleep.

CONCLUSIONS

First, we show that sleep consistently benefits the consolidation of declarative memory. We do not find that only certain types of declarative memory benefit from sleep, but that a broad range of tests are promoted by sleep, if tested in a sufficiently large sample. In addition, a broader coverage of different memory tasks can also reduce the risk of type II errors and help detect the small to medium sized effects of sleep that are usually reported. Second, consolidation was not improved in a reduced-interference wake control group. Thus, the effect of sleep on memory consolidation is active in nature and not merely caused by a lack of interference. Finally, the beneficial effect of sleep we found in all three experiments was not mediated by time spent in certain sleep stages nor by the amount of activity in a specific EEG band during sleep. Therefore, we believe that it is the intricate interplay of sleep-related physiological processes that allows memory consolidation to be particularly strong during sleep.

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SUPPLEMENT

Table 1. Effects of session order for all scales and total score. Order of conditions was balanced over sessions in all experiments separately. Note that performance in the second session was not always superior to that of the first session.

Scale	*Experiment 1*			*Experiment 2*			*Experiment 3*				
	Day1 - Day2 Mean ± SEM	t_{17}	p	Day1 - Day2 Mean ± SEM	t_{17}	p	Day 1 Mean ± SEM	Day 2 Mean ± SEM	Day 3 Mean ± SEM	$F_{2,22}$	p
Verbal	-0.38 ± 0.17	-2.27	0.04	-0.27 ± 0.16	-1.67	0.11	-0.15 ± 0.22	0.13 ± 0.23	0.13 ± 0.29	0.76	0.48
Nonverbal	0.01 ± 0.23	0.03	0.97	0.32 ± 0.20	1.61	0.13	0.06 ± 0.20	-0.03 ± 0.29	0.08 ± 0.17	0.12	0.89
Single item	-0.21 ± 0.23	-0.95	0.36	-0.16 ± 0.26	-0.62	0.54	0.12 ± 0.25	0.09 ± 0.35	-0.14 ± 0.18	0.48	0.63
Associated items	0.22 ± 0.17	1.36	0.19	0.41 ± 0.14	2.91	0.01	0.03 ± 0.20	0.07 ± 0.24	-0.01 ± 0.18	0.08	0.92
Recall	-0.10 ± 0.15	-0.69	0.50	0.12 ± 0.16	0.78	0.45	-0.02 ± 0.21	0.21 ± 0.18	-0.02 ± 0.16	0.94	0.41
Recognition	0.04 ± 0.21	0.17	0.87	0.17 ± 0.14	1.22	0.24	0.02 ± 0.23	-0.09 ± 0.26	0.08 ± 0.19	0.19	0.83
Cued recall	0.60 ± 0.21	3.25	0.005	0.88 ± 0.27	3.26	0.005	0.04 ± 0.32	0.37 ± 0.34	-0.20 ± 0.24	1.56	0.23
Free recall	-0.34 ± 0.18	-1.85	0.08	-0.13 ± 0.13	-0.98	0.34	-0.04 ± 0.19	0.15 ± 0.20	0.04 ± 0.16	0.43	0.65
Total score	-0.05 ± 0.21	-0.23	0.82	0.41 ± 0.17	0.96	0.35	-0.03 ± 0.23	0.08 ± 0.34	0.02 ± 0.25	0.08	0.92

Significance threshold according to Holm–Bonferroni method for groups of independent tests at $\alpha_{corr} = 0.006$
Significance threshold according to Holm–Bonferroni method for all tests in table at $\alpha_{corr} = 0.002$

STUDY 2:

EVIDENCE FOR TWO DISTINCT SLEEP-RELATED LONG-TERM MEMORY CONSOLIDATION PROCESSES

Monika Schönauer, Melanie Grätsch, & Steffen Gais

MS, MG, and SG planned and designed the experiments. MS and MG collected the data. MS, MG, and SG analyzed the data. MS and SG wrote the manuscript.

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ABSTRACT

Numerous studies examine the effect of a night's sleep on memory consolidation, but few go beyond this short time-scale to test long-lasting effects of sleep on memory. We investigated long-term effects of sleep on typical memory tasks. During the hours following learning, participants slept or stayed awake. We compared recall performance between wake and sleep conditions after delays of up to 6 days. Performance develops in two distinct ways. Word pair, syllable, and motor sequence learning tasks benefit from sleep during the first day after encoding, when compared with daytime or nighttime wakefulness. However, performance in the wake conditions recovers after another night of sleep, so that we observe no lasting effect of sleep. Sleep deprivation before recall does not impair performance. Thus, fatigue cannot adequately explain the lack of long-term effects. We suggest that the hippocampus might serve as a buffer during the retention interval, and consolidation occurs during delayed sleep. In contrast, a non-hippocampal mirror-tracing task benefits significantly from sleep, even when tested after a 4-day delay including recovery sleep. This indicates a dissociation between two sleep-related consolidation mechanisms, which could rely on distinct neuronal processes.

INTRODUCTION

Sleep represents an important part of daily life. Whereas early theories of sleep function emphasized mainly recuperation and energy conservation, more recently, its role in cognitive performance has come into focus. Astonishingly, only few aspects of cognition have proven to be consistently affected by sleep, the most prominent of which are probably sustained attention and memory. Sustained attention is impaired by lack of sleep (Killgore, 2010); memory performance is enhanced by sleep (Diekelmann & Born, 2010). Systems memory consolidation is one mechanism by which sleep can support memory formation. By reactivation and consequent strengthening, neuronal traces of newly learned memories are thought to be integrated into existing memory networks and made more durable (Rasch et al., 2007; Stickgold & Walker, 2013). Reactivation is supposed to originate in the hippocampus. Hippocampal reactivation then leads reactivation in neocortical or striatal areas (Ji & Wilson, 2007; Lansink et al., 2009). This mechanism can therefore be assumed to underlie mainly hippocampus-dependent memory (Inostroza & Born, 2013). However, it has been proposed to also mediate consolidation of some procedural tasks with hippocampal contributions, possibly linked to explicit aspects of these tasks (Cohen et al., 2005; Geyer et al., 2013; Robertson et al., 2004; Schönauer et al., 2014; Walker et al., 2005). Reactivation of learning-related neural activity during sleep can be observed not only in the hippocampus, but in many of the regions involved in learning (Maquet et al., 2000). Whether consolidation in all memory systems relies on the same neuronal processes is still unclear.

When considering typical experimental designs used to study the effects of sleep on declarative memory, large gaps in our knowledge become apparent. Mostly, participants have to learn some kind of material before a period of sleep or wakefulness, and they are asked to retrieve this material afterwards. The duration of the retention interval usually lies between 1 and 24 hours. Often, retention periods filled with sleep are directly compared with periods filled with wakefulness. While appropriate for many research questions, some central positions cannot be analyzed using this experimental design. First, it is difficult to distinguish between effects of sleep on consolidation of previously learned memory and effects of sleep on following memory retrieval: memory retrieval may be impaired because of fatigue after a night of sleep deprivation. Confounds include effects of prior sleep on

following memory encoding and circadian factors when comparing morning-evening vs. evening-morning settings. Finally, because many studies use short retention intervals, only little is known about long-term effects of sleep on memory. Examining long-term effects of sleep on memory can give a more comprehensive view of the extended consolidation process and its neuronal dynamics. It can thus shed further light on the specific mechanisms that mediate consolidation in different memory systems.

As mentioned above, most studies on declarative memory test performance within the first 24 h after learning. Only occasionally, experimental designs include recovery sleep, mainly with the intention to avoid effects of acute fatigue in designs using sleep deprivation. Just a few studies systematically explore longer retention intervals after sleep deprivation, and most of these are quite old (Rasch & Born, 2013). The longest interval tested for non-emotional declarative memory – six days between learning and recall – was investigated by Graves (1937). She tested whether learning in the evening (sleeping after learning) or learning in the morning (staying awake after learning) influenced retention after 24, 48, 72, 96 or 144 h. She used nonsense syllables as learning material and the savings method as performance measure, i.e. the reduction in the number of relearning repetitions required for perfect list reproduction. Graves found a long-range effect of sleep on syllable recall developing after 72 h, but none before that. Apart from being only a single-participant study – testing the author herself – and not using a standardized method of presentation, this study confounds circadian effects with effects of sleep. The finding was replicated by another study, which used a very similar study design and the same task, but employed a larger group of participants and better-controlled experimental conditions (Richardson & Gough, 1963). These authors also find a similar delay in the onset of effects. They find no difference between the sleep and wake conditions after 24 and 48 h, but only after 144 h. These results stand in contrast to a large body of recent literature which stresses immediate effects of sleep on memory performance (Diekelmann & Born, 2010).

Apart from these older findings, some more recent studies examined memory performance following consolidation in sleep or wakefulness after 2- or 3- day intervals. Gais et al. (2007) saw a significant sleep effect on word-pair learning after a 44-h retention interval comprising two nights of sleep or one night of sleep

deprivation and one night of recovery sleep. In a comparable study design, Gais et al. (2006) found a positive effect of sleep on foreign language vocabulary after a 48-h interval containing two undisturbed nights of sleep or one night of sleep deprivation and one night of recovery sleep. However, no significant effect of sleep vs. sleep deprivation on behavioral performance was found after 3 days for spatial memory in a virtual maze task (Orban et al., 2006). Sterpenich et al. (2007) tested recognition in a remember/know paradigm and found a significant positive effect of sleep on recollection of neutral and emotionally positive images when comparing three nights of sleep with one night of sleep deprivation and two recovery nights. In the same study, emotionally negative material did not show long-term benefits of sleep. Smith (1995) briefly reports of a study that did not find effects of sleep deprivation after learning on word recognition and figure reproduction one week later.

Regarding retention intervals longer than a few days, evidence is exceptionally scarce. There are several fMRI studies that assessed performance after 6-months delays, demonstrating clear differences in recall-related brain activity, but finding no significant differences in performance between participants who slept or were sleep deprived after learning (Gais et al., 2007; Rauchs et al., 2008; Sterpenich et al., 2009). Only one study reports that three hours of sleep after learning dramatically increase recognition memory for emotional texts in an unannounced test four years after the original experiments (Wagner et al., 2006). In the same experiment, non-emotional texts did not benefit from sleep.

In the domain of non-declarative memory, effects induced by one night of sleep deprivation can be long lasting: participants will not benefit from practicing a visual discrimination task if they are sleep deprived for one single night after learning the task, even if performance is measured after several recovery nights. The benefit of sleep, on the other hand, persists even after a week (Stickgold et al., 2000). Similarly, a motor adaptation task shows sleep-induced improvements three days after a night of sleep or sleep deprivation (Maquet et al., 2003). Also, Smith (1995) reports that memory in a number of procedural tasks was impaired one week after REM sleep deprivation. Together, there is some evidence for a long-lasting effect of sleep on procedural and emotional memory, whereas findings for neutral declarative memory are mixed. The number of studies investigating long-term effects of sleep is

very small considering the total number of publications on this topic, and results remain inconclusive as to the existence of long-lasting effects of sleep.

In two separate studies, we investigate whether sleep after learning, compared with sleep deprivation or day-wake periods of equal length, shows a lasting beneficial effect on memory performance. We tested retention intervals of up to six days. The first experiment tested verbal word pair learning and a procedural mirror-tracing task. We seek to answer three questions. First, is there an effect of sleep after learning that is it still detectable after four nights? We know from previous studies using the same material that an effect should be observed after one night. Only little data is available on long-term effects, however. Results on declarative memory are inconsistent when going beyond a timescale of two days from learning. Given findings on tasks with strong procedural components (Maquet et al., 2003; Smith, 1995; Stickgold et al., 2000), we expect clear long-term effects for mirror-tracing performance. Second, because previous experiments often tested directly after sleep or wakefulness, some effects of sleep can be interpreted either as effects of sleep on memory consolidation or as effects of sleep deprivation on following memory recall. Therefore, we introduce an additional condition that controls for effects of sleep deprivation before memory recall. Third, we used semantically related word pairs as well as unrelated word pairs as learning material because in previous studies, it remained open which type of material preferably benefits from sleep. While in an early study by Plihal and Born (1997) and a number of experiments following the same experimental procedures related word pairs benefitted from reactivation in sleep, a newer study by Payne et al. (2012) shows that unrelated, but not related word pairs are remembered better over sleep than wakefulness. The second experiment was based on an older study that described an effect of sleep that not only persisted but increased over time (Richardson & Gough, 1963). We used the same nonsense syllable material and procedure as that study, testing different time intervals to investigate how effects develop. Furthermore, we tested performance on a finger sequence tapping task. Additionally, because Richardson & Gough (1963) used a night-sleep/day-wake design and did not control for circadian effects, we added a night-wake control condition.

EXPERIMENT 1

METHODS

GENERAL PROCEDURE

21 healthy, young participants (aged 24.3 ± 2.7 years [mean ± s.d.]) participated in three experimental conditions. They were non-smokers, regular sleepers, and did not take any regular medication except contraceptives. They were not allowed to use caffeine 12 h before and during the experiment. Each condition consisted of one learning and two testing sessions. In the first session of each condition, which took place in the evening, 2 h before bedtime, participants learned word lists and practiced a mirror-tracing task (Plihal & Born, 1997). During the following night, they either slept or stayed awake, depending on the experimental condition. In the morning after this first night, at 7 a.m., memory was tested. For the next two nights, participants slept normally. During the fourth night, participants again slept or stayed awake and were tested in the following morning. Together, over the three conditions, participants were once sleep deprived after learning, once sleep deprived before testing, and once allowed to sleep during all four nights (see Figure 1). All participants gave informed consent before participating in the study.

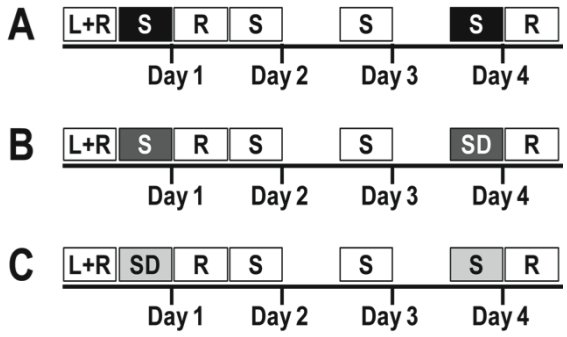

Figure 1. Design of Experiment 1. Subjects participated in three experimental conditions. Each condition consisted of one learning session in the evening including initial performance assessment (L+R), and two testing sessions (R) in the mornings of day 1 and day 4. Participants were allowed to sleep (S) during all four nights in condition A, were sleep deprived (SD) before delayed testing on day 4 in condition B, or after learning on day 1 in condition C. Figure reprinted with permission.

TASKS

The first task was a paired-associate word list learning task that consisted of 4 lists of 20 pairs. Two lists contained semantically related pairs (e.g. breakfast – crumb, insect – horsefly), the other two contained unrelated pairs (e.g. volcano – gravy, fork – flower). Participants were presented with 4 pages of 5 pairs for 15 seconds each. After initial stimulus presentation, we showed the first word of each pair in random order, and they had to name the matching word aloud. If less than 70% of correct answers were given on one of the four lists, presentation of this list was repeated until that threshold was reached. This procedure ensured that the same learning criterion was reached for all lists. Participants needed on average 1.1 ± 0.2 repetitions for related pairs and 1.8 ± 0.5 repetitions for unrelated pairs ($p<0.001$). Recall was tested twice, once after the first night, once after the fourth night. Each time, we tested half of the material, i.e., one related and one unrelated list.

We additionally tested procedural memory using a mirror-tracing task. Here, participants had to trace a figure while seeing their hand, the stylus and the figure only in a mirror. Tracing speed, number of times the stylus moved off the figure (errors) and time the stylus stayed outside the figures (error time) were measured electronically. To avoid fast within-session adaptation, participants first had to practice the task on a simple star figure until they could complete this figure in less than 60 s and with less than 9 errors. Then they had to trace one of the three actual figures as fast as possible. In each condition, a different figure was used. The figures differed in the preferential direction and shape of angles. Participants had to stay within the boundaries of the lines, which were 1 cm wide. As it is common practice in numerous studies, we tested two tasks in each experiment (Ellenbogen et al., 2006; Plihal & Born, 1997; Smith, 1995). According to Brown & Robertson (2007), it is possible that the second task interferes with the consolidation of the first for subjects in a wake condition. This can enlarge the effect of sleep compared to wakefulness, similar to what has been found for interference within declarative memory (Ellenbogen et al., 2006).

In a third task, long-term memory access was tested. Here, participants had to name as many female first names and male first names starting with a certain letter. Letters were selected to have similar frequency in lists of German first names (E, S, L

for female names, B, J, M for male names). There was no speed component, but participants were told to finish within about 5 min.

SLEEP

Participants slept at home. Sleep duration was recorded by the participant with a sleep log and confirmed by actimetric recordings. In nights during which participants had to stay awake, participants stayed in the laboratory under the supervision of the experimenter and played board games. Participants were allowed to leave the lab after 8 a.m. in the morning to follow their usual daily activity. Daytime naps were not allowed and activity was monitored using actimetry.

STATISTICAL ANALYSES

The main analysis is based on a mixed general linear model with the within-subject factors delay (1 day, 4 days) and condition (sleep on night 1, sleep deprivation on day 1, sleep deprivation before day 4 recall). Analysis was done in SPSS 21. Note that degrees of freedom can be decimals in mixed models analyses. All tests are based on a two-sided significance level of 0.05. All values are given as mean ± s.e.m.

RESULTS

DECLARATIVE MEMORY

We first asked whether sleep after learning affects retrieval of word pair memory 1 or 4 days afterwards. Whereas there was a significant difference in retention of word pairs on day 1 between sleep (S) and sleep deprivation (SD; $F_{1,34.0} = 16.8$, $p < 0.001$ for interaction pre/post × S/SD), we no longer observed this difference on day 4 after S or SD ($F_{1,34.7} = 0.65$, $p = 0.43$ for interaction pre/post × S/SD). The interaction between delay (day 1, day 4) and sleep (S, SD) is significant, confirming that measurements actually differ between time points and sleep affects early more than late long-term memory performance ($F_{1,37.6} = 32.9$, $p < 0.001$; see Figure 2). SD before recall has no significant effect on memory performance. If anything, participants in the SD condition were slightly better than in the other two conditions ($F_{1,32.0} = 2.4$, $p > 0.1$).

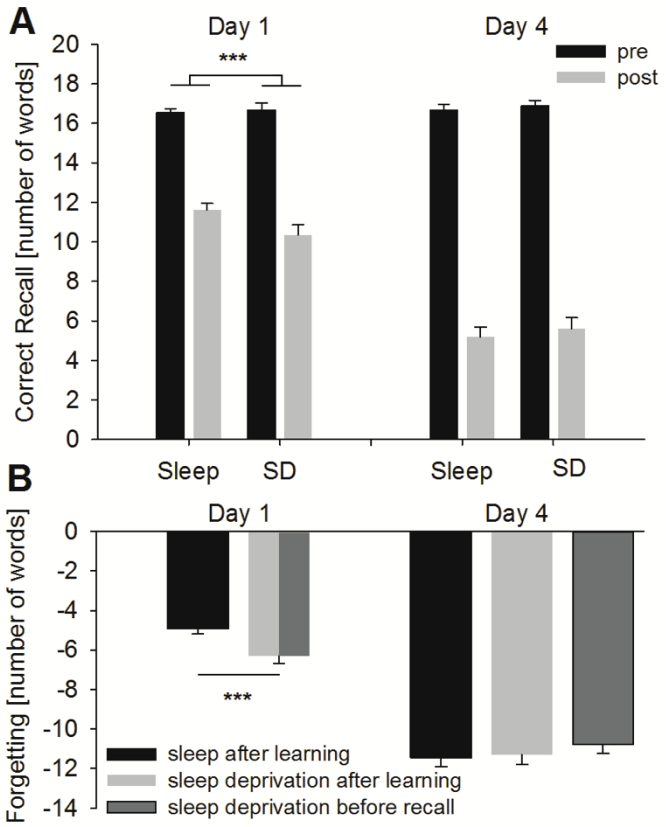

Figure 2. Memory performance for word pairs after retention intervals of 1 and 4 days. (A) Absolute values for word pair recall immediately after learning (pre) and after the retention interval (post). (B) Forgetting of word pairs over the retention intervals. A significant positive effect of sleep can only be seen after the first night. The observed effect does not seem to be attributable to fatigue induced by sleep deprivation because sleep deprivation during the night before recall on Day 4 has no effect on memory performance. Figure reprinted with permission.

Word pairs with a semantic relation are usually remembered more easily than word pairs with no such relation. To test whether sleep affects semantically related and semantically unrelated word pairs differently, we compared both types of material after S and SD in the 1-day and the 4-day retention conditions. On day 4, no significant interaction between S vs. SD deprivation and semantically related vs. unrelated word pairs was found ($F_{1,40.8} = 0.51$, $p = 0.48$), as could be expected from the missing effect of sleep per se. Interestingly however, on day 1, the benefit of semantic relation (difference in memory performance between semantically related and unrelated items) was completely absent after sleep deprivation. Numerically, unrelated pairs were even remembered better ($F_{1,33.4} = 2.2$, $p = 0.15$). A comparison of semantically related vs. unrelated word pairs between S and SD was significant on

day 1 ($F_{1,38.5} = 9.5$, $p = 0.004$; see Figure 3), showing that only semantically related word pairs gained from sleep.

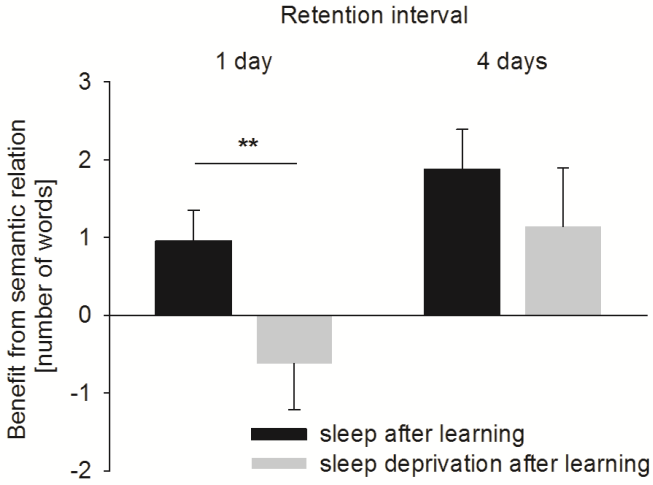

Figure 3. Semantically related word pairs benefit more from sleep than semantically unrelated words. This effect can only be seen on the first day after sleep deprivation, as no significant effect of sleep exists after four days. Benefit from semantic relation is calculated as the difference in memory performance between semantically related and unrelated items. Figure reprinted with permission.

An effect of sleep deprivation on word fluency was not found in the data. Production of names was similar after S and SD nights (S: 15.3 ± 0.9 names, SD: 16.1 ± 1.1, $F_{1,39.7} = 0.90$, $p = 0.35$).

PROCEDURAL MEMORY

In the procedural mirror-tracing task, results differed clearly from word pair memory. Here, significant effects were seen both on day 1 and on day 4. Consolidation, as measured by the reduction in the number of errors between learning and testing, was significantly stronger after S than SD (day 1: $F_{1,20} = 5.2$, $p = 0.03$; day 4: $F_{1,20} = 7.7$, $p = 0.01$; see Figure 4). Similarly, reduction in error time was significantly larger after S than SD (day 1: $F_{1,20} = 22.2$, $p < 0.001$; day 4: $F_{1,20} = 7.1$, $p = 0.01$). Improvement in tracing speed was also numerically higher after S than SD, but not significantly so (day 1: $F_{1,20} = 1.0$, $p = 0.33$; day 4: $F_{1,20} = 1.0$, $p = 0.33$). For all three measures, interactions between S/SD and day 1/day 4 were not significant (all $p > 0.46$). In this procedural task, however, SD before testing on day 4 has a small, non-significant detrimental effect (see Figure 4). Speed and error performance differ significantly neither from S nor from SD (all $p > 0.1$). Therefore, a

detrimental effect of fatigue on performance cannot be excluded in any experiment in which participants were sleep deprived directly before performance testing.

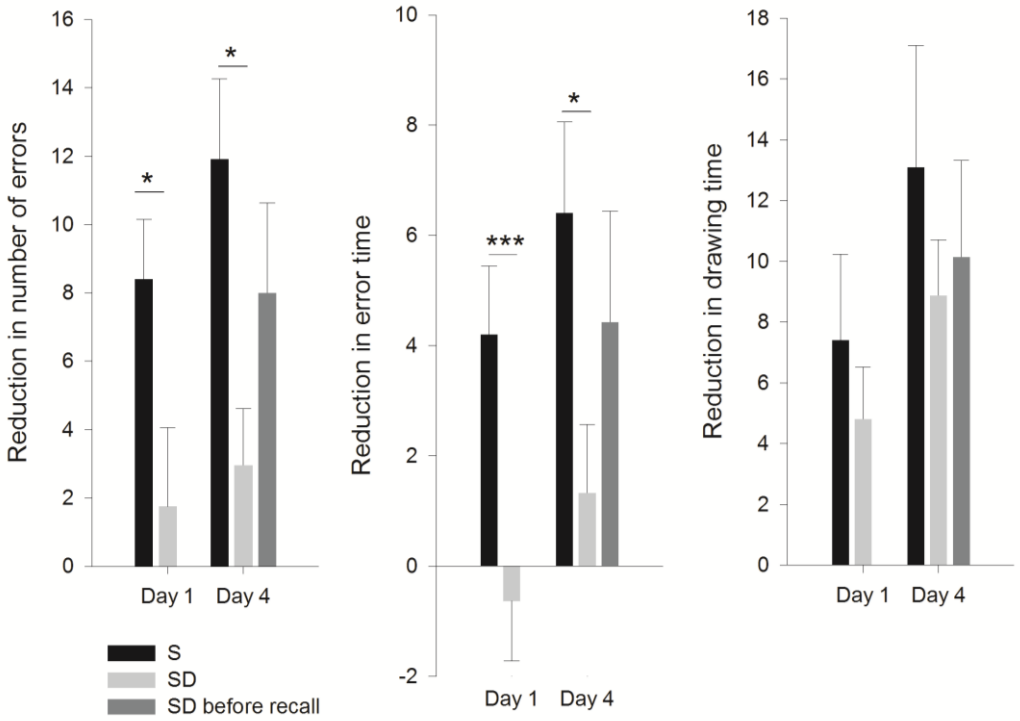

Figure 4. Results of the mirror-tracing task. Significant improvements from sleep after training can be seen both on Day 1 and on Day 4. Thus, the benefit from sleep seems to be persistent. Sleep deprivation before testing has no significant effects, although the values lie slightly below those of the sleep condition. Figure reprinted with permission.

SLEEP

Sleep during the four nights between learning and retesting was documented in sleep logs and verified by actimetry. Overall, participants slept 7 hours 40 min (± 7 min) during these nights.

EXPERIMENT 2

METHODS

GENERAL PROCEDURE

36 healthy, young native German speakers (aged 22.5 ± 3.6 years) were assigned to one of three experimental groups, each comprising of three experimental conditions. Each condition consisted of a learning session and a testing session. Depending on the condition, learning took place at 9 a.m. before a day of wakefulness (DW), at 9 p.m. before a night of sleep (NS), or at 9 p.m. before a night of sleep deprivation (NW). Testing took place 12 h, 72 h or 144 h after learning for the three groups, respectively (see Figure 5). Order of conditions was fully balanced across groups and subjects.

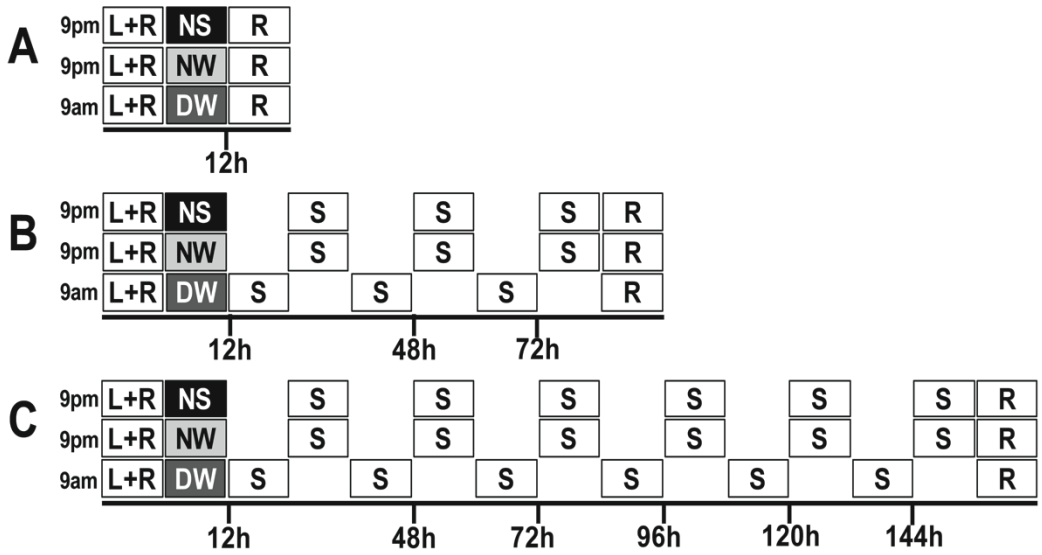

Figure 5. Design of Experiment 2. Subjects were assigned to three experimental groups (A, B, C). All subjects had to participate in three different experimental conditions, consisting of a learning session including initial performance assessment (L+R) and delayed testing (R). Depending on the condition, learning took place at 9 a.m. before a day of wakefulness (DW), at 9 p.m. before a night of sleep (NS), or at 9 p.m. before a night of sleep deprivation (NW). Group A was tested 12 h after learning in all three conditions, group B after 72 h, and group C after 144 h. Figure reprinted with permission.

Potential participants with sleep disorders were not admitted to the study. Only subjects were allowed to participate who had a regular sleep rhythm (e.g., no sleep pathologies, no crossing of time zone borders, no shift work, and no other sleep

restrictions). Participants were asked not to use caffeine or centrally active medication on the days of the experiments. All gave informed consent before participating in the study.

TASKS

First, participants learned a list of 10 meaningless consonant-vocal-consonant (CVC) syllables until they were able to reproduce the whole list without errors three times in a row (Richardson & Gough, 1963). Syllables were randomly chosen from a list of syllables that had been rated to be non-meaningful and of medium difficulty in a pre-test. All 10 syllables were presented in a fixed order one after another for 1.6 s each. Then, learning was tested in a typed free recall procedure. If list reproduction was not perfect, presentation started again from the beginning. The number of presentations until full recall marked initial performance. Later, during testing, participants were first asked to recall as many syllables as possible (free recall score). Additionally, a relearning score was obtained as the number of presentations required until the participant could again reproduce the list without errors three times in a row. A saving score was calculated as the percentage of trials saved during retesting compared with initial learning.

In addition, participants performed a simple sequential finger tapping task of five elements with the fingers of their non-dominant hand (Walker et al., 2002). Three sequences were assigned to the three conditions (4-2-3-1-4, 2-4-1-3-2, 2-3-1-4-2). The sequences were balanced across conditions and groups. Participants were instructed to follow the sequence as fast and as accurately as possible. The sequence was displayed as a string of numbers on a computer screen; position within the sequence was indicated by asterisks below. The number of correct sequences (speed) and the number of errors (accuracy) per 30 s was calculated. Learning consisted of 12 trials of 30 s. The last three trials were used to determine initial learning performance. Testing after sleep or wakefulness consisted of three trials of 30 s as well. After each trial, there was a pause of 30 s.

Verbal and non-verbal IQ was measured with the Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B) (Lehrl, 2005) and the Zahlen-Verbindungs-Test (ZVT) (Oswald & Roth, 1987), respectively, two German standard test batteries. There were no noticeable results (average verbal IQ in MWT: 109 ± 2; average numerical speed IQ in ZVT: 115 ± 2.8).

SLEEP

Participants filled out sleep logs during the five days prior to the experiments. During sleep deprivation nights, participants were playing games or watching non-arousing movies under constant supervision of an experimenter. For all periods between learning and testing during which the participant was not under direct supervision of the experimenter, a sleep log had to be kept, and activity was controlled by actimetry.

STATISTICAL ANALYSES

Analyses were done in SPSS 21, based on a mixed general linear model with one within-subject factor (condition: night sleep, night wake, day wake) and one between-subject factor (delay: 12 h, 72 h, 144 h). Note that degrees of freedom can be decimals in mixed models analyses. All tests are based on a two-sided significance level of $\alpha = 0.05$. All values are given as mean ± s.e.m.

RESULTS

DECLARATIVE MEMORY

First, we tested whether declarative memory recall benefitted from sleep after learning, and whether such an effect would be detectable for longer periods of time. We found a significant effect of condition (NS, NW, DW) on the number of syllables remembered in the 12-h group ($F_{1,10} = 8.7$, $p = 0.01$; see Figure 6). This effect was due to significantly enhanced memory recall after the short 12-h interval in the sleep condition compared with the day wake condition ($t_{10} = 2.9$, $p = 0.02$). Conditions did not differ in the 72-h and 144-h groups ($F_{1,11} = 0.6$, $p = 0.45$; and $F_{1,10.4} = 0.7$, $p = 0.40$, respectively). Similarly, a significant effect of condition was found on the number of trials required to re-learn the task (saving score) in the 12-h group ($F_{1,10} = 6.2$, $p = 0.03$), which was based on higher recall scores in the sleep condition compared with both the night wake ($t_{10} = 2.0$, $p = 0.07$) and day wake conditions ($t_{10} = 2.0$, $p = 0.08$). Again, no differences between conditions was found in the 72-h and 144-h groups ($F_{1,11} < 0.1$, $p = 0.96$; and $F_{1,11} = 0.4$, $p = 0.56$, respectively).

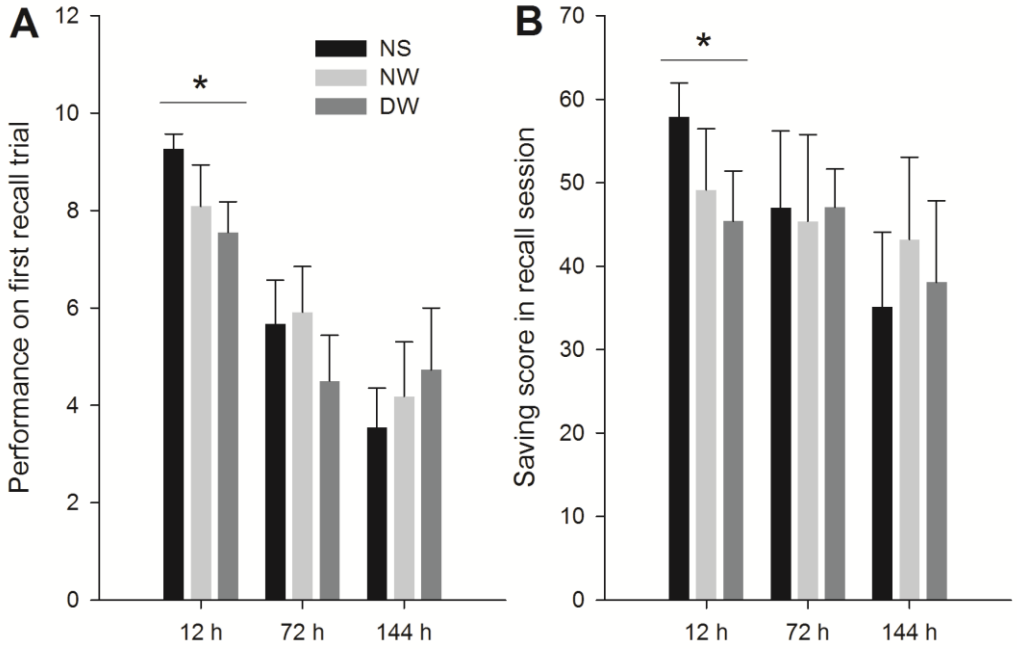

Figure 6. Retention of consonant-vocal-consonant syllables. (A) Recall performance on the first trial. (B) Reduction in number of trials needed to achieve perfect performance compared with initial learning. Similar to word pair learning, a significant positive effect of sleep can only be found on the first day after learning. Again, fatigue cannot explain differences between night sleep (NS) and wakefulness, because performance in the night wake condition (NW) does not differ from performance in the day wake condition (DW), although fatigue is much higher after a night of wakefulness. Figure reprinted with

PROCEDURAL MEMORY

Results of the finger tapping task showed a similar pattern as the syllable recall task. Again, we find a significant effect of sleep only in the 12-h group ($F_{2,10} = 5.7$, $p = 0.02$; see Figure 7). If sleep followed learning participants show a larger increase in the number of correctly typed sequences. In the 72-h and 144-h groups performance in the sleep condition is no longer superior to performance after staying awake ($F_{2,10} = 1.2$, $p = 0.33$; and $F_{2,4.9} = 0.3$, $p = 0.75$, respectively). This lack of effect can be attributed to a recovery of performance in the wake conditions, with a similarly large increase in the number of correctly typed sequences as the in the 12-h group sleep condition and both sleep conditions in the 72-h and 144-h groups, suggesting that participants catch up on the boost in performance caused by sleep following learning during the first recovery night.

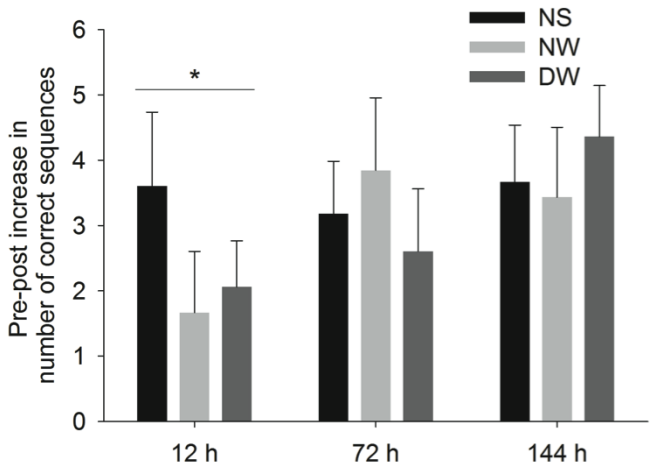

Figure 7. Performance in the finger tapping task shows the same pattern as the declarative memory tasks. A consolidation related increase is found after sleep on the first day, but this benefit of sleep disappears after further nights of recovery sleep. However, fatigue, which is higher after a night of wakefulness (NW) than after daytime wakefulness (DW), does not seem to explain reduced performance in the 12-h condition. Figure reprinted with permission.

Initial learning performance was identical in all groups and conditions: there was no interaction between conditions (NS, NW, DW) and delay group ($F_{4,33} = 0.5$, $p = 0.74$), and no main effect between delay groups ($F_{2,33} = 0.2$, $p = 0.80$). However, a difference in initial performance between conditions cannot be excluded: we find an inconclusive main effect of condition ($F_{2,33} = 2.1$, $p = 0.14$; see Table 1), and a borderline significant post hoc comparison between the night sleep and the day wake condition ($t_{33} = 2.0$, $p = 0.06$; $t_{33} = 1.4$, $p = 0.16$ for night wake vs. day wake). Thus, we cannot exclude circadian influences on initial motor learning, with better learning performance in the evening than in the morning.

Table 1. Detailed results of the finger-tapping task.
(S: night sleep, SD: night wake, W: day wake; mean ± s.e.m.)

		Number of correct sequences		
Duration		pre	post	difference
12 h	S	20.3 ± 1.3	23.9 ± 2.2	3.6 ± 1.1
	SD	20.6 ± 1.5	22.3 ± 2.2	1.7 ± 0.9
	W	19.2 ± 2.1	21.2 ± 2.4	2.1 ± 0.7
72 h	S	21.5 ± 1.2	24.6 ± 1.5	3.2 ± 0.8
	SD	20.9 ± 1.4	24.7 ± 2.2	3.8 ± 1.1
	W	19.2 ± 1.7	21.8 ± 2.3	2.6 ± 1.0
144 h	S	19.1 ± 1.7	22.8 ± 1.9	3.7 ± 0.9
	SD	17.6 ± 1.5	21.0 ± 2.1	3.4 ± 1.1
	W	18.4 ± 1.7	22.7 ± 2.3	4.4 ± 0.8

SLEEP

According to their sleep logs, participants slept 7h 51 min (± 9 min) on average during the night after learning, which was verified by actimetry. Sleep length did not differ between the delay groups ($F_{2,32} = 0.3$, $p = 0.74$). During the days before the experiment, participants slept on average 7h 41 min (± 6 min). Sleep durations before the experiment also did not differ between groups ($F_{2,33} = 0.6$, $p = 0.55$).

DISCUSSION

Because evidence for persistent effects of sleep on memory consolidation is scarce, we tested whether a period of sleep during retention intervals of different lengths improves memory performance at delayed testing compared with periods of wakefulness. For all tasks, we see significantly enhanced performance immediately after intervals of no more than 24 hours when comparing sleep with wakefulness. Contrary to our expectations, this difference remains significant after longer retention intervals only for the mirror-tracing task. Here, a positive effect of sleep can still be seen after three additional nights of sleep. No such effect was found for the declarative memory tasks or the finger sequence tapping task. For these tasks, the performance benefit seen immediately after periods of sleep is lost after subsequent nights.

We see a persistent long-term effect of sleep on the mirror-tracing skill. Even after three recovery nights, performance is better if participants were allowed to sleep after learning mirror-tracing than if they were sleep deprived. Therefore, we can conclude that there are sleep-dependent processes that have to occur during a specific time window after learning in order for memory enhancement to occur for this task. Actually, accuracy of mirror-tracing improved little or not at all if participants did not sleep after learning. On the behavioral level, this finding is similar to that of Stickgold et al. (2000), who reported an improvement in visual discrimination skill only when participants were allowed to sleep during the first night after training. Their study is particularly remarkable because it is one of the rare studies showing a process of memory consolidation that strongly requires sleep, i.e., it shows no improvement without sleep. In the present study, mirror-tracing skills improved during training, and this improvement remained stable between test sessions. However, only if participants slept after training, additional off-line improvements were seen. This is another similarity to the findings of

Stickgold et al. who found that visuo-motor skill training leads to an improvement only if it was followed by sleep. Whether the off-line improvements we observe in the mirror-tracing task depend on similar underlying mechanisms as sleep-dependent improvements of the visuo-motor skill remains open. It must be noted that visual discrimination and motor learning are skills which recruit very different neuroanatomical substrates. However, both tasks rely at least partially on neocortical plasticity (Inoue et al., 1997; Schwartz et al., 2002) and for both, no hippocampal contribution has been shown, yet. We therefore believe that it is possible that they rely on similar sleep-related synaptic consolidation mechanisms.

Although our results do not show long-term benefits of one night of sleep for all memory tasks, they do not exclude an essential role of sleep in memory consolidation in these tasks. Even in those tasks that do not show long-term effects, restoration of performance seen after sleep following initial wakefulness can be explained by active consolidation processes as well as by relief of fatigue. Intriguingly, all tasks, which did not show long-term effects, have been assumed to rely on a strong hippocampal contribution. This is obvious for word pair learning and nonsense syllables, but even the finger tapping task has been shown to activate the hippocampus during learning (Schendan et al., 2003; Walker et al., 2005). The role of the hippocampus in finger tapping could encompass explicit aspects of sequence learning (Devito & Eichenbaum, 2011), but recent research shows that it could also be related to implicit aspects of the task, with hippocampal activation present, even when the participants were completely unaware of the sequential structure of the task (Albouy et al., 2008; Gheysen et al., 2011; Rose et al., 2002; Schendan et al., 2003). This common contribution of the hippocampus can explain why finger tapping shows a similar time course of consolidation as the declarative memory tasks. A major function of the hippocampus is supposed to be the short-term buffering of new information (McClelland et al., 1995; Rolls & Treves, 1994). The model of complementary learning systems assumes that the hippocampus is a fast learning system, which acquires information more quickly, but at the same time also forgets more quickly (McClelland et al., 1995). It is likely that information is buffered until systems consolidation of new memories can occur, even over a prolonged period of wakefulness. Such a buffer would certainly make sense because unique and perhaps vital new memories should not be lost when encoding is followed by a lack of sleep. Therefore, effects of sleep loss can be compensated in

hippocampal-dependent tasks. Acquisition of procedural tasks without hippocampal contributions, on the other hand, is usually slow and relies on a large number of repetitions. Consolidation of this type of memory seems to benefit lastingly from sleep and suffers from prolonged periods of wakefulness after encoding.

The difference in behavior between the mirror-tracing task and the other, hippocampal-dependent tasks lends to the conclusion that there are qualitatively different sleep-related memory consolidation processes. Currently, there are no generally accepted theories that can explain how different memory tasks depend on different consolidation processes. There are, however, two models of how sleep can influence memory consolidation, one relying on processes of systems consolidation (Gais & Born, 2004), the other on mechanisms of synaptic consolidation (Tononi & Cirelli, 2003). Reactivation theory suggests that hippocampal learning activity is replayed during sleep, leading to an active strengthening of these traces and their integration into neocortical networks (Rasch & Born, 2007). External reactivation has been shown to boost memory consolidation in both declarative (Rasch et al., 2007; Rudoy et al., 2009) and finger sequence tasks (Schönauer et al., 2014). Synaptic models of consolidation, on the other hand, assume that molecular changes induced during learning have delayed consequences, which could be mediated or modulated by sleep (Dumoulin et al., 2013). Systemic reactivation and synaptic consolidation are thought to be concurrently involved in consolidation of hippocampal-dependent memories (Mascetti et al., 2013), with trace reactivation early during sleep preparing cortical synapses for later occurring plasticity processes (Diekelmann & Born, 2010). Non-hippocampal procedural tasks like mirror-tracing, on the other hand, have not been shown to rely in a similar way on reactivation and systems interaction between different memory networks. It is tempting to speculate that hippocampal buffering and trace reactivation during sleep mediates the recovery of memory performance after the first night of sleep in the declarative memory tasks and the finger sequence tapping task. In contrast, in tasks without hippocampal involvement, synaptic consolidation may need to occur within a defined time window after encoding in order to be effective. While it is very speculative which mechanism underlies which sleep-related effect, findings clearly speak for the existence of at least two distinguishable, sleep-related mechanisms, which affect memory consolidation (Geyer et al., 2013).

Our findings obviously raise the question whether the effects of sleep on memory, which have been reported in numerous studies during the last decade, can be explained fully by consolidation processes, i.e. by additional strengthening of memory traces during night sleep, or whether general or specific fatigue, caused by prolonged wakefulness and task-related strain, respectively, contribute to observed effects. Although present experiments cannot resolve this question, we have reason to believe that the missing long-term effects are due to delayed action of sleep on hippocampally-buffered memory. In general, there is very little evidence that fatigue, as induced by less than 40 h of sleep deprivation, can actually impair declarative memory recall (Quigley et al., 2000). In the first experiment, no significant impairment of word pair recall by sleep deprivation before recall was found, and also recall of names from long-term memory was not impaired by sleep deprivation. Furthermore, in the second experiment, no difference was found between daytime and nighttime wakefulness, although nighttime wakefulness (i.e. sleep deprivation) is much more fatiguing. Actually, the night wake group numerically even performed slightly better than the day wake group on syllable recall, which might result from an additional circadian influence on memory performance. Similarly, in another study, the length of a prior wake period did not influence recall performance (Gais et al., 2006). Fatigue has been discussed extensively as a cause for apparent sleep-related improvements with regard to procedural memory (Keisler et al., 2007; Sheth et al., 2008; Song et al., 2007). However, for procedural mirror-tracing performance, our data show a definite, long-lasting improvement, which must be independent of fatigue. Therefore, we conclude that for procedural memory, active, sleep-dependent consolidation processes exist. These may interact with influences of fatigue. For hippocampally-buffered memory, we assume that delaying the sleep period mainly postpones sleep-related consolidation processes, because there is little indication that fatigue induced by temporary sleep loss significantly impairs recall performance.

In our data, we find that sleep-related improvement is only seen for semantically related word pairs, but not for unrelated pairs. This observation is in line with the findings of Plihal and Born (1997) who also use related word pairs, but does not support the findings of Payne et al. (2012), who report effects exclusively in semantically unrelated material. In view of the literature, which shows sleep effects with both types of material, we assume that semantically related and unrelated

word pairs alike benefit from sleep. Whether an effect of sleep becomes apparent may be modulated by strength of initial encoding, which depends on the learning skill of the participants and difficulty of the material. Encoding strength in turn can modulate consolidation during sleep either positively or negatively (Drosopoulos et al., 2007; Tucker & Fishbein, 2008). Whether complex interactions of stimulus material and experimental design, or a mere lack of statistical power is responsible for divergent findings, remains open.

CONCLUSIONS

In conclusion, our data confirm that sleep has an enduring influence on memory performance. We find evidence for two distinct sleep-related memory consolidation processes, which differ with regard to their development over time. On the one hand, we observe short-term enhancement of performance after the first interval of sleep or wakefulness in both declarative and procedural memory tests that have previously been associated with hippocampal activity during learning. These effects are only temporary and disappear after recovery sleep. Because there is little evidence that memory retrieval is impaired by fatigue in our and previous studies, and because fMRI studies have shown long lasting consolidation-related changes in brain activity without overt changes in performance (Orban et al., 2006), we believe that the lack of enduring behavioral effect is rather due to temporary memory buffering and delayed consolidation than to fatigue after wakefulness. On the other hand, for a hippocampal-independent motor learning task, sleep provides long-lasting benefits, which remain stable even after several recovery nights. We suggest that these two consolidation processes are based on two different mechanisms. Whereas the actual mechanisms are not known, it can be speculated that more complex forms of consolidation rely on memory trace reactivation and systems interactions whereas purely implicit motor memory only requires strengthening of synaptic connections, which has to occur shortly after learning.

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STUDY 3:

INFERRING WHAT HAS BEEN LEARNED FROM THE SLEEPING BRAIN'S ACTIVITY

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MS, AP, and SG planned and designed the experiments. MS, AA, and AP collected the data. MS, SA, HJ, and SG analyzed the data. MS, SA, HJ, and SG wrote the manuscript.

ABSTRACT

Neuronal learning activity is replayed during subsequent sleep. The dynamics of this memory trace reactivation in humans are still poorly understood. We employed multivariate pattern classification (MVPC) methods to determine based on electrical brain activity during sleep what type of images participants had viewed in an evening learning session before sleep. We find significant and generalizable learning-related processing in the electroencephalogram (EEG) of rapid eye movement (REM) and non-REM (NREM) sleep. It occurs during specific time windows, which are congruous to critical periods of synaptic plasticity. Its spatial distribution over the scalp and its frequency composition differ between NREM and REM sleep, speaking for at least two distinct mechanisms underlying memory processing in these states. We conclude that MVPC is a useful tool to enlighten the connection between continuous electrical brain activity and behavior.

MAIN TEXT

Sleep strengthens new memories (*1, 2*). We assume that a reactivation of newly formed memory traces in the sleeping brain is one mechanism that contributes to this effect. Such replay of neuronal activity patterns has been found in single cell recordings of the hippocampus and neocortex in animals (*3, 4*), where it has recently been shown to promote neuronal plasticity (*5*). In humans, PET studies have found learning-related activity on the level of brain regions during sleep (*6, 7*). Furthermore, reactivating memories by presenting auditory or olfactory cues during sleep that were associated with a memory beforehand improves later recall performance in humans (*8, 9*), and reactivation of hippocampal cells is associated with greater memory strength and precision in rats (*10, 11*).

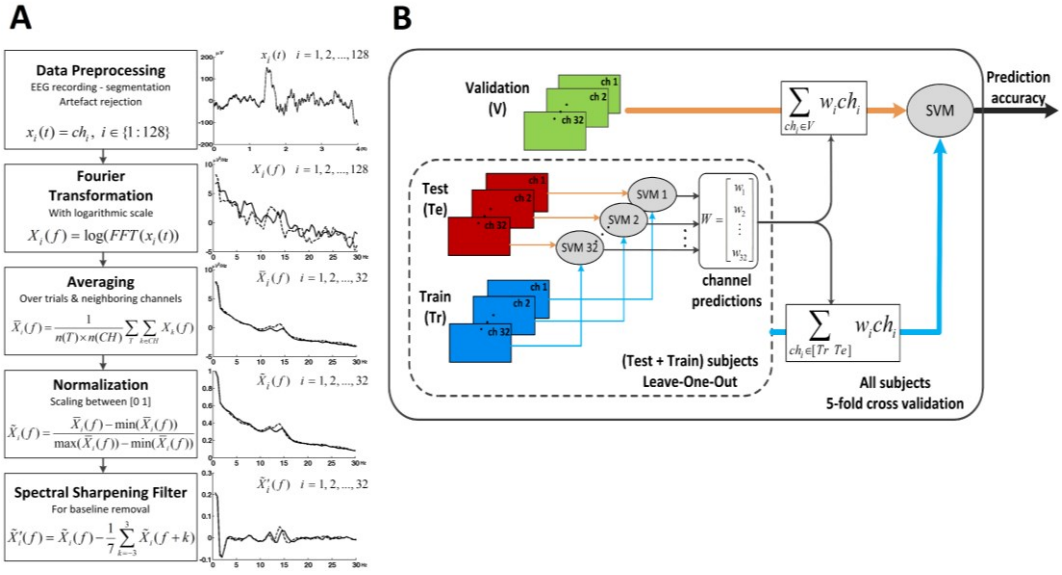

Figure 1. Algorithm used for data preparation and analysis. (A) After artefact rejection, power spectra of 4s-segments of 128-channel sleep EEG data were calculated. To reduce the dimensionality of the data and to increase the signal-to-noise ratio, segments and channels were averaged. Finally, data were normalized to make channels comparable, and a spectral sharpening filter was applied to remove common aspects of the spectra and enhance differences between neighboring frequency bins. (B) During MVPC analysis, training data was strictly separated from validation data. During training, it was again an important goal to reduce dimensionality of the data. Therefore, single channels were weighted according to their individual performance in separate classifiers. A weighted average of data from all channels was then used to train a classifier to distinguish between face and house stimulus conditions. Finally, classification was tested on independent validation data (also see supplementary methods).

In the present study, we tested whether the human sleep EEG contains information about what has previously been learned. If the content of memory is replayed during sleep, we assumed that the brain's electrical activity reflects what has been learned before sleep. To detect such replay activity, we had 32 subjects learn either pictures of faces or of houses before an 8-h period of nighttime sleep. Brain activity was recorded with high-density EEG during a whole night of sleep. We employed multivariate pattern classification (MVPC) methods to test whether electrical brain activity contains information specific to the previously learned material. Specifically, we tried to distinguish brain activity during sleep following learning of these two types of stimuli (see supplementary methods and Fig. 1). If MVPC can determine from the sleep recording which type of stimulus a subject has learned before sleep, this can be taken as a sign of stimulus-specific reprocessing of the learned material during sleep. In particular, if only specific parts of the night contain such information, an actively regulated process can be assumed.

Our main interest was to detect differences in brain activity that are caused by foregoing learning. In particular, we were interested in time and sleep-stage specific activity, because it has been discussed in previous literature whether replay occurs during specific periods of the night or whether it is linked to specific sleep stages (*1, 10, 12*). However, EEG differs greatly between sleep stages and between sleep and wakefulness, and activity can only be directly compared within and not between these states. We therefore divided the night into five 90-min intervals, which approximately reflect the naturally occurring NREM-REM-sleep cycle, and analyzed sleep stages separately. Our results show that human sleep EEG can be used to determine the type of stimuli learned before sleep (Fig. 2A). To confirm statistical significance, we used randomization statistics. This ensured that datasets with random labeling of the data, which do not contain information, do not by chance result in similarly high prediction rates (Fig. 2C). Furthermore, we show a good generalizability of our finding. The concordance between classification rates for the training dataset and the independent validation dataset is high for all sleep periods that are significant in randomization tests (Fig. 2B). Thus, patterns detected during classifier training can be generalized to new data.

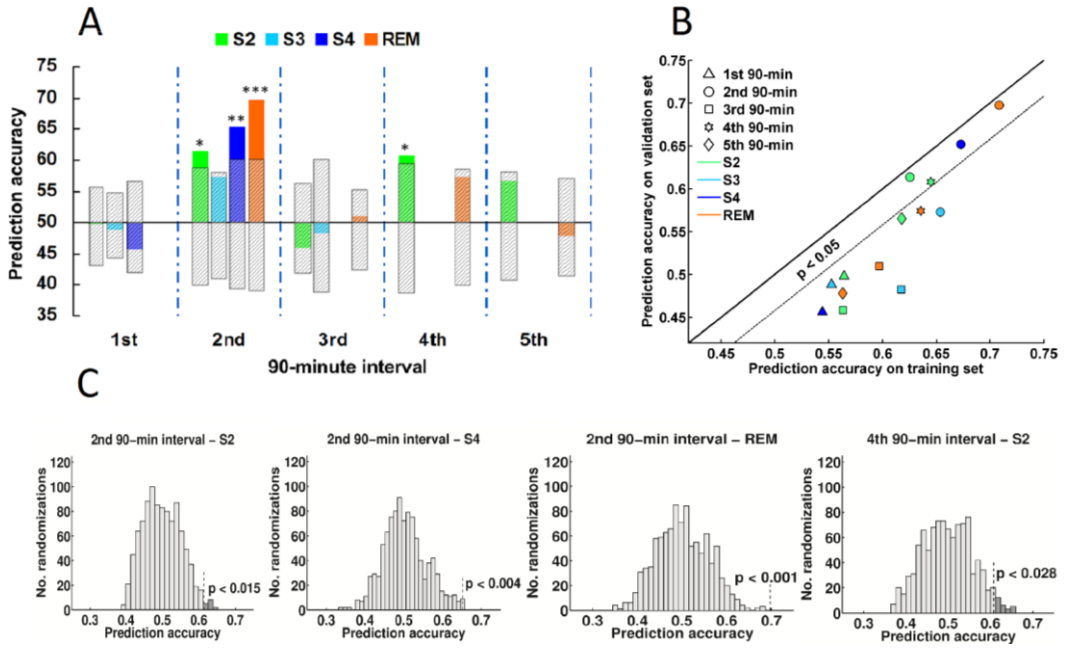

Figure 2. Classification results. (A) The type of material learned before sleep can be determined from sleep EEG during the second and fourth 90-min interval of the night. During these periods, classification for all sleep stages is significant or approaches significance. (B) Similarity of classification accuracy in the training and validation data sets is a good indicator that generalizable information could be extracted during classification and that training data was not overfitted. This is the case for data from the second (circles) and fourth (stars) 90-min intervals of the night. Data from the first (triangles) and third (squares) 90-min intervals show low training accuracy and even lower accuracy in validation data, signifying that no information about previous learning could be extracted from these periods of the night. (C) Significance was tested using randomization tests. The entire analysis was repeated 1001 times on the actual data set with randomly shuffled condition labels. The resulting distribution of prediction accuracies can be used to determine the exact significance levels of predictions for the data set with original condition labels.

Our data show that the sleep EEG reflects previous learning, and moreover, both NREM and REM sleep contain relevant information. Notably, because brain activity in REM and NREM sleep is very different, these two components of the sleep EEG reflect different modes of information processing. That we find both states to hold activity related to previous learning resolves a long discussion on whether NREM or REM sleep accounts for the effects of sleep on memory (*13*). Recently it has been shown that external reactivation of memories during NREM sleep benefits retention, and that this effect is highly specific to individual items of memory (*8, 9*). However, many other papers link memory consolidation and reactivation to REM sleep (*7, 12, 14-19*). Our findings integrate these views and suggest that both types of sleep play their specific roles in memory processing during sleep.

Processing of learning material during sleep occurs during two distinct periods in the second and fourth 90-min interval of the night (Fig. 2). To investigate this pattern on a more fine-grained scale, we split the night into smaller 4.5-min pieces. These independent segments of data were then analyzed using the same procedure as above, and a time course was plotted for the whole night (see supplementary methods). Again, we find two periods of the night, congruent with the 90-min sleep cycles, during which brain processing seems to be more strongly related to previous learning. During other periods, no learning-related information can be detected (Fig. 3). We show that reprocessing is cyclic in nature and its occurrence seems to depend rather on timing than on sleep stages. Another important implication of this finding is that the activity we detect is not simply ongoing post-learning activity or selective fatigue in certain brain areas, but must be a process that is explicitly activated at specific points during sleep.

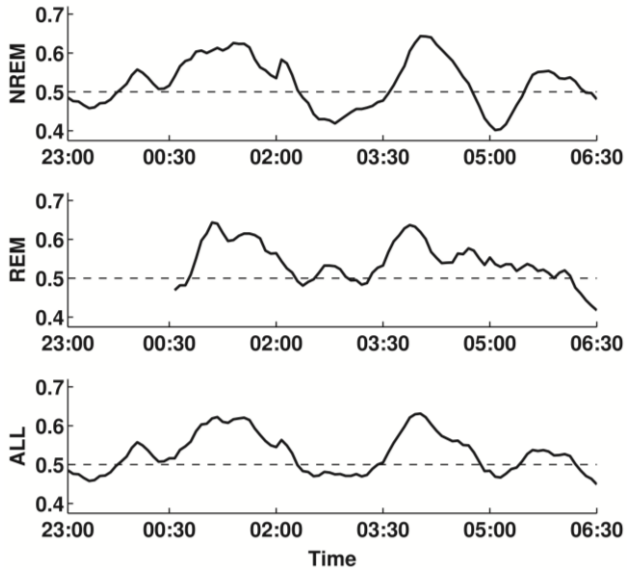

Figure 3. Time course of prediction accuracy across the night. Separate analyses were performed for NREM and REM sleep. Both show an oscillatory pattern with two distinct peaks around two and five hours after sleep onset. Timing is more relevant to when memory reprocessing occurs than sleep stage.

The finding that reprocessing during sleep exhibits peaks around three and around six hours after learning fits particularly well with the concept of 'sleep windows'. These have been proposed to be specific periods during which sleep has to occur

after learning to strengthen memory. If sleep is prevented during these periods, memory performance deteriorates (*20, 21*). Our finding of discrete windows for memory reprocessing also agrees with animal data showing that discrete critical periods exist during memory consolidation, when memory is particularly sensitive to disruption (*22*). E.g., inhibition of protein synthesis 15 min and 3 h after learning, but not 1 h after learning abolishes learned behavior in hippocampal one-trial avoidance learning (*23*). Similarly, in drosophila, time windows of different behavioral memories and corresponding neuronal traces develop over several hours after conditioning (*24*), a process that has been related to systems memory consolidation in humans (*25*).

Apart from the precise temporal details of activity, we were interested in frequency and location of reprocessing during sleep. For this, we analyzed feature weights provided by the classifier to distinguish between classes. Figure 4A shows the relative contribution of different frequency bands to stimulus classification. It is apparent that the frequencies relevant for classifying learning conditions differ between sleep stages. Activity in the frequency range of sleep spindles (12-16 Hz) can distinguish previous learning conditions only in NREM sleep. Consistent with this finding, it is known that spindle activity increases after learning. Sleep spindles have been associated with hippocampal sharp-wave-ripple activity, which in turn accompanies memory trace reactivation during sleep (*26-28*). Here, we show additionally that NREM spindle activity holds information about the kinds of stimuli that were previously learned and that sleep spindles, in this way, directly reflect memory reprocessing (Fig. 4B). Slow frequencies below 4 Hz were informative both in NREM and REM sleep. Interestingly, these were as important in REM sleep as in NREM sleep, although discrete slow waves do not normally occur in REM sleep. Still, slow potential drifts can occur and seem to contain information relevant to previous learning. Most interestingly, theta-band activity (4-8 Hz) during REM sleep showed higher importance than in the other sleep stages. This supports hypotheses about the role of REM sleep theta in memory processing that have only recently received renewed attention (*12, 29*).

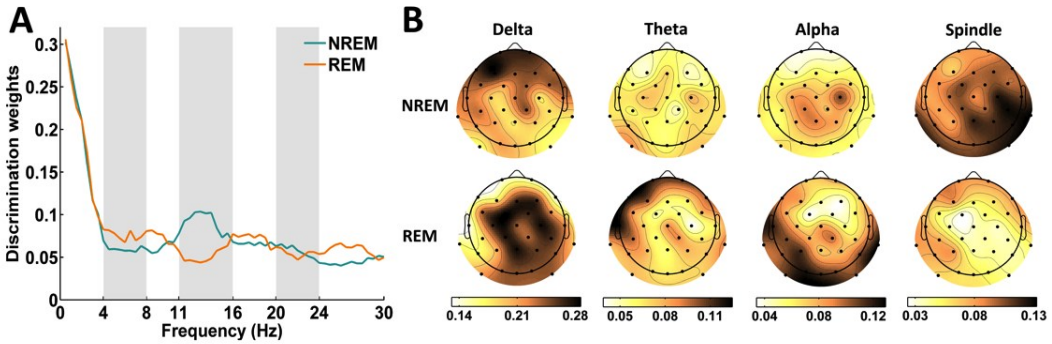

Figure 4. Frequency contribution to memory reprocessing in NREM and REM sleep. (A) Discrimination weights show that spindle activity in the range between 11 and 16 Hz is most predictive in NREM sleep. In REM sleep, in contrast, theta and alpha frequencies contributed more to correct classification. Slow frequencies below 4 Hz contribute to classification in both sleep stages. (B) The topography of predictive channels clearly differs between NREM and REM sleep. In NREM sleep, frontal delta power and right parieto-temporal spindle activity are most informative for classification. REM sleep shows a more complex pattern. Here, slow oscillations of central electrodes, frontal and temporal theta as well as occipital alpha contributed most to discrimination between learning conditions.

Together, we show that all sleep stages have specific contributions to memory processing during sleep, and that a single task induces learning-related activity in several sleep stages. Apart from establishing that the classifier focuses on frequency bands that had already proved relevant previously (spindles and slow oscillations), our results provide valuable indications for future studies. We propose that, apart from slow-wave and spindle activity, particular consideration should be given to REM sleep theta. Increases in frontal theta power have been linked to successful memory encoding and retrieval (30, 31), and theta is thought to be responsible for controlling, maintaining, and storing memory content during wakefulness (32). Moreover, theta band activity has been investigated previously in the context of sleep and memory (33). A recent study has shown increased frontal theta power after reactivating a verbal learning task by cueing during sleep (34). Together, these findings support the view that theta holds an active role in memory reprocessing.

The relevance of different frequency bands varies depending on scalp location. Figure 4 shows the pattern of classification weights over the scalp for informative frequency bands. In NREM sleep mainly frontal slow-wave activity and right parieto-temporal sleep spindle activity are predictive for the learning condition. Frontal slow-waves have previously been shown to correlate with performance gains observed after memory was externally reactivated by cueing during sleep (*34*). Sleep spindles have also been linked to memory performance and have been shown

to occur in a locally specific manner (*35, 36*). Parietal sleep spindles accompany task specific reactivation seen in fMRI (*37*). Compared to NREM sleep, we see a more differentiated pattern in REM sleep. Frontal theta activity, frontal and temporal beta activity, as well as occipital-temporal alpha activity all contribute to the distinction between face and house conditions. In contrast to NREM sleep, central slow-waves have higher predictive power than frontal ones, speaking for a different slow-wave-related process in REM sleep than in NREM sleep and against an effect of REM sleep eye movements. Together, memory-related processes during REM sleep seem to be more complex, with a more differentiated frequency pattern over the scalp than during NREM sleep. This speaks for several distinct, region- and sleep stage-specific mechanisms by which memory is reprocessed during sleep.

Memory reprocessing occurs in all sleep stages. This finding combines recent and previous views which stress the importance of either light NREM sleep, SWS or REM sleep for memory consolidation (*38-41*). There are two main suggestions regarding the interaction of NREM and REM sleep. The sequential hypothesis of sleep posits that the succession of sleep stages is relevant to its memory function. In this model, both sleep stages have specific and substantially different roles in the processing of memories (*42*). Other models assume that independent and separate processes contribute to memory processing during NREM and REM sleep. Again, both sleep stages are assumed to have different functions, but these pertain to different aspects or forms of memory (*43*). Our observation of learning-related activity during NREM and REM sleep emphasizes that all sleep stages are relevant to memory processing. Our data cannot directly answer the question how these stages interact and whether they have to be consecutive. However, relevant activity occurs in close temporal proximity over different stages, and the same memory task triggered learning-related activity in both NREM and REM sleep EEG. It therefore seems likely that both sleep stages cooperate in some way in the processing of memories.

Although the separate functions of NREM and REM sleep are unknown, there are a number of hypotheses regarding the contributions of different sleep stages to memory consolidation. Cortical processing in terms of activity and long-range connectivity differs between sleep stages, allowing local memory reactivation in SWS and network-wide information integration in REM sleep (*44, 45*). It has also been proposed that SWS serves to downscale synaptic potentiation (weakening

unused connections) whereas REM sleep rather increases the strength of synaptic connectivity (*46, 47*). We are, however, only beginning to understand how sleep acts on a synaptic level, and our understanding is far from complete (*5, 12, 13, 48*).

We used a multivariate pattern classification approach to infer the content of a previous learning session from electrical brain activity during sleep. Instead of looking for a single feature that can distinguish between conditions, these methods take into account and compare the whole temporospatial pattern of activity. We show that both NREM and REM sleep participate in reprocessing of previously learned material. Our results demonstrate the existence of at least two distinct temporal processing windows, which coincide with the timing of synaptic plasticity processes after learning. Differences in topographical and frequency patterns, moreover, indicate different roles of NREM and REM sleep in the reactivation process. Our results, thus, shed light on the dynamics of memory trace reactivation in humans. Pattern classification methods may in future pave the way towards a better understanding of the covert mechanisms which mediate the link between electrical brain activity and behavior.

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SUPPLEMENT

MATERIAL AND METHODS

SUBJECTS

EEG recordings from 32 healthy subjects with no history of neurological or psychiatric disorders were analyzed in this study. All participants were students, between 18 and 30 years old, native German speakers and non-smokers. They were right handed as measured by Edinburgh Handedness Inventory-test (*1*). Subjects were regular sleepers with a habitual sleep duration of 6-9 h. They did not do shift work or changed time zones in the six weeks prior to the experiment. Participants were told to refrain from drinking alcohol, coffee and tea and from taking any drugs that can affect the central nervous system on the days of the experiment.

EXPERIMENTAL DESIGN

Participants spent three nights in the sleep laboratory. The first night served as an adaption night, during which subjects were accustomed to the laboratory and to sleeping under experimental conditions (e.g. wearing an EEG cap). In the other two experimental nights, which were spaced at least 5 days apart, subjects completed an intensive image learning task, studying pictures of either faces or houses. After having EEG electrodes attached, they learned the task from 9 p.m. to 10.30 p.m., and memory was tested afterwards. Then, they went to bed at 11.30 p.m. After 8 h of sleep, they were woken up and memory was tested again at 7.30 a.m. All subjects participated in two sessions, each time learning only one type of images, in a counterbalanced fashion.

LEARNING TASK

Subjects learned a set of 100 images of faces or houses. In 30 repetitions, with random order, individual images were always presented in one of the four quadrants of the screen, and participants had to learn to associate the images with the quadrant in which it was presented. During testing, 100 learned and 100 new images were presented in random order. First, participants had to indicate by keypress whether they had seen the image before (with left hand on main keyboard: 1-sure, 2-probably, 3-probably not, 4-surely not). Then, for responses 1 and 2, the quadrant in which it had been presented was probed (with right hand on numerical pad: 1-lower left, 3-lower right, 7-upper left, 9-upper right). Two sets of images

were used: 300 pictures of houses were taken from German online real estate sites, 300 pictures of neutral faces were taken from Minear & Park (*2*).

EEG RECORDING

Sleep EEG was recorded using an active 128 channel Ag/AgCl-electrode system (ActiCap, Brain products, Gilching, Germany) with 1 kHz sampling frequency and a high-pass filter of 0.1 Hz. Electrodes were placed according to the extended international 10–20 electrode system. For the purpose of sleep scoring, recordings were segmented into 30-s epochs and stages were determined on electrodes C3/C4 according to standard rules by two independent raters (*3*).

DATA PREPARATION

For further analysis, EEG data was split into 3-s segments. Artefact rejection was done in a semiautomatic process using custom MATLAB scripts. Based on the distributions of several parameters of the raw data and power spectrum, thresholds were chosen for each recording individually that made sure that only a minimal number of artefacts remained in the data. Epochs containing artefacts were removed from the dataset, channels that contained too many epochs with artefacts were removed and interpolated using routines provided by EEGLAB. Artefact-free epochs were then transformed into the frequency domain using Fourier transformation. To get smooth spectra, Welch's method was used for this, averaging over 10 Hamming windows of 2-s length with 95% overlap, resulting in a final data resolution of 0.5 Hz. Data was used up to a maximum frequency of 30 Hz.

The following steps for data preparation had three specific aims: (1) to increase signal-to-noise ratio, (2) to reduce the dimensionality of the data, and (3) to adapt the signal better to between-subject classification. First, we averaged electrode signals within a radius of approximately 3 cm around the 32 evenly spread locations of an extended 10-20-system. This decreases the number of redundant features, increases signal-to-noise ratio without significant loss of spatial resolution, and increases spatial similarity between subjects. Next, we averaged over all available artefact-free epochs for each 90-min cycle and sleep stage separately to have the most reliable estimate of spectral properties. This also makes sure that the number of epochs per subject does not differ in the analysis, which would bias the classification. Subjects were only included in the analysis of a cycle and stage if they had at least 40 artefact-free epochs (i.e. 120 s of data) for that data point. The

number of subjects available for each 90-min cycle and sleep stage can be found in supplementary table S2. Only cycles and stages with at least 12 subjects were analyzed. Finally, to remove amplitude differences between channels, which are caused by the distance of each channel to the reference electrode, spectra from all channels were separately normalized between zero and one. This also removed between subject differences in general spectral power.

Because baseline EEG power spectra are very similar to each other and differences between conditions are of smaller magnitude, it is necessary to enhance these differences within the spectra. Therefore, in a final data preparation step, we applied a spectral sharpening filter to remove the baseline spectrum and emphasize differences between neighboring frequencies. This was done by subtracting the moving average of five neighboring frequency bins from the signal. This amplifies the changes within the power spectra. It can be noted that this procedure is valid because neighboring data points represent frequencies from the same signal, which are highly correlated.

MULTIVARIATE PATTERN CLASSIFICATION (MVPC)

The aim of the present study was to test whether EEG activity during sleep contains information about the kind of previously learned visual material. In order to be able to discover all information in the pattern of brain activity, we used a multivariate classification approach instead of typical multiple univariate tests, which only detect those features of the data that by themselves can distinguish between conditions.

Sleep EEG recordings from 64 nights were analyzed using a classification algorithm developed on the basis of linear support vector machines (SVM), which we employed to detect the presence of material-specific information in the data. Because our EEG recordings with 128 channels times 60 frequency bins pose problems associated with high dimensional, low sample size data, we implemented a procedure that aimed at reducing the number of features while simultaneously increasing signal-to-noise ratio (see Figure 1 in main text). By averaging during data preparation, we already reduced the number of channels to 32. During classification, we used a stepwise procedure, which first regarded every channels as an independent classifier and then combined outcomes of this first step for the actual classification.

First, data was split into independent training and validation sets. We then trained one linear SVM per EEG channel on all subjects of the training set except one to determine how well each channel can be used to distinguish data coming from the 'face' or the 'house' learning condition. Classification was cross-validated on each subject in a leave-one-out procedure, and resulting accuracies were averaged over all cross-validation runs. The average classification accuracy from each channel was then used to calculate a weighted average of data. The main SVM was trained on this weighted training set and classification accuracy tested on the independent validation set. The main rationale behind weighted averaging of channels is to reduce feature space dimensionality which is crucial for high dimensional, low sample size data. This two-step process was cross-validated using 280 repetitions of a 5-fold procedure, which covers the whole dataset with five independent validation sets.

We used randomization tests to test for significance. These tests generate the distribution of the null hypothesis by randomly shuffling the original data and repeating this process a large number of times. Here, we randomly shuffled condition labels, i.e. the two conditions of each subject were randomly labeled as 'face'/'house' or as 'house'/'face'. We then calculated classification accuracies for the randomly labeled data. This was repeated 1001 times to estimate the whole random distribution. Significance was calculated by determining the percentage of times randomly labeled data produced a classification accuracy that was equal or higher to the one found in real data.

In the first analysis, we split the night into five 90-min sleep cycles separately for all sleep stages to assess the temporal dynamics of memory reprocessing (see main text). To determine a more fine-grained time course of classification accuracy, we split the night into smaller 4.5-min pieces. These independent segments of data were then analyzed in the same way as before. To reduce measurement noise we used a 22-min sliding-window in this analysis.

To assess which features of the sleep EEG are particularly predictive, we analyzed classification weights. We averaged the absolute values of classification weights over all repetitions of the training procedure, resulting in an averaged 32 (channels) × 60 (frequencies) weight matrix. To examine frequency contributions to memory reprocessing, we further averaged these values over all channels (see

Fig. 4A in main text). The topography of predictive channels (see Fig. 4B in main text) was obtained by averaging classification weights for each channel over different frequency ranges.

SUPPLEMENTARY RESULTS AND DISCUSSION

A number of current theories assume that memory traces are reactivated during sleep, and several animal studies have already provided evidence for such a process. However, it is difficult to show reactivation directly in humans. Electroencephalographic activity during sleep is completely unlike that during wakefulness. The signals differ both in the time domain and in the frequency domain, i.e., amplitudes and power spectra cannot be compared between sleep and wakefulness. This is owing to different modes of generation and transmission of electrical activity during sleep (*4, 5*). Previous data have shown that reactivation can be modified in time (e.g. time compression) or in location (e.g. neocortical replay following hippocampal activity)(*6, 7*). Signs of reactivation can thus be transformed by a large number of operations, making the search space virtually infinite. Because wake-to-sleep classification is thus problematic, and within-subject, between-session classification is confounded by various session differences (e.g. recording artefacts), we opted for a between-subject classification approach. This allowed us to detect whether brain activity in the same state of consciousness differs between two experimental conditions. If brain electrical activity during sleep can distinguish between two preceding learning conditions, sleep EEG activity must be caused by foregoing learning activity. Furthermore, activity that is present only at specific points in time indicates that the underlying process is related to reactivation rather than prolonged ongoing activity.

One of the challenges of sleep data analysis is the size of the data and the difficulty to record large sample sizes. In addition, high-density EEG recordings present the problem of multivariate analysis. For multiple univariate analyses, which are commonly used (e.g. in classical fMRI analysis) the goal of an analysis is to find single features that are strong enough independently to distinguish between conditions. If, however, it is of interest whether the whole pattern of data contains relevant information to distinguish conditions, a multivariate approach is needed. There are some methods that can deal with large numbers of data dimensions, a prominent one is MVPC. However, high dimensional, low sample size data, like EEG

recordings, pose specific problems for classical statistical testing as well as for MVPC (*8, 9*). For this kind of data, it is important to reduce the number of features to approach approximately the number of samples. If the signal across features is highly correlated, as it is the case for EEG data, averaging can serve this purpose. Averaging is therefore vital to reduce dimensionality of the data as well as to increase signal-to-noise ratio. We developed a two-step procedure that uses spatial averaging and a channel-based weighted average to improve classifiability of our data (Fig. 1, also see supplementary methods).

To make sure that findings are significant and generalizable, we used two approaches. First, we generated randomly labeled data, which, per se, cannot contain any information, and compared the performance of the classifier on these random data with its performance on the original observed data (see supplementary figure 1). This test allows to determine the probability of an outcome given that the data contain no actual information and thus provides exact significance values. Because this process, which repeats the whole analysis for each random iteration, is very calculation intensive, we completed only 1001 repetitions. Thus, the limit of precision of given significance levels is $p<0.001$. In the case of REM sleep of the 2nd 90-min sleep cycle, none of the random iterations produced higher prediction rates than the real data.

The second approach to ensure generalizability of predictions was to compare prediction rates of training and validation rates. If prediction is higher during training than during validation testing, this is a sign of overfitting of the classifier to the training data set. In this case, the classifier uses random feature characteristics to separate classes in the training data, which are not predictive for new data. Ideally, prediction rates should be similar for training and validation data. In that case, the classifier can extract as much information as possible from the training set, and the learned pattern can be generalized to new data. It can be seen in Fig. 2B (main text) that for data from the 1st (triangles) and 3rd (squares) 90-min sleep cycle training accuracy was low (<0.625), but prediction accuracy for the validation set was still worse. Thus, EEG from these sleep periods does not contain information pertaining to previous learning experience. On the other hand, EEG from the second (circles) and fourth (stars) 90-min sleep cycle consistently shows higher training

and validation accuracies, and in some cases shows near perfect generalization between training and validation.

SUPPLEMENTARY FIGURE

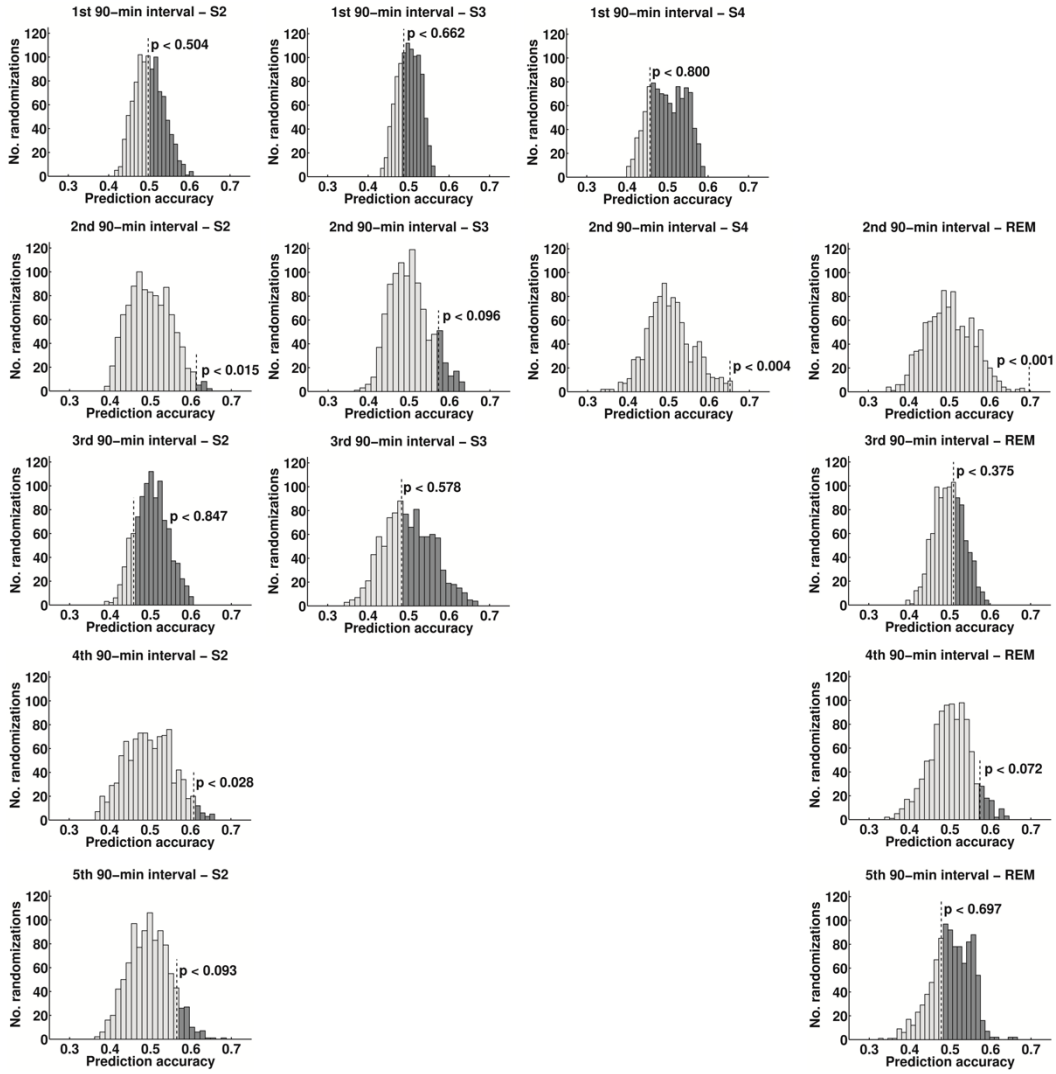

Figure S1. Randomization statistics for classification in all sleep cycles (rows) and stages (columns). Dark grey area shows number of randomizations when prediction accuracy on randomly labeled data exceeded the prediction accuracy obtained on correctly labeled data.

SUPPLEMENTARY TABLE

Supplementary Table S1. Number of participants for classification in different sleep cycles and sleep stages. Only data points with N≥12 were analyzed.

N	*S2*	*S3*	*S4*	*REM*
1st 90-min interval	31	30	18	0
2nd 90-min interval	32	20	12	18
3rd 90-min interval	29	16	6	24
4th 90-min interval	24	9	2	19
5th 90-min interval	20	0	0	18

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STUDY 4:

STRENGTHENING PROCEDURAL MEMORIES BY REACTIVATION IN SLEEP

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MS and SG planned and designed the experiments. MS and TG collected the data. MS and SG analyzed the data. MS and SG wrote the manuscript.

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ABSTRACT

There is robust evidence that sleep facilitates procedural memory consolidation. The exact mechanisms underlying this process are still unclear. We tested whether an active replay of prior experience can underlie sleep effects on procedural memory. Participants learned a finger-tapping task in which key presses were associated with tones during practice. Later, during a consolidation interval spent either sleeping or awake, we presented auditory cues to reactivate part of the learned sequence. We show that reactivation strengthens procedural memory formation during sleep, but not during wakefulness. The improvement was restricted to those finger transitions that were cued. Thus, reactivation is a very specific process underpinning procedural memory consolidation. When comparing periods of sleep with and without reactivation, we find that it is not the time spent in a specific stage of sleep per se, but rather the occurrence of reactivation that mediates the effect of sleep on memory consolidation. Our data show that longer sleep time as well as additional reactivation by cueing during sleep can enhance later memory performance.

INTRODUCTION

After memories have been encoded, they undergo a phase of consolidation. It has been shown that sleep facilitates this process (Diekelmann & Born, 2010). The exact mechanism by which this happens, however, is still under debate. A process of active systems consolidation has been proposed for declarative, hippocampus-dependent memory (Diekelmann & Born, 2010; Dudai, 2004). In that framework, off-line reactivation and replay of prior waking experience during sleep strengthen hippocampal memory traces and integrate information into neocortical long-term memory networks. Studies in rodents have shown that neuronal firing patterns observed at encoding are reactivated during postlearning sleep in both the hippocampus and the neocortex (Peyrache, Khamassi, Benchenane, Wiener, & Battaglia, 2009; Euston, Tatsuno, & McNaughton, 2007; Ji & Wilson, 2007; Ribeiro et al., 2004; Louie & Wilson, 2001; Nadasdy, Hirase, Czurko, Csicsvari, & Buzsaki, 1999; Wilson & McNaughton, 1994). Hippocampal activity precedes replay in neocortical sites and may thus hold a crucial role in the orchestration of memory trace reactivation and integration (Peyrache et al., 2009; Ji & Wilson, 2007).

Apart from this evidence for a replay of neuronal activity during sleep in rodents, studies in humans have shown that declarative memory consolidation benefits from reactivation. Navigation-related hippocampal activity after maze learning is reexpressed during postlearning sleep, and the amount of this reactivation predicts later memory performance (Peigneux et al., 2004). It is also possible to enhance later memory recall of declarative information by presenting cues of the learning task during sleep (Rudoy, Voss, Westerberg, & Paller, 2009; Rasch, Buchel, Gais, & Born, 2007). For example, if an odor context is associated with learning a spatial card location task and the odor cue is again presented during sleep, cueing during sleep led to superior memory than sleep without additional reactivation of the learning context. Interestingly, presentation of the odor during wakefulness was not effective (Rasch et al., 2007). These results indicate that, for declarative memory formation, sleep is a critical period during which reactivation of learned information reinforces consolidation (for a review, see Diekelmann & Born, 2010).

Procedural memory consolidation can also benefit from sleep (Korman et al., 2007; Fischer, Hallschmid, Elsner, & Born, 2002; Walker, Brakefield, Morgan, Hobson, &

Stickgold, 2002). However, which aspects of procedural memory benefit from sleep is still not entirely clear. It has been shown that consolidation of procedural memory can also happen efficiently over periods of wakefulness (Song, Howard, & Howard, 2007; Press, Casement, Pascual-Leone, & Robertson, 2005). Awareness can modulate whether a task is influenced by sleep or not (Robertson, Pascual-Leone, & Press, 2004). And movement-related benefits on finger-tapping tasks do not seem to require sleep for consolidation, whereas consolidation of goal-related sequence information improves over sleep (Albouy, Fogel, et al., 2013; Cohen, Pascual-Leone, Press, & Robertson, 2005). Experimental evidence for memory reactivation of procedural content in sleep is still scarce. PET studies in humans have shown that brain regions that engage in learning a motor task are also more active during postlearning sleep (Destrebecqz et al., 2003; Peigneux et al., 2003; Maquet et al., 2000). In a recent study, Antony, Gobel, O'Hare, Reber, and Paller (2012) showed that reactivation also holds a functional role in procedural memory consolidation. In their study, participants learned to tap two differently pitched melodies on four keys of a keyboard. One of these melodies was later replayed during sleep, leading to a performance increase for the cued sequence. These findings show the possibility to bias memory processing during sleep.

The aim of this study was to test the hypothesis that reactivation enhances off-line memory consolidation for procedural memory, to investigate whether reactivation was specific to sleep, and to describe the relation between external reactivation by cue presentation and endogenous reactivation by sleep. We tested participants on a finger sequence task for which each finger was associated with a corresponding piano tone, so that the participants effectively played a short, repetitive tune. In the first experiment, half of the tone sequence was replayed to the participants during a 3-hr period of sleep or wakefulness. We hypothesized that later reproduction of the replayed half of the sequence would be better than the nonreplayed half, but only when replay took place during sleep. In two additional experiments, we addressed the question of how much sleep is required for procedural improvement to occur. It has been shown for the declarative memory domain that presenting external cues of a previous learning task condenses the time course of consolidation (Diekelmann, Biggel, Rasch, & Born, 2012). Therefore, in additional experiments, participants slept after learning either for 3 hr as in the first experiment or for a whole 8-hr night. Controls stayed awake during intervals of corresponding length. Because we

suppose that reactivation occurs normally during sleep and longer periods of sleep should provide more reactivation, our hypothesis was that the beneficial effect of a short period of sleep together with external reactivation would be similar to the beneficial effect of a longer period of sleep without reactivation.

METHODS

PARTICIPANTS AND GENERAL PROCEDURE

One hundred sixty-two healthy, paid volunteers participated in three experiments. They were 18–30 years old (mean age = 22.3 ± 2.4 years), nonsmokers, and righthanded. They did not take any medication or ingest caffeine on the day of the experiment. Participants were not professional musicians, nor did they practice any musical instrument regularly in the last 4 years. All participants had a regular circadian rhythm and were not extreme morning or evening types, as assessed by the Munich Chronotype Questionnaire (Roenneberg et al., 2007). They had no long-distance flights within 6 weeks before the experiment. Potential participants not conforming to these requirements were not allowed to participate in the experiments.

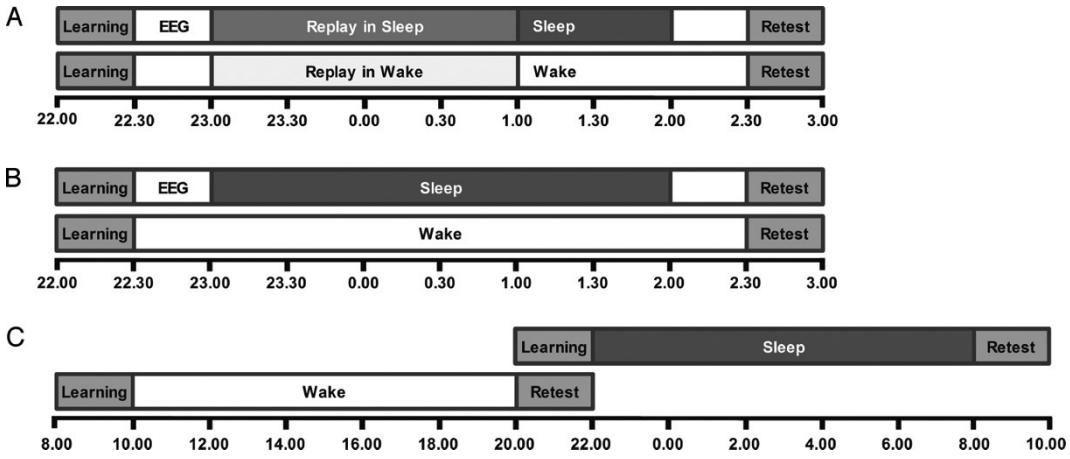

Figure 1. (A) In the first experiment subjects practiced a finger-tapping task with tones associated to each key press and then either slept or stayed awake for 3 hours before being retested on the same task. During the first two hours of this time, half of the practiced sequence was replayed to them. (B) In two further experiments, we tested the effect of sleep on the learned task without additional external reactivation. The second experiment followed the exact same procedure as the first one, except that no tones were replayed to the subjects during the consolidation interval. (C) In the third experiment, subjects slept for a whole night or stayed awake during the day. The sleep group practiced the task in the evening and was retested in the morning, whereas the wake group practiced the task in the morning and was retested in the evening. Figure reprinted with permission.

In the first experiment, 64 participants (32 men) participated in one experimental session, which took place between 22.30 hr and 03.30 hr (Figure 1). First, participants learned a procedural finger sequence task, during which tones were played. Performance was tested immediately after learning and again after a 4-hr interval. They were randomly assigned to one of two experimental groups: 30 min after finishing the task, half of the participants went to bed to sleep for 3 hr whereas the other half stayed awake during the same time. Wake participants were playing board games and having conversations when at risk of falling asleep, but they also had periods of quiet wakefulness, in particular at the beginning of the night when the experimenter was still occupied with other tasks related to the experiments. Thus, the wake control included phases of both quiet and active wakefulness. During the first 2 hr of sleep or wakefulness, the sequence of tones from the learning task was played to the participants. Half an hour before the delayed test, participants in the sleep group were awoken. In the second experiment, 34 participants (16 men) followed the same experimental procedures and times, but without presentation of the tones during the intervening interval of sleep or wakefulness. Again, half of the participants slept, whereas the other half stayed awake during the consolidation interval. Another 64 participants (32 men) followed the same experimental procedure in the third experiment. Again, no tones were presented between learning and retest. In this study, the intervening period was prolonged to include a full night of sleep or a full day of wakefulness. Half of the participants learned the motor task 1 hr after getting up in the morning. They were tested in the evening, 12 hr later. In-between, participants followed their normal daily routine, but they were not allowed to sleep during that time. The other half learned the task in the evening, 13 hr after getting up. They were tested in the next morning 12 hr after learning, which was 1 hr after getting up, and, thus, included a whole night of sleep. Participants' activity was monitored using actimetry in both conditions.

TASK AND STIMULATION

Participants were instructed that they had to learn a short finger tapping sequence. Four gray empty circles were presented on a black computer screen background along the horizontal middle axis. The circles were filled sequentially following a 12-element sequence. Participants had to react as fast and as accurately as possible to the filled circle target location by pressing a key on a computer keyboard with the corresponding finger of their nondominant left hand. With each correct reaction, a

piano tone was played. Tones were assigned in a rising fashion to the keys and locations. Pitches were chosen according to a pentatonic scale so that any combination of keys would result in a harmonic, nonaversive melody. Tones were played at a sound pressure level of 57 dB. This level was chosen because it was clearly audible, but not intrusive or aversive, and it did not disturb participants during the retention interval. Furthermore, as previous work on external auditory stimulation during sleep has used comparable sound pressure levels, we expected this level to be sufficient to drive reactivation (Rudoy et al., 2009). If an error was made and the wrong key was pressed, no sound was played. Immediately after participants reacted to a given location, the next target location was indicated by a filled circle on the screen according to a fixed sequence. Sounds were presented over speakers during learning and over in-ear headphones fixated with medical tape during the night. The maximal sound pressure level was measured before each experiment with a digital sound level meter) at the location of the ear or with the headphones directly plugged into the sound level meter.

During the learning session, participants practiced one of two 12-element sequences: 423121432413 or 412431421323, balanced across groups. The sequences were chosen based on previous literature on motor sequence learning (Destrebecqz et al., 2003) and are balanced for first-order dependencies. Therefore, each possible transition between the four finger locations occurred only once in the 12-item string. We additionally chose the sequences in a way that the seventh item corresponded to the first item of each sequence. Thus, both the first and second half of the sequence could be replayed in an iterative loop, generating only transitions that were practiced in this order during learning. Participants completed 15 experimental blocks each containing eight repetitions of the sequence. The last three blocks were used to determine initial performance. During retest, participants completed another three blocks of eight repetitions of the previously learned sequence.

In the first experiment, during the first two hr of the 3-hr sleep period or the matching wake interval, tones corresponding to one half of the previously learned sequence were replayed to the participants. Reactivation started immediately after lights off and at corresponding times in the wake interval. Tones for reactivation were presented at a sound pressure level of 57 dB, the level at which tones were

presented during learning, which is clearly audible but not too intrusive. The tones were played continuously over 2 hr, paced in a 1-sec rhythm. This tempo was chosen to allow the tone transitions to be easily processed, while still being pleasant to listen to and unobtrusive during sleep. Balanced over groups and sequences, either the first or second half of the sequence was cued. In the additional experiments, no tones were presented during the consolidation interval.

STATISTICAL ANALYSIS

Statistical testing for the first experiment relied on two-factorial repeated-measures ANOVA with one withinsubject factor (Cued/ Uncued) and one group factor (Sleep/ Wake). Initial performance, as measured during the last three blocks of training, was entered as a covariate to account for individually different levels of performance. Subsequently, sleep and wake groups were analyzed separately with paired t tests to compare cued and uncued parts of the sequence. For the additional experiments, univariate ANOVA was used with one group factor (Sleep/Wake) and again the initial performance, as measured during the last three blocks of training, as a covariate. All analyses used typing accuracy during the retesting blocks as dependent measure. All tests were two-tailed with an α-level of 0.05. Participants had to type a fixed number of sequences in each block. Therefore, the total number of errors made when typing the whole sequence or half sequence during the last three blocks of training and during the three retesting blocks was used as a measure of accuracy. The mean time needed per key press was taken as a measure of speed.

Nine of the 162 participants had to be excluded based on unusually slow RTs or large numbers of errors. This left n = 29 participants in the wake group and n = 28 participants in the sleep group in the first experiment, all n = 18 (wake) and n = 16 (sleep) participants in the second experiment, and n = 31 (wake) and n = 31 (sleep) participants in the third experiment.

SLEEP AND EEG RECORDINGS

Sleep was recorded polysomnographically in the sleep lab in the 3-hr sleep group. EEG was recorded from two scalp electrodes (C3, C4 according to the International 10–20 System) against a nose reference. Bipolar EOG and EMG were recorded as well. Recordings were scored off-line by two independent raters according to standard criteria (Rechtschaffen & Kales, 1968). Discrepant scorings were decided

with the aid of a third rater. Sleep spindles were determined and counted by means of a previously published algorithm (Gais, Molle, Helms, & Born, 2002). Participants in the whole night of sleep condition in the third experiment slept at home. Here, sleep duration was assessed via wrist actigraphy (ActiSleep Monitor, ActiGraph, Pensacola, FL) and questionnaire data. Activity was recorded on all three spatial axes and integrated with a resolution of 1 min. Data were plotted and scored manually with an accuracy of 10 min. Activity data were compared manually with questionnaire data. Any discrepancies were either solved together with the participant or led to the exclusion of the participant from analysis. Actigraphy was used in the same way to confirm that participants in the wake group did not sleep during the daytime interval.

Subjective state was obtained using visual analogue scales with the endpoints "alert–not alert," "motivated– not motivated," "interested–not interested." In addition, a 10-min version of the psychomotor vigilance task was used to assess potential differences in fatigue and vigilance between groups (Dinges & Powell, 1985).

RESULTS

In the first experiment, we compared performance on the half of the sequence that was replayed during the consolidation interval with the other half, which was not replayed. We also compared participants who slept during the interval with a wake control group. In the sleep group, participants made significantly fewer errors in the cued part of the sequence than in the uncued part (1.2 ± 0.4 vs. 2.1 ± 0.3 errors [mean ± SEM], $F(1, 27) = 6.1$, $p = .02$; see Figure 2). Reactivation during wakefulness had no such effect (2.3 ± 0.3 vs. 1.9 ± 0.3, $F(1, 28) = 0.6$, $p = .44$). A significant interaction confirmed that replaying the sequence of sounds to the participants improved performance only during sleep, $F(1, 53) = 6.6$, $p = .01$ for Sleep/ Wake × Cued/ Uncued. We could not show a significant effect of sleep on the uncued part (sleep: 2.1 ± 0.3, wake: 1.9 ± 0.3 errors, $t(55) = 0.1$, $p = .88$) and no significant main effect of condition Sleep/Wake, $F(1, 53) = 1.2$, $p = .27$. We found no differences in tapping speed between conditions (Sleep/ Wake × Cued/ Uncued: $F(1, 53) = 0.1$, $p = .77$; sleep group: 455 ± 10 msec in the cued vs. 452 ± 11 msec in the uncued half; wake group: 443 ± 10 msec in the cuedvs. 442 ± 11 msec in the uncued half).

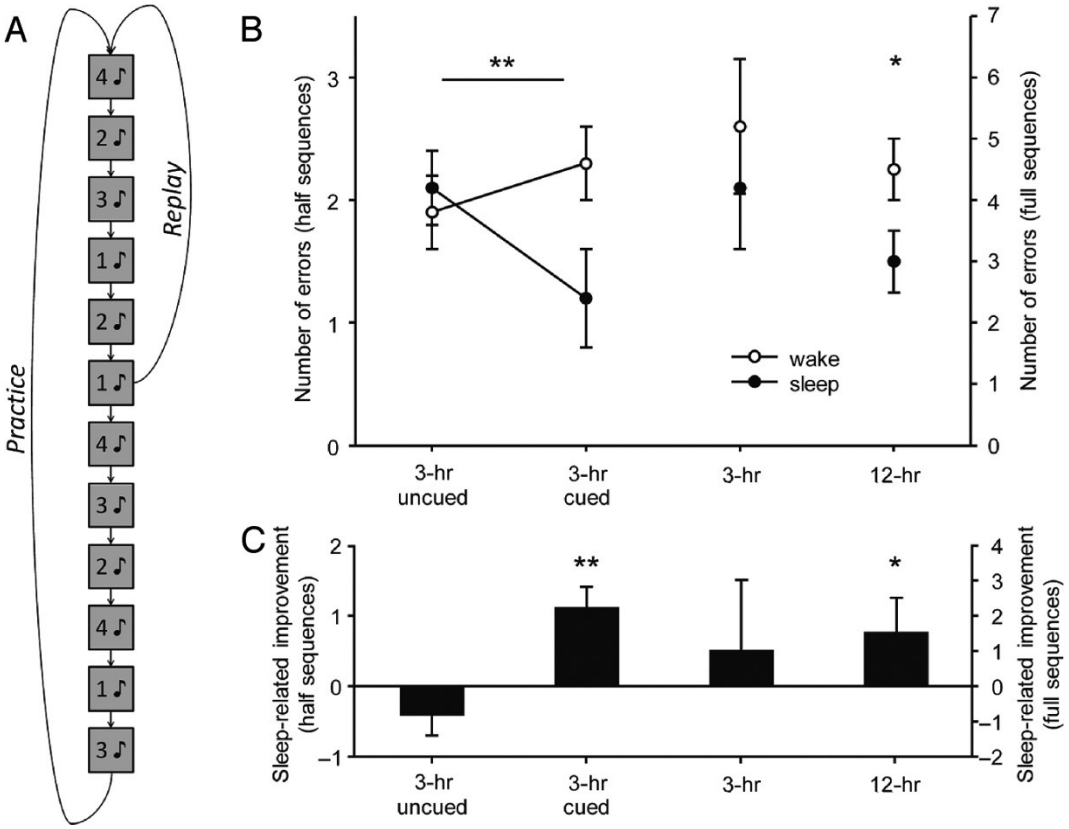

Figure 2. (A) Subjects practiced one of two 12-item tapping sequences. Each key was associated with a tone of a pentatonic scale. The sequences could be divided into halves without changing the transition sequence. During the 3-hour consolidation interval of the first experiment, either the first or the second half of the sequence was replayed to the subjects. (B) Absolute number of typing errors made after periods of sleep or wakefulness. The significant interaction shows that a 3-h sleep period enhanced consolidation of the finger tapping sequence significantly only for the part of the sequence that was cued. (C) Comparing sleep periods with the matching wake control conditions shows that a 3-h period of sleep without external reactivation was not sufficient to improve consolidation of this task significantly. A longer sleep period of 8 h, however, improves consolidation, similar to the shorter sleep period with additional external reactivation. Errors are given with respect to half sequences for the first experiment (left vertical axis), and to full sequences for the second and third experiment (right vertical axis). Values display differences between the sleep and wake groups' performance during retesting. Bars show s.e.m. * signifies an α-level of $p < 0.05$, ** an α-level of $p < 0.01$. Figure reprinted with permission.

Table 1 gives an overview over performance measured as number of errors for all experiments, groups, and conditions, averaged over the last three blocks of training and the three retesting blocks. Corresponding information for RTs can be found in Table 2.

Table 1 – Performance measured as errors during the last three block of training and the three retesting blocks for all experiments, groups, and conditions

		3h sleep, with reactivation *(per half-sequence)*		***3h sleep, no reactivation*** *(per full sequence)*	***8h sleep, no reactivation*** *(per full sequence)*
		Cued	Uncued		
Sleep	Pre	3.8 ± 0.8	3.5 ± 0.4	9.6 ± 1.7	9.0 ± 1.0
	Post	1.2 ± 0.3	2.0 ± 0.3	4.2 ± 1.2	3.1 ± 0.5
Wake	Pre	4.2 ± 0.6	4.4 ± 0.7	9.7 ± 1.2	8.6 ± 0.9
	Post	2.3 ± 0.5	2.0 ± 0.4	5.2 ± 1.2	4.4 ± 0.8

Table 2 – Average reaction times in ms during the last three blocks of training and the three retesting blocks for all experiments, groups, and conditions

		3h sleep, with reactivation		***3h sleep, no reactivation***	***8h sleep, no reactivation***
		Cued	Uncued		
Sleep	Pre	509 ± 19	506 ± 20	461 ± 21	465 ± 12
	Post	463 ± 19	461 ± 22	414 ± 19	392 ± 10
Wake	Pre	493 ± 17	485 ± 15	445 ± 14	520 ± 12
	Post	436 ± 17	434 ± 14	405 ± 14	423 ± 8

Over the five groups of three blocks of the training session, tapping speed improved in parallel for all conditions, $F(4, 52) = 20.6$, $p < .001$ (see Figure 3A), whereas, at the same time, the number of errors increased significantly, $F(4, 52) = 4.7$, $p = .003$ (see Figure 3B). After the consolidation interval, we observe further improvement in speed and, concurrently, a steep decline in the number of errors. Although the improvement in speed did not differ between conditions, the improvement in accuracy was most pronounced in the cued half of the sequence for the sleep group, $F(1, 55) = 4.6$, $p = .04$ for Sleep/ Wake × Cued/ Uncued (Figure 3; again the value represents an average over three blocks).

In two additional experiments, participants slept for either 3 or 8 hr between learning and retesting, without external replay of the sound cues. Control groups stayed awake for the same duration. Compared with wakefulness, the 3-hr sleep period did not suffice to affect memory consolidation. There was no significant difference in the number of errors between 3-hr sleep and 3-hr wakefulness conditions (4.2 ± 1.1 vs. 5.2 ± 1.0, $F(1, 31) = 0.4$, $p = .51$; note that these numbers refer to full sequences, not half-sequences as above). We again found no differences in tapping speed between groups (sleep: 407 ± 9 msec, wake: 410 ± 7 msec, $F(1, 31) = 0.1$, $p = .81$). In contrast, participants who slept for a full night were significantly better than participants who stayed awake during the day. After the longer sleep

interval participants made significantly fewer errors at later retesting than after the wake interval (3.0 ± 0.5 vs. 4.5 ± 0.5, $F(1, 59) = 4.1$, $p = .047$). Again, tapping speed did not differ across groups (sleep: 407 ± 7 msec, wake: 408 ± 7 msec, $F(1,59) < 0.1$, $p = .95$).

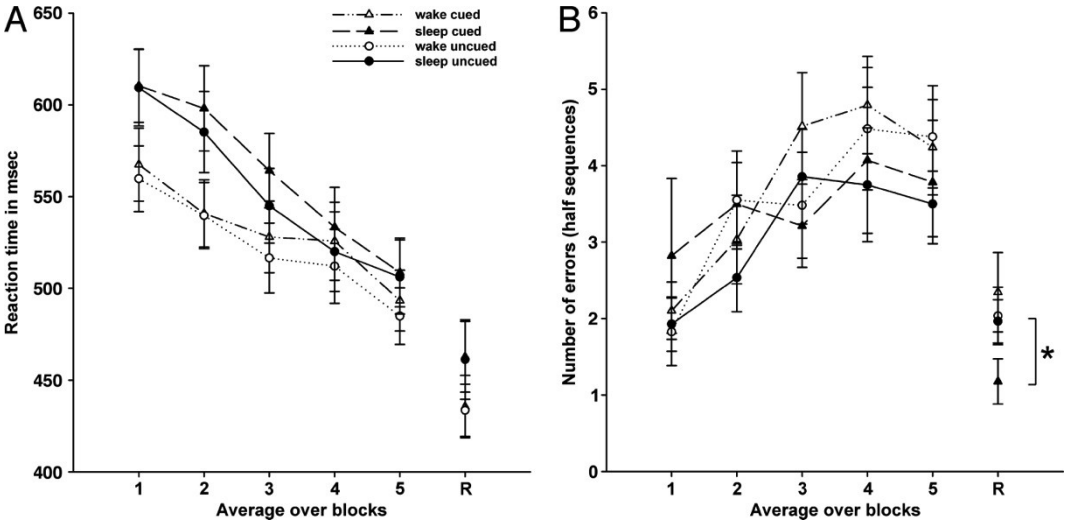

Figure 3. Tapping speed and errors across the six groups of three blocks of training and retesting in experiment one. (A) Tapping speed improves in parallel for all groups and parts of the sequences during training (1-5) and over the consolidation interval as measured during retesting (R). (B) Errors increase with faster reaction times and fatigue during training (1-5), but show a steep decline over the consolidation interval as measured during retesting (R). One can clearly observe the significant benefit achieved by additional external reactivation in the sleep group, which is spaced apart from all other conditions and lies even below the lowest error rate observed during training. Errors are given with respect to half sequences. Bars show s.e.m. * signifies an α-level of < 0.05. Figure reprinted with permission.

Wake conditions of all three experiments show a comparable number of errors, irrespective of reactivation or duration, $F(2, 75) = 0.2$, $p = .81$ (see Figure 2). Pairwise comparisons between all of these conditions did not reveal any significant difference (all $p > .55$). Conversely, benefits from a long sleep interval are similar to benefits from external reactivation. No difference was found between 3-hr sleep with reactivation and a full night sleep condition, $F(1, 57) = 1.0$, $p = .32$. Sample size independent estimates of effect sizes for the sleep–wake comparisons show that, in the 3-hr condition without additional reactivation, sleep exhibits only a small, nonsignificant effect on performance of the 12-element finger-tapping task (Cohen's $d = 0.21$). The significant effect of sleep in the full-night condition without reactivation is $d = 0.37$. We observe the largest effect of sleep in the 3-hr condition with additional reactivation, which is $d = 0.52$ for the cued part of the sequence.

Thus, additional external reactivation seems to bolster the inherent reactivation processes active during sleep and to substitute for a longer sleep period.

The distribution of sleep stages for the 3-hr sleep periods with and without replay of sound stimuli can be found in Table 3. There was no significant difference in total sleep duration between 3-hr groups with and without reactivation. However, participants who received external reactivation cues spent less time in SWS and spent more time in Stage 2 sleep. No differences were seen in time spent in REM sleep or Stage 1 sleep and spindle activity. Regarding alertness, motivation, and interest in the experiment self-report scales showed no difference between the sleep and wake condition; all $p > .25$. Number of lapses, as measured by the PVT, was comparable between sleep and wake groups in all experiments (all $p > .61$).

Table 3 – Sleep duration and sleep stage information (mean ± s.e.m.)

	3h sleep, with reactivation	*3h sleep, no reactivation*	*t*	*p*	*8h sleep, no reactivation*
Time asleep	2h 45min ± 3min	2h 49min ± 3min	0.8	0.45	7h 39 min ± 10 min
Wake	10 ± 3%	5 ± 2%	1.4	0.17	
S1	8 ± 1%	9 ± 1%	0.5	0.63	
S2	39 ± 2%	26 ± 2%	3.9	<0.01	
SWS	34 ± 3%	52 ± 4%	3.8	<0.01	
REMS	9 ± 1%	8 ± 2%	0.8	0.44	
Spindle density	2.4 ± 0.2	2.3 ± 0.1	0.4	0.70	

To determine whether sleep-related improvements could be caused by sleep stage-specific processes, we further analyzed correlations between sleep parameters and postsleep task performance. We found no correlation between the amount of time spent in individual sleep stages and performance. Neither the number of errors after sleep nor the improvement between the last three blocks of training and the three retesting blocks, nor the benefit by reactivation, that is, the difference in improvement between the cued and the uncued part, were related to sleep parameters. The number of sleep spindles per 30-sec epoch of sleep (spindle density) for S2 and SWS was also unrelated to later task performance (see Table 4).

Table 4 – Correlations between sleep parameters and memory performance

		*3h sleep, with reactivation**		*3h sleep, no reactivation*	
		r	p	r	p
Number of errors after sleep	S2	-0.11	0.57	0.25	0.36
	SWS	0.17	0.38	-0.15	0.58
	REMS	0.17	0.38	0.31	0.24
	Spindle density	0.15	0.46	-0.07	0.80
Improvement over sleep	S2	0.12	0.54	-0.13	0.62
	SWS	0.05	0.80	0.12	0.67
	REMS	0.25	0.21	-0.38	0.14
	Spindle density	-0.01	0.96	-0.27	0.32
Benefit by reactivation	S2	0.02	0.92		
	SWS	0.09	0.64		
	REMS	0.30	0.12		
	Spindle density	0.04	0.85		

** the values are given for the cued part of the sequence only*

DISCUSSION

Presenting sound cues during sleep that had been related to procedural sequence tapping beforehand improved later performance of this task. This indicates that reactivation of procedural memory traces can indeed be a mechanism that supports memory consolidation. Notably, the beneficial effect of external reactivation occurred only during sleep. Presenting the same cues during wakefulness had no influence on the consolidation process at the behavioral level. Additionally, external reactivation showed a similar effect as extending sleep: A short 3-hr period of sleep with reactivation cues was as beneficial for consolidation as a whole night of sleep without. Thus, we suggest that presenting cues of a previous learning task drives an internal and naturally occurring mechanism of memory reactivation, resulting in an accelerated consolidation process. Astonishingly, we did not find any significant relationship between sleep parameters and memory performance after sleep. Actually, participants who received sound cues during sleep demonstrated enhanced consolidation although they had less SWS than participants who were not presented with the cues. This unpredicted finding indicates that it might not be the stage of sleep per se which determines how well we consolidate procedural memory, but perhaps rather the availability of sufficient reactivation during sleep.

This study shows that effects of reactivation are highly specific for the material being cued. Instead of cueing the entire context of a finger sequence using differently

pitched tunes, as has been done previously (Antony et al., 2012), we explicitly designed the task in a way that it was possible to cue only a portion of a sequence. We used sequences of tones that only differed in the order of elements. The rationale behind this approach was that this method allows cued and uncued conditions to be entirely symmetrical. Comparing nights with and without cueing could obviously result in unspecific changes in sleep structure, which might influence performance. Using two melodies made from different tones could result in a general reactivation of the context. In our tapping sequence of 12 unique finger transitions, only those transitions belonging to the replayed part of the sequence showed a cueing-related benefit. Transitions in the uncued part of the sequence, although composed of the same tones as the cued part, did not benefit from the procedure. Thus, cueing can target highly specific content, like individual aspects of a memory. Because of this material specificity, our findings cannot be explained in terms of attention, fatigue, or other postintervention effects, as those would affect both the cued and uncued half of the sequence. They rather indicate a direct influence of cueing on memory consolidation.

Some previous studies suggest that rest and recovery from fatigue mediate performance gains in procedural learning after sleep (Mednick, Makovski, Cai, & Jiang, 2009; Rickard, Cai, Rieth, Jones, & Ard, 2008). Our data show that such recovery occurs in both the sleep and wake groups. However, we also show another process, which is related to reactivation and which leads to stronger improvement in the sleep group than in the wake group. Thus, our study identifies an active, sleep-specific mechanism that goes beyond recovery (Korman et al., 2007; Korman, Raz, Flash, & Karni, 2003; Walker, Brakefield, Seidman, et al., 2003). Reactivation might also underlie changes in the neuronal representation of procedural memory on the systems level observed after sleep (Korman et al., 2007; Fischer, Nitschke, Melchert, Erdmann, & Born, 2005; Walker, Stickgold, Alsop, Gaab, & Schlaug, 2005), similar to what has been suggested for declarative memory (Gais et al., 2007; Takashima et al., 2006).

It has recently been demonstrated in rats that presenting acoustic cues for a spatial learning task during sleep biases the content of replay, but not the number of reactivations (Bendor & Wilson, 2012). After presentation of acoustic cues, hippocampal reactivation events showed more frequent replay of those spatial

memory episodes that were associated with the cue than those that were not. Similarly, in our experiments, reactivation enhanced consolidation only for those finger transitions associated with the cue beforehand. This enhancement might even have come at the expense of the uncued transitions, which do not show any benefit of sleep at all. Because of this specificity, the observed off-line gains in performance cannot be explained in terms of global processes that affect the brain on a wider scale, but might rather be related to cueing biasing the content of neuronal replay. Moreover, longer sleep periods enhanced consolidation more strongly than shorter ones. We suggest therefore that sufficient reactivation of the specific memory trace is required for the observed sleep-related benefits on memory consolidation, and this depends on the availability of external or internal cueing and the duration of the sleep period. Whether this influence is dose dependent cannot be concluded from the present data. However, regarding sleep times and interventions, it is tempting to speculate that more reactivation, either by externally driving the inherent process or by longer sleep times, leads to stronger memory consolidation during sleep.

In the framework of systems consolidation in the declarative domain, hippocampal networks are thought to be reactivated during sleep, leading to stabilization of the neural trace and integration of information into neocortical networks (Diekelmann & Born, 2010). Studies on declarative memory have also shown that external reactivation enhances (Diekelmann, Buchel, Born, & Rasch, 2011; Rasch et al., 2007) and accelerates (Diekelmann et al., 2012) the consolidation of memories. Rodent studies demonstrate that the sequences of hippocampal place cell activation observed during learning are replayed during postlearning sleep together with a reactivation in neocortical areas (Peyrache et al., 2009; Ji & Wilson, 2007; Nadasdy et al., 1999; Wilson & McNaughton, 1994). Although the standard model of memory ascribes only declarative memory to the hippocampus, a number of fMRI studies find activity in the hippocampus during motor tasks using explicit and implicit sequences (Walker et al., 2005; Schendan, Searl, Melrose, & Stern, 2003). Additionally, studies indicate that hippocampal activation during sleep can also lead reactivation in subcortical structures like the striatum, which is a central area for procedural learning (Lansink, Goltstein, Lankelma, McNaughton, & Pennartz, 2009; Pennartz et al., 2004). Furthermore, it has been shown that the interaction between the hippocampal and striatal systems during training predicts the subsequent consolidation of a finger-tapping task (Albouy, Sterpenich, et al., 2013) and that

caudate activity increases when task performance becomes more consistent (Albouy et al., 2012). Although activation of the hippocampus and striatum are competitive during learning, they become cooperative during the night, and this interaction optimizes later behavior (Albouy et al., 2008). We have not measured explicit sequence recall; however, it is very likely that the melody allowed the participants, at least partially, to memorize the sequence explicitly. This finding is therefore compatible with findings that mainly explicit sequence learning benefits from sleep (Robertson et al., 2004). The idea of a hippocampal–striatal interaction would suggest that automatization of the task would lead to a more implicit representation of the task after sleep, which depends more on the striatum. To sum up, we propose that hippocampal–striatal interactions during sleep contribute to consolidation in the present procedural memory task. However, this remains to be tested in further studies.

Our data show no relation between sleep macro- and microstructure and the observed effects of sleep on procedural memory consolidation. Previous animal studies investigating reactivation in the hippocampus and neocortex, and human studies on memory reactivation suggest that reactivation occurs primarily in SWS (Antony et al., 2012; Rudoy et al., 2009; Ji & Wilson, 2007). However, that finding might be biased because most of these studies focused their investigations on SWS. Beneficial effects of reactivation in other sleep stages may thus have been overlooked. In contrast, we presented reactivation cues not only during SWS but for 2 hr during all sleep stages. Whereas previous studies, which cued only during SWS, find a correlation between the amount of SWS and task performance (Antony et al., 2012), we did not find such a correlation. In fact, it can be argued that our data even speak somewhat against the idea that SWS is central to consolidation of this task, because by introducing the sound cues, we actually decreased the amount of SWS while improving performance. Thus, our findings, although not contradicting possible effects of reactivation during SWS, also provide no indication that reactivation is restricted to SWS.

Cueing in the present experiments was not restricted to SWS. We therefore cannot distinguish whether cue presentation was effective during light or deep sleep. To exclude that hearing the cues before falling asleep was sufficient to improve task performance, we compared reactivation during sleep with reactivation during

wakefulness. Both the sleep and wake condition comprised periods of quiet wakefulness during which participants listened to the sound cues. An interaction analysis shows that the effect of reactivation differs significantly between sleep and wake groups. Furthermore, Antony et al. (2012) presented cues solely during sleep, and they still observed an effect of a reactivated as compared with a nonreactivated melody, which hints further at a unique role of sleep in memory consolidation. The effect of cueing seems therefore to be nonconscious and sleep-specific.

Our choice of stimulating during the whole sleep period and rely on a wake control group was based on a number of considerations. First, because the present task is procedural in nature and at the time of data acquisition no other data were available, we could not be certain that cueing during SWS would show the expected effect. Additionally, restricting cue presentation to SWS does not prove that cueing effects are SWS related. Only by providing an interaction between sleep and wake conditions, it can be ascertained that effects are limited to sleep. Finally, if cue presentation starts only after sleep onset, there is a higher chance of the participant awakening. Embedding the cues into white noise, on the other hand, might have impaired stimulus discernibility. Potential influences of habituation are assumed to be negligible because repeated stimulation has been found to elicit reliable responses even during sleep (Atienza, Cantero, & Escera, 2001), and, if present, they should affect both sleep and wake conditions equally. Habituation should also lead to less severe disturbance of sleep, because no unexpected sounds occur during sleep. In fact, this experimental design did not disturb participants' sleep, as indicated by similar amounts of time in wake and Stage 1 sleep as in nights without cue presentation.

Comparing effect sizes for the different experimental groups also leads to the conclusion that the magnitude of the effect primarily depends on sleep duration and external cueing, rather than on specific sleep stages. A shorter 3-hr period of sleep with a high proportion of SWS only induces a small, nonsignificant effect. In contrast, a longer 8-hr interval, which contains chiefly more Stage 2 and REM sleep than the shorter interval, significantly enhances performance compared with a wake interval. We thus suggest that it is internal and external reactivation in sleep, which mediates the observed effects on memory consolidation.

Our findings complement a number of previous studies, which see effects of reactivation on consolidation of different material. However, Rasch et al. (2007) also tested cueing of a procedural finger-tapping task and failed to find an enhancing effect. We believe that this was because of the type of cueing used in that study. A slow stimulus like the odor used in that study is more suited to cue the temporal and spatial context of a task, which relates to its declarative components, than individual finger movements. Similar effects of stimulus specificity can also be observed in conditioning, where odor cues can provoke slow responses like taste aversions, but are unable to elicit fast reactions like startle responses.

For procedural learning, sleep-related effects are found for measures of speed and accuracy (Witt, Margraf, Bieber, Born, & Deuschl, 2010; Brown & Robertson, 2007; Walker, Brakefield, Hobson, & Stickgold, 2003; Fischer et al., 2002). Figure 3 shows that speed and accuracy both provide sensitive measures of performance in the present task. Speed increases with training and even more over the retention interval, which speaks for a recovery function of these periods. Accuracy, on the other hand, deteriorates with training, probably because of fatigue. After the retention period, improvement in speed is accompanied by a restoration of accuracy in the 3-hr conditions without reactivation and an improved accuracy with reactivation as well as in the 8-hr sleep condition. Thus, similar to the study of Antony et al. (2012), our results show a significant effect of sleep on accuracy only. We suggest that the lack of a sleep effect on speed, both in the cued and the uncued condition, is because of tone presentation during learning, which has resulted in a tendency to type the sequence more rhythmically and with a consistent speed.

Together, we show that reactivation during sleep can constitute an efficient way to selectively strengthen learned skills. Reactivation during wakefulness did not show the same effect, suggesting that sleep has certain memory-supporting properties, which are not present in the awake state. An explanation based solely on homeostatic recovery from fatigue cannot account for our observation that reactivation only enhanced the replayed parts of the motor sequence task. A surprising finding was that the effect of reactivation was independent of the time spent in specific sleep stages. Although cueing reduced SWS, memory consolidation was enhanced. Our results therefore indicate that it might be rather the amount of

internal and external reactivation and not the time spent in a specific sleep stage that determines the strength of memory consolidation.

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STUDY 5: SLEEP INTEGRATES SEPARATE REPRESENTATIONS OVER MULTIPLE MEMORY SYSTEMS

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MS and SG planned and designed the experiments. MS, FP, and JW collected the data. MS, FP, MC, and SG analyzed the data. MS and SG wrote the manuscript.

This manuscript has been submitted for publication.

ABSTRACT

It has been suggested that beyond stabilization and enhancement, sleep changes the quality of memory. We examined how performance changes over periods of sleep or wakefulness in a feedback-driven classification task. We tested explicit and implicit memory separately, as well as cooperation and competition between both. We find that sleep improves performance and changes the relational structure between memory representations. Explicit and implicit memory were anticorrelated after wakefulness, but showed a positive relation after sleep. Brain activity reflected this finding. Over sleep, hippocampal activity increases in the implicit task, whereas the striatum becomes more active in the explicit task. Additionally, cooperation between both regions increases. Thus, sleep not only improves memory performance, but also leads to integration of explicit and implicit aspects of memory.

MAIN TEXT

Numerous studies show that sleep has a beneficial effect on the consolidation of newly acquired memories (Diekelmann & Born, 2010). Sleep has been shown to support the stabilization of declarative memory (Ellenbogen et al., 2006; Gais et al., 2006) as well as the enhancement of procedural memory (Walker et al., 2002; Geyer et al., 2013) and has thus been deemed a state of high importance to the offline evolution of newly encoded memory traces (Stickgold, 2013). Not only has it been shown that information can be selectively strengthened (Wilhelm et al., 2011), it has also been suggested that sleep-dependent memory processing may change the quality of the memory trace (Stickgold & Walker, 2013). Sleep is thought to help item integration and multi-item generalization, leading to a functionally different representation of memories. While data on stabilization and enhancement of memory by sleep is ample (Diekelmann & Born, 2010), there are only few studies examining the effect of sleep on multi-item generalization (Stickgold & Walker, 2013). Generalization over items can happen either by extraction of gist from sets, or by extraction of rules from relations. Data from artificial grammar learning (Gomez et al., 2006; Hupbach et al., 2009; Nieuwenhuis et al., 2013), statistical learning (Durrant et al., 2011; Durrant et al., 2013) and transitive interference (Ellenbogen et al., 2007) suggest that sleep in fact helps to extract hidden relations between studied items, leading to offline rule extrapolation. In this way sleep may help to form a new framework for explaining contingencies we encounter in everyday life.

During classification learning, two kinds of representations are generated. Detailed episodic memory of individual items allows explicit extraction of relevant features and application to new items or a new context. Implicit statistical learning leads to a non-declarative model of common relations between items, which shapes behavior without awareness and develops gradually over a large number of learning trials. While explicit memory is generally assumed to rely on the hippocampal system, statistical learning tasks rely heavily on the striatum (Shohamy, 2011). Both memory systems can interact during task training and the significance of systems for a specific task can shift over time (Poldrack et al., 2001; Poldrack & Rodriguez, 2004; Wimmer & Shohamy, 2012).

The aim of the present experiments was to directly compare the contribution of sleep to the evolution of both the explicit and the implicit trace acquired during categorization learning. We investigated a form of implicit contingency learning in a typical feedback-driven classification task during which subjects learned a complex decision rule (Fig. 1).

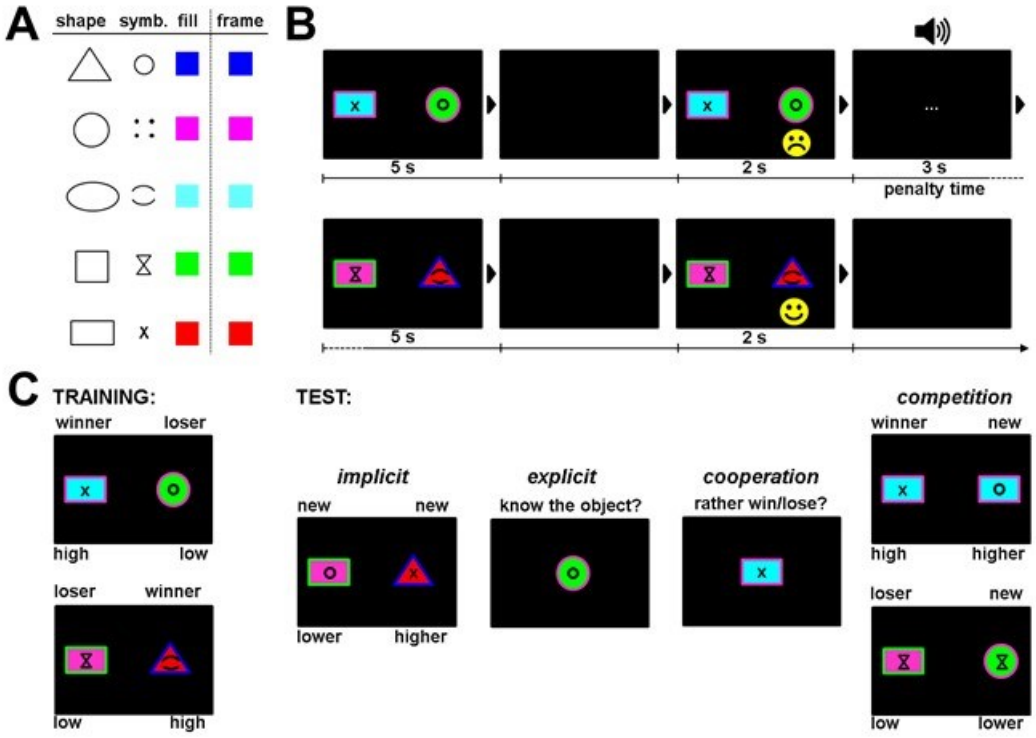

Figure 1. (A) Object dimensions and possible dimension characteristics. Shape, symbol, and fill were the dimensions relevant for the hidden rule. (B) In 625 training trials, subject had to choose the better of two objects based on feedback they received on their decisions. An arbitrarily chosen hierarchy between the different object dimensions defined the value of each single object. Correct decisions were accompanied by positive feedback, symbolized by a happy face. Incorrect decisions were accompanied by negative feedback in form of an unhappy face, a negative sound, and a 3s penalty period. (C) During training, the same 50 objects always either won or lost in all comparisons. Thus, subjects could learn the task using two different strategies: An item-based strategy memorizing the properties of winning and losing objects, or a rule-based strategy by gradually developing a feeling for the underlying complex hierarchical rule. During testing, we separated implicit rule extrapolation (sets of new items) from explicit item knowledge (training item recognition). We further tested memory trace cooperation during decisions whether items would rather win or lose in comparison to other items. To separate contributions of implicit and explicit strategies to our decision task, we paired former winning items with higher-ranking new items and former losing items with lower-ranking new items to assess preferential strategy use during memory trace competition.

Over a large number of trials, they had to decide which of two objects ranked higher according to a hidden hierarchy. During training, the same set of 25 objects always either won or lost in all comparisons. Thus, two different memory traces were established during learning: explicit knowledge about individual items, and an implicit tendency to answer according to rule-related regularities. Importantly, the rule was too complex to derive it intentionally from learned exemplars out of explicit memory. After periods of sleep or wakefulness, we tested explicit item knowledge and implicit rule proficiency separately. We additionally tested cooperation and competition between implicit and explicit memory using tasks that could be solved using both implicit and explicit memory, or using only either implicit or explicit memory (see supporting online methods).

Eighty-seven participants learned the classification task and were tested 12 hours later. Half of the subjects (n=44) learned in the evening and were tested in the morning after having slept for a whole night. The other half (n=43) learned in the morning and stayed awake during the day, before being tested in the evening. To account for effects of time-of-day and simple passage of time on memory, an additional 58 subjects learned either in the morning (n=29) or in the evening (n=29), with the test immediately following the encoding session. In a third experiment, 40 subjects followed the 12-h night-sleep (n=21) versus 12-h day-wake (n=19) protocol, but were tested during functional magnetic resonance imaging (fMRI) to determine brain activity changes underlying the effects of sleep.

First, we tested effects of sleep on implicit and explicit memory. Implicit rule knowledge was tested by comparing how well participants could apply the hidden regularities to new items that they had not seen during training. A night of sleep significantly aided implicit rule learning compared to a day of wakefulness ($p=0.02$, $F_{1,67}=5.9$, Fig. 2). Circadian effects were not observed ($p=0.19$, $F_{1,46}=1.7$; Suppl. Fig. 4). Explicit recognition memory for old items, on first sight, was not affected by sleep ($p=0.33$, $F_{1,67}=0.9$). However, a benefit of sleep for explicit memory may have been masked by circadian effects. In the morning, explicit item memory was enhanced compared to evening performance ($p=0.06$; Fig. 2 and Suppl. Fig. 4). When considering the interaction with circadian effects, a significant effect of sleep on explicit recognition memory becomes apparent ($p=0.02$, $F_{1,116}=5.8$). These results are in line with previous findings that sleep benefits implicit rule learning as well as

explicit declarative knowledge, with less consistent effects when the latter is tested via recognition (Diekelmann et al., 2009; Nieuwenhuis et al., 2013).

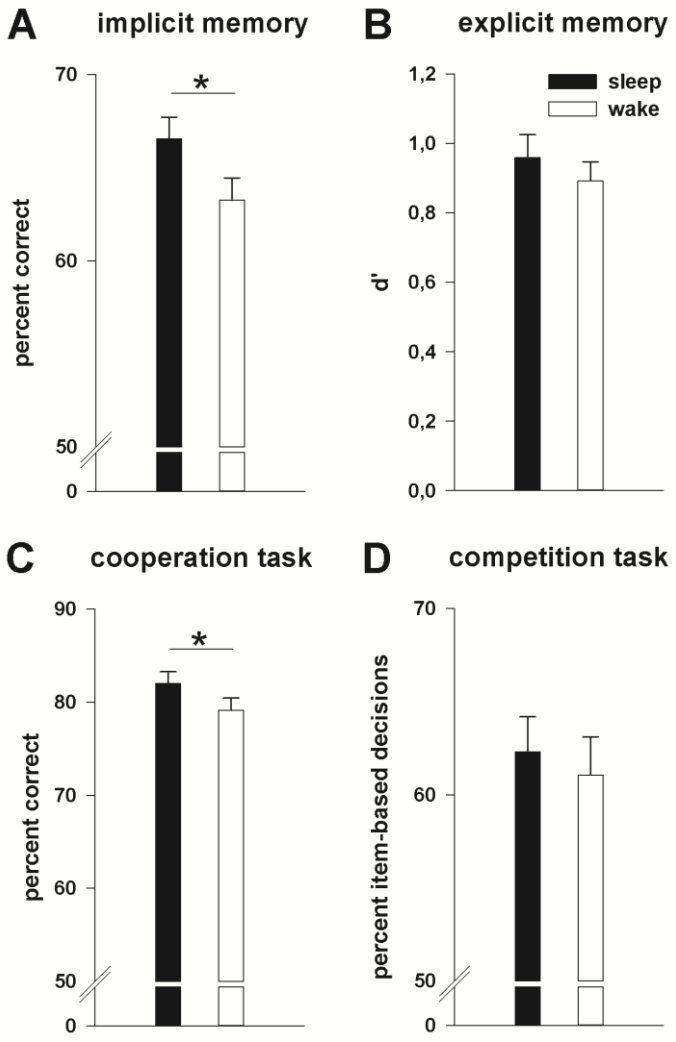

Figure 2. (A) Sleep significantly benefitted implicit memory measured by the extrapolation of the implicit rule underlying the classification task. (B) Both groups could discriminate learned items equally well. Note, however, that a marginally significant circadian effect may have masked effects of sleep on explicit item recognition (Suppl. Results and Suppl. Fig. 2B) (C) Participants who slept could decide significantly better whether an item would rather win or lose in comparison to other items, a measure which reflects both explicit item and implicit rule knowledge and thus assess memory trace cooperation. (D) Sleep did not bias participants towards either a more item-based or a more rule-based decision strategy when old winner or loser items were paired with higher- and lower-ranking items to create competition between explicit and implicit knowledge. * $p < 0.05$

We assessed two other aspects of memory to test cooperation and competition between memory traces. In a task where explicit and implicit knowledge had to be used cooperatively, subjects had to judge whether an item would rather win or lose. Here, again, we find a positive effect of sleep over wakefulness ($p=0.02$, $F_{1,67}=6.3$). Finally, to have a sensitive measure of whether sleep selectively affects explicit or implicit memory, we set old winning items against higher-ranking new ones and old losing items against lower-ranking new ones. Thus, subjects would choose differently based on their item knowledge or based on their rule knowledge. Any differential change in memory strength between sleep and wakefulness should be observable in this competitive test, however, no such effect emerged ($p=0.64$, $F_{1,67}=0.2$).

We further examined how the structure of memory develops during consolidation in sleep and wakefulness. Using structural equation modeling, we analyzed the interrelations between training performance and the four aspects of retest performance (Fig. 3 and Suppl. Results). Nested-models comparisons demonstrate that the structural relations between different aspects of memory remain unchanged over a 12-h consolidation interval containing only wakefulness when compared with immediate testing ($p>0.70$), but they change significantly during a 12-h interval containing sleep ($p=0.04$). Thus, while longer periods of wakefulness do not modify memory structure, memory representations evolve and change over sleep.

A closer look at path weights in the structural equation model highlights the unique role of sleep in offline memory processing. Implicit and explicit knowledge are strongly negatively correlated at immediate testing ($r=-0.59$) and stay so after periods of wakefulness ($r=-0.46$). However, over sleep this negative relation becomes positive ($r=0.15$). Furthermore, sleep reduces the close association between initial learning and retest performance for explicit and implicit memory tasks, indicating that these measures are influenced by an additional source of variance over sleep, but not during wakefulness: nested models analyses show that the assumption of independence between training and test performance is violated in the wake and immediate control groups (all $p<0.001$), whereas models assuming independence in the sleep group do not deviate from observed data (all $p>0.21$). We

suggest that this independence of training performance develops as a sign of memory trace reactivation and consolidation during sleep (Rasch et al., 2007).

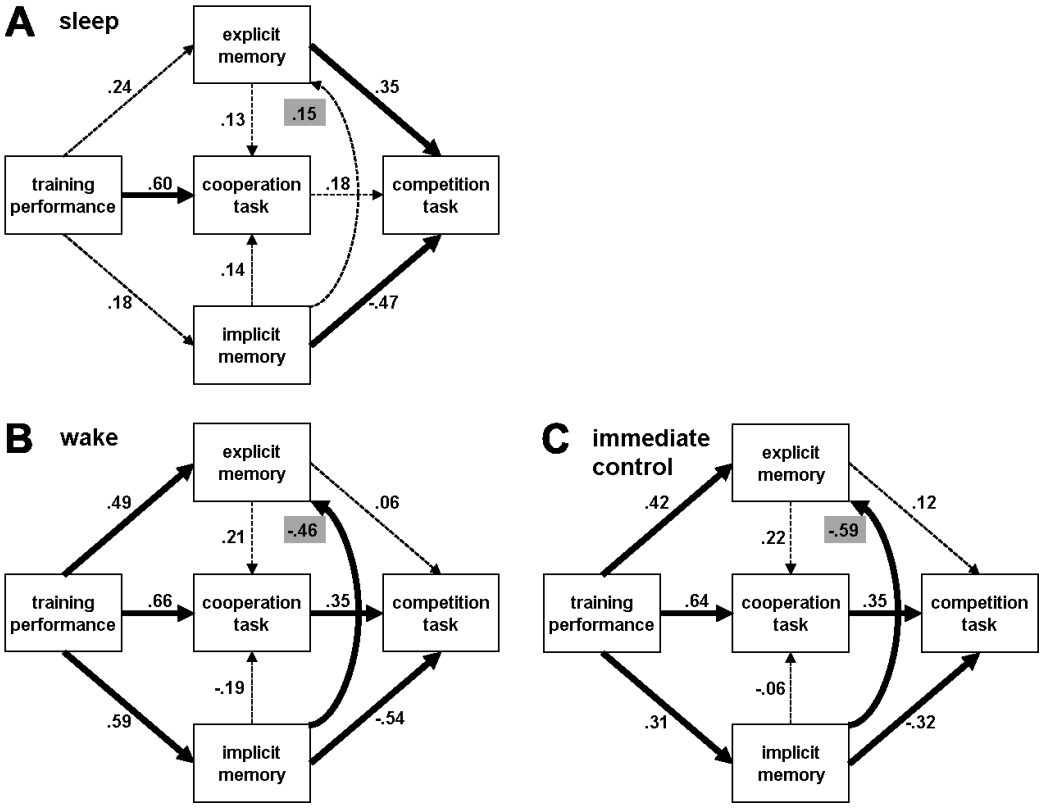

Figure 3. We fitted a model where the level of initial learning can mediate the strength of both the explicit and implicit trace. Both explicit and implicit memory can influence decisions in the cooperative and competitive tasks. Competition between the explicit and implicit traces was measured as percent item-based recall in a conflict situation. The wake and immediate control groups show very similar path strengths between all measures, whereas the sleep group deviates from this pattern. Differences between groups must originate from memory processing during sleep-dependent consolidation. Bold lines: Significant correlation at $p < 0.05$. All values reported for the comprehensive model.

Finally, whereas performance in the cooperative task was positively related to explicit and negatively related to implicit performance after short or long delays without sleep, they both contribute positively after an interval of sleep (Fig. 3). This cooperation of systems can be causal to the increased performance after sleep reported above. Interestingly, an additional test of explicit rule knowledge during debriefing showed that participants in the sleep group more frequently understood that the rule required an integration of item properties across multiple item dimensions than participants who stayed awake (χ^2=4.4, p=0.04; Suppl. Results). Together, these behavioral analyses show that sleep changes the structure and

quality of memory, whereas no such changes occur over corresponding periods of wakefulness.

Since sleep induces cooperation between implicit and explicit aspects of memory, we hypothesized that this should also be reflected on the level of brain activity. Previous studies using similar rule learning tasks related explicit memory to hippocampal activity and implicit knowledge to striatal activity (Poldrack et al., 2001; Doeller et al., 2006). In a subsequent fMRI experiment, we scanned participants during testing after sleep and wake conditions (see supplemental information for behavioral data). Congruent with our behavioral results, showing integration of implicit and explicit memories, implicit memory recall evoked more activity in the hippocampus after sleep than after wakefulness (Fig. 4A and Suppl. Table S1). Similarly, explicit memory retrieval after sleep activated the caudate nucleus more strongly than after wakefulness (Fig. 4B and Suppl. Table S2). Thus, participants who slept during the consolidation interval recruit structures usually related to explicit memory during the implicit task, and structures related to implicit memory during the explicit task. This finding strongly supports recent ideas that implicit striatal and explicit declarative memory are less strictly separated and can interact more flexibly than has previously been thought possible (Albouy et al., 2008; Sadeh et al., 2011).

With regard to the cooperative task, participants who slept activate both the hippocampus and the caudate nucleus more strongly than those who stayed awake (Fig. 4C and Suppl. Table S4). Finally, to investigate whether hippocampus and striatum complement each other, i.e. either one or the other solving an item, or cooperate with each other, i.e. both contributing to the same items, we performed a psychophysiological interaction analysis. This analysis revealed increased functional connectivity between these two regions for correct answers after sleep compared with wakefulness (Fig. 4D and Suppl. Table S5). Both regions are therefore concurrently active during successful memory retrieval in this task. We conclude that sleep promotes not only stronger activity in both the hippocampal and the striatal memory systems per se, but an actual cooperation between these two systems.

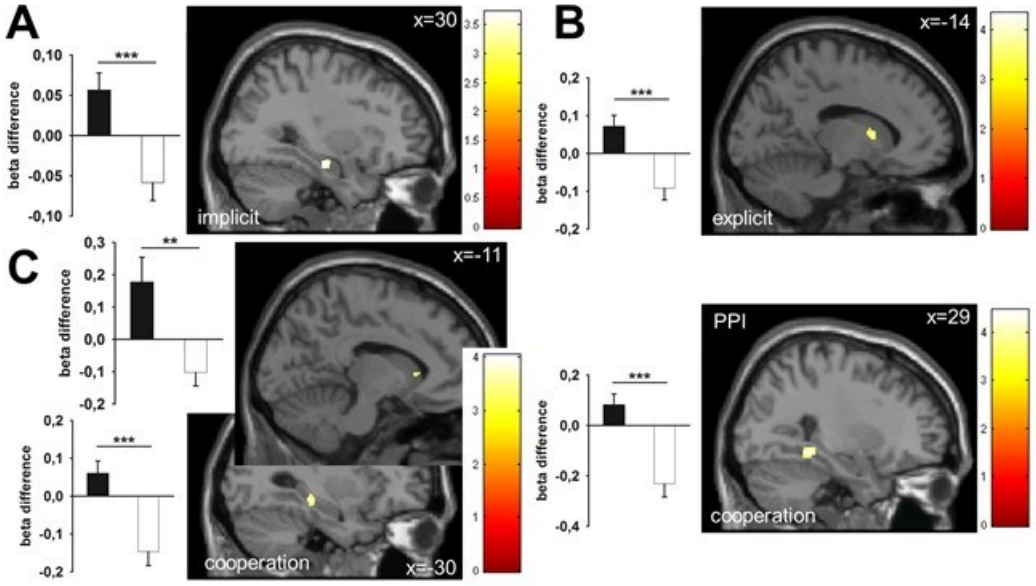

Figure 4. (A) Implicit memory recall activates the hippocampus after sleep (sleep-wake × correct-incorrect). (B) Explicit memory recall activates the caudate nucleus after sleep (sleep-wake × correct-incorrect). (C) In the cooperative task, we see higher activation in both the hippocampus and the caudate nucleus after sleep (sleep-wake × correct- incorrect; left panel). (D) We also observe an increased connectivity between these structures during correct memory recall (S-W; right panel). Black bars represent beta values in the sleep group, white bars show beta values in the wake group.

Similar cooperative interactions between hippocampus and striatum have been observed previously in an implicit oculomotor sequence learning task (Albouy et al., 2008). Our present results generalize those findings. After sleep, normally hippocampal-dependent explicit memory activates the striatum and normally striatal-dependent implicit rule knowledge activates the hippocampus. We show that periods of sleep, but not of wakefulness, introduce changes in the structure of memory on the behavioral level as well as in terms of brain activity. Whereas strengthening of memories might be achieved by a number of different synaptic mechanisms, inducing cooperation between memory systems requires an active systems consolidation process, during which memory traces are co-activated. Such activation has been shown during sleep within the hippocampus (Rasch et al., 2007; Fuentemilla et al., 2013), but also in an interaction between hippocampus and striatum (Lansink et al., 2009). Together, sleep strengthens all parts of our memory representation. Our results show that rather than favoring one representation over the other, sleep integrates different aspects of memory that otherwise would remain separate, thereby enabling an improved performance.

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SUPPLEMENT

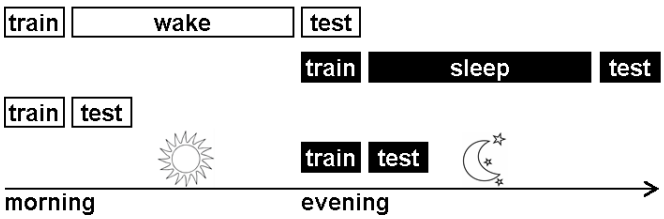
METHODS

PARTICIPANTS

185 healthy young adults aged between 18 and 30 years (24 ± 2 [mean ± SD]) took part in the three experiments of this study. Experiments were approved by the ethics committee of the Department of Psychology at LMU Munich. All participants gave informed consent and were paid or received course credit for their participation. They were German native speakers, did not have any neurological conditions, had a regular sleeping pattern of 6–10 hours a night, and did not have shiftwork or overseas flights in the 6 weeks prior to the experiment. All were non-smokers and did not take any medication other than oral contraceptives. Color vision was tested with standard Ishihara screening plates. On the days of the experiment, participants abstained from drinking alcohol and from consumption of caffeine.

GENERAL DESIGN AND PROCEDURE

The main experiment followed a 12-h day-wake vs. 12-h night-sleep design (see Suppl. Fig. S1). Participants learned a feedback-driven classification task either in the morning, 1 hour, or in the evening, 13 hours after their habitual getting-up time. Performance was tested after a 12-h consolidation period, during which they had either a full night of sleep or a normal day of wakefulness.



Supplementary Figure S1. Experimental design. The top bars show the time-course of the day-wake vs. night-sleep design in which we tested effects of sleep on rule learning performance and brain activity, using fMRI. The bottom bars show the corresponding time-courses for the circadian control experiment where participants' performance was tested immediately after learning in the morning or in the evening.

Participants were randomly assigned to either the sleep or wake condition; sex was balanced across conditions. Activity during the consolidation period was monitored using actimetry. Additionally participants kept sleep logs during the two nights preceding the experimental session and on the day of the experiment itself to verify regular sleeping patterns. Participants in the sleep condition slept on average 7h 14min ± 7min during the night after learning the classification task. At the end of every visit to the laboratory, participants performed a 10-min psychomotor vigilance task (PVT) (*1*). Fatigue did not differ between conditions (number of lapses – learning: sleep 1.3 ± 0.4, wake 1.1 ± 0.3; t_{55}=0.4, p=0.68; retrieval: sleep 1.0 ± 0.2, wake 1.0 ± 0.4; t_{55}=0.1, p=0.94; median reaction times – learning: sleep 289ms ± 5ms, wake 293ms ± 6ms; t_{55}=0.6, p=0.58; retrieval: sleep 282ms ± 5ms, wake 291ms ± 5ms; t_{55}=1.1, p=0.24). In an experiment designed to control for circadian influences, participants learned the task either in the morning or in the evening, but they were retested immediately after training to control for circadian effects on performance. A third experiment followed the same design as the main experiment, but here performance was tested in the MRI scanner.

TASK

During classification task learning, two stimuli were presented on a black screen, and participants had to decide which of these items has a higher rank, i.e. wins the comparison (see Fig. 1A in main text). The rank of the stimuli was defined by a hidden rule reflecting a linear combination of values assigned to stimulus properties. Stimuli differed on four dimensions (shape, symbol, fill color, frame color). Each dimension had five possible values. For every participant, we assigned a new arbitrary hierarchy to the five values of the dimensions shape, symbol, and fill color. Frame color served as a distractor. According to the hierarchy, scores between 1 and 5 were assigned to each value. A score between 3 and 15 was calculated for each object by adding these values. Because of this small range of scores for the 625 possible different stimuli, this task was difficult to learn and it was impossible for the subjects to deduce the underlying value system.

During training, the participants' task was to decide which of the two presented items ranked higher according to this score, and to indicate the result via keypress with the left or right index finger, respectively (see Fig. 1B in main text). They performed 625 of these decision trials. In each trial, the two items were shown for a

minimum of 2 seconds. If participants did not react within 5 seconds, the stimuli vanished and a black screen was shown until a decision was made. The short presentation time and the large number of trials made it virtually impossible to keep all eight different features of one display in working memory. This procedure prevented participants from actively trying to discover the hidden rule underlying the classification task by intensely studying, comparing, and excluding different alternatives based on the stimuli present on screen. After each reaction, a symbolized happy or sad face showed up for 2 seconds, depending on whether the answer was correct or not. If the items had already disappeared when the decision was made, they reappeared together with the smiley to give feedback on the response. The sad face was additionally accompanied by an aversive sound and followed by an empty screen and a penalty delay of 3s, emphasizing the error.

During the 625 training trials, 50 items were shown in varying combinations in such a way that 25 items were always the higher ranking ones (winner items) and 25 items were always the lower ranking ones (loser items). Thus, participants could solve the task either by explicitly remembering for single items if they were winners or losers (item-based), or by implicitly learning the underlying hidden rule that determined the item's rank (rule-based). Instructions made participants aware of both possible solutions. They were also informed that they would be asked to recognize the stimuli later.

In the retrieval session, four different aspects of memory were tested. Implicit memory for the hidden rule was tested by pairing two new items. Participants had to decide, which item ranked higher. These decisions could only be based on implicit memory for the underlying rule. Explicit item memory was tested in a recognition task by presenting those 50 items that had appeared during learning and 50 new ones. Participants indicated whether an item was old or new. Additionally, we used a cooperative memory task that could be solved with the help of both explicit and implicit memory. Participants were asked for individual, previously presented items to judge whether these items would rather win or lose in comparison to other items. In this task, best performance can be achieved by relying on both explicit and implicit memory representations. Finally, in a forced-choice task, we created a competition between item-based memory and rule-based memory. All 50 stimuli were presented together with new items. These new items were chosen in a way

that winner items would now lose according to the underlying rule, and, vice versa, loser items would now win. We thus induced a competition between the rule-based and the item-based solving strategy. Participants could decide based on what they implicitly learned about the hierarchy of the stimuli's property values, or they could explicitly remember individual items as winners or losers. A higher number of item-based responses indicates stronger explicit memory, whereas a higher number of rule-based responses indicates stronger implicit rule knowledge. This task is particularly sensitive to relative changes in the use of these two memory systems. During all retrieval tests, no feedback for correctness of decisions was given. Participants were questioned about their explicit knowledge of the hidden underlying rule during debriefing at the end of the retrieval test.

STATISTICAL ANALYSIS

We compared differences in memory performance between participants who slept after learning and participants who stayed awake. Implicit rule knowledge was measured as the percentage of correct decisions. Explicit recognition was analyzed in terms of memory sensitivity d' which is calculated as Z(hits)–Z(false alarms). Memory trace cooperation was assessed by the percentage of correct decisions whether items would rather win or lose. Memory trace competition was measured as percentage of item-based decisions. Chance performance was at 50% for the implicit memory test and the cooperation task. In the competitive task, 50% meant that neither item-based nor rule-based decisions were favored. Statistical testing relied on univariate ANOVAs. Training performance was entered as a covariate to account for differences in initial learning level. All tests were two-tailed with an α-level of 0.05. All results are given as mean ± SEM.

Explicit knowledge of underlying rules was dichotomized according to complexity. Verbal reports were scored either as complex rules integrating item properties over multiple feature dimensions or as simple ones considering only a single item property. Differences were analyzed using χ^2 tests.

We further analyzed the relationships between different aspects of memory using structural equation modeling with manifest variables. Path analysis allows analyzing the co-dependent relations between multiple parameters using partial and semi-partial correlations. We fitted a comprehensive model using multi-group analysis including the sleep group, the wake group and the control group, which was tested

immediately after learning. Data fit and parameter estimates were calculated using maximum likelihood estimation. We report standardized estimates throughout the manuscript. Model fit was tested using χ^2, root mean square error of approximation (RMSEA), and standardized root mean square residual (SRMR) as absolute fit indices, which reflect the average deviation per person and degree of freedom from the observed data (small values indicating good fit of the data with the proposed model), and the comparative fit index (CFI), which, as a relative fit index, quantifies the difference of the postulated model to a maximally restricted baseline model that assumes all correlations to be zero (large values indicating good fit of the data with the proposed model). P-values for model fit were determined using a 500-fold Bollen-Stine bootstrap. We assured that modification indices showed no correlated residuals, which would suggest that specification of additional paths was required in the proposed model. To test for differences in the structure of memory between groups, we restricted parameters and tested nested models against the comprehensive model including all plausible paths. A model is said to be nested within another model when its set of freely estimated parameters is a subset of those estimated in the baseline model, and it is thus restricted compared with this model. Nested-models allow comparing goodness of fit for different theoretical models directly, providing objective and robust criteria which inform about comparative model fit. A nested model is assumed to fit the data well when χ^2-differences between the baseline model and the restricted model are small and data fit in the nested model does not deviate significantly from data fit in the baseline model.

FMRI ACQUISITION AND ANALYSIS

Whole-brain functional T2*- weighted MRI data were acquired at the Max Planck Institute of Psychiatry, Munich, with a 3-T MR750 scanner (GE) using a 12-channel head coil and a single-shot echo planar imaging (EPI) sequence (voxel size: 1.88 x 1.88 x 3.5 mm^3; matrix size: 128 x 128 x 42; repetition time 2,500 ms; echo time 30 ms; flip angle 90°; interleaved slice acquisition). Data were analyzed in SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8). Preprocessing included realignment to the first volume, spatial normalization to a standard EPI template, and smoothing with an 8 mm FWHM Gaussian kernel. Data were analyzed using mixed-effects models. At the first level, brain responses to stimulus presentation were convolved with a standard hemodynamic response function, and were

modeled together with 12 nuisance regressors related to head movements (6 movement vectors derived from rigid body realignment and their respective first order derivatives) in a GLM for fixed effects for each individual subject. The first four scans were discarded to allow for magnetic saturation effects. High-pass filtering was implemented in the matrix design by using a cutoff period of 128 s to remove low-frequency drifts from the time series. Serial correlations in the fMRI signal were estimated by using a first-order autoregressive plus white noise model and a restricted maximum likelihood (ReML) algorithm. The effects of interest consisted in the hemodynamic response to correct and incorrect decisions in the different retrieval tasks. This was tested by linear contrasts, generating statistical parametric maps. These summary statistic images were further smoothed (6-mm FWHM Gaussian Kernel) and entered in a second-level analysis.

Data of all participants were combined in a full factorial random effects model with the factors performance (correct decisions/incorrect decisions) and group (sleep/wake). Main effects and interactions were then calculated and tested by using one-sided t contrasts. ReML estimates of variance components were used to allow for unequal variance and possible deviations from sphericity. Psychophysiological interactions (PPIs) were estimated for the cooperation task by using a seed region in the caudate that was based on caudate activation in the ([sleep – wake] × [correct – incorrect]) contrast.

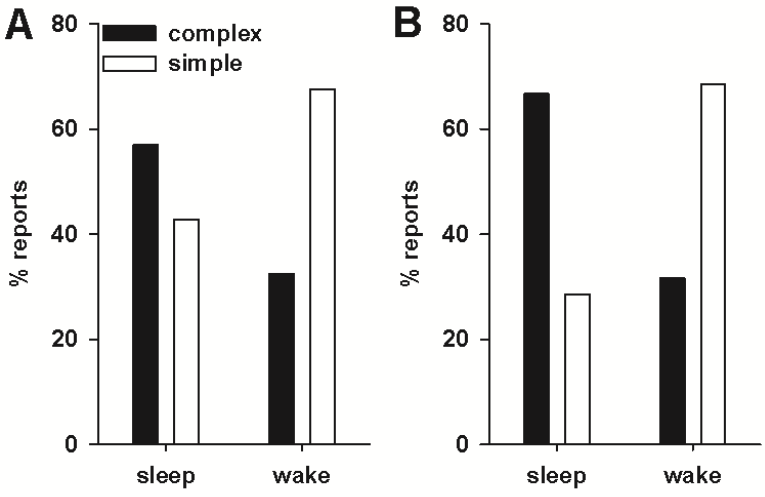
Only peaks reaching the statistical threshold of $p_{uncorr} < 0.001$ with the cluster exceeding a voxel count of 10 are reported in the results section and tables. For regions with a priori hypotheses, correction for multiple testing was performed for small volumes of interest (SVC). As regions of interest we used anatomical masks of the hippocampus and caudate nucleus, bilaterally. Peaks reaching the statistical threshold of $p_{SVC} < 0.05$ are additionally reported in the results section and tables. All coordinates are given as standard Montreal Neurological Institute (MNI) coordinates and correspond to the maxima of the reported cluster of activation. Coordinates were labeled by using the Anatomy 2.0 toolbox (available at http://www.fz-juelich.de/inm/inm-1/DE/Forschung/_docs/SPMAnatomy Toolbox/SPMAnatomyToolbox_node.html). Functional images are displayed on a single subject T1 image.

SUPPLEMENTARY RESULTS

BEHAVIORAL FINDINGS OF THE MAIN EXPERIMENT

Participants who slept after learning were significantly better at applying the implicit rule to new items (sleep: 66.6% ± 1.2%, wake: 63.3% ± 1.2%; $F_{1,67}$=5.9, p=0.02). No difference was found for explicit item recognition memory (d' – sleep: 0.95 ± 0.07, wake: 0.89 ± 0.05; $F_{1,67}$=0.9, p=0.334). This lack of result may be due to circadian effects counteracting the beneficial effect of sleep (see results on circadian control experiment below). Participants who slept performed significantly better in the cooperation task (sleep: 82.0% ± 1.3%, wake: 79.1% ± 1.3%; $F_{1,67}$=6.3, p=0.02). Sleep favored neither an item-based nor a rule-based decision strategy when these were directly competing against each other (item-based responses – sleep: 62.3% ± 1.9%, wake: 61.1% ± 2.0%; $F_{1,67}$=0.2, p=0.635; see Fig.2 in main text).

After the retrieval tests, participants were questioned about their explicit rule knowledge. Participants who slept were significantly more likely to report complex rules that involved integration of item properties across more than one dimension, whereas those who stayed awake more frequently reported simple rules that considered only one item property (sleep – complex: n=20 [57.1%], simple: n=15 [42.9%]; wake – complex: n=12 [32.4%], simple: n=25 [67.6%]; χ^2=4.4, p=0.04; see Suppl. Fig. S2A).

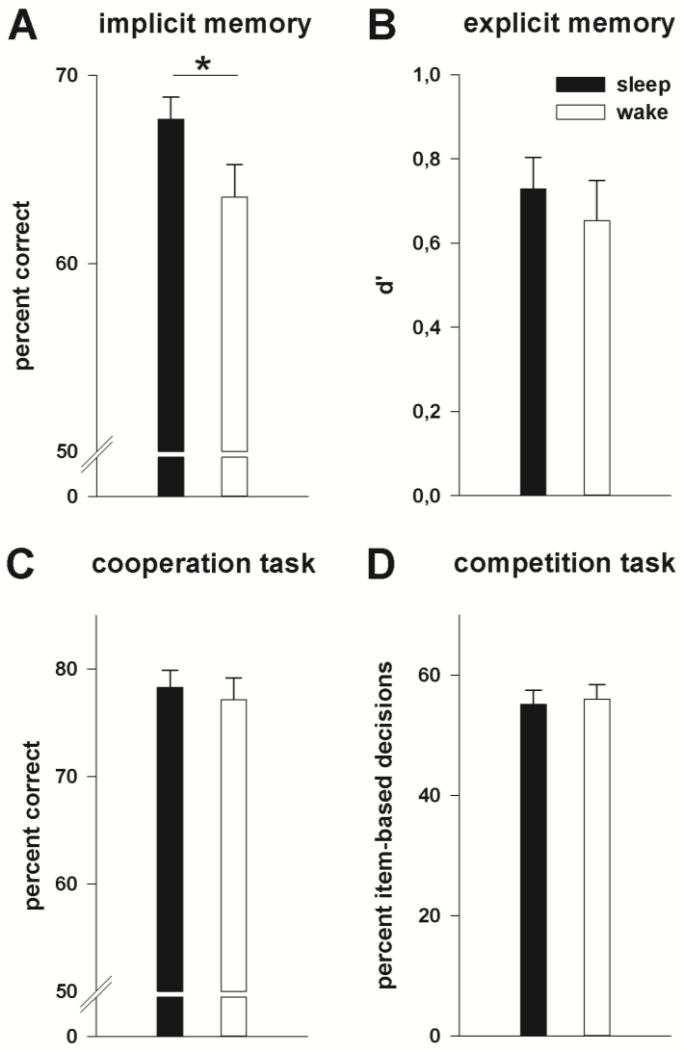


Supplementary Figure S2. Percentage of subjects reporting a complex rule, comprising the integration of at least two feature dimensions for (A) the main experiment and (B) the additional fMRI experiment.

BEHAVIORAL FINDINGS OF THE FMRI EXPERIMENT

We were able to replicate the behavioral results of the main experiment in 40 participants who underwent fMRI scanning during the test session. Participants who slept performed better during implicit memory recall, explicit memory recall and on the cooperation task. Because of the smaller sample size, results reached significance only for implicit rule memory (implicit rule learning - sleep: 67.7% ± 1.2%, wake: 63.5% ± 1.7%, $F_{1,37}$=4.4, p=0.04; explicit recognition memory d' – sleep: 0.73 ± 0.07, wake: 0.65 ± 0.10, $F_{1,37}$=0.4, p=0.54; cooperation task – sleep: 78.3% ± 1.6%, wake: 77.1% ± 2.0%, $F_{1,37}$=0.4, p=0.51; item-based responses in the competitive task – sleep: 55.1% ± 2.3%, wake: 56.0% ± 2.4%, $F_{1,37}$=0.1, p=0.80; see Suppl. Fig. S3).

Again, participants who slept were significantly more likely to report complex rules that involved an integration of values for item properties across more than one item dimension (sleep – complex: n=14 [66.7%], simple: n=6 [28.6%]; wake – complex: n=6 [31.6%], simple: n=13 [68.5%]; χ^2=5.8, p=0.02; see Suppl. Fig. S2B; note that data from one participant is missing for this analysis).

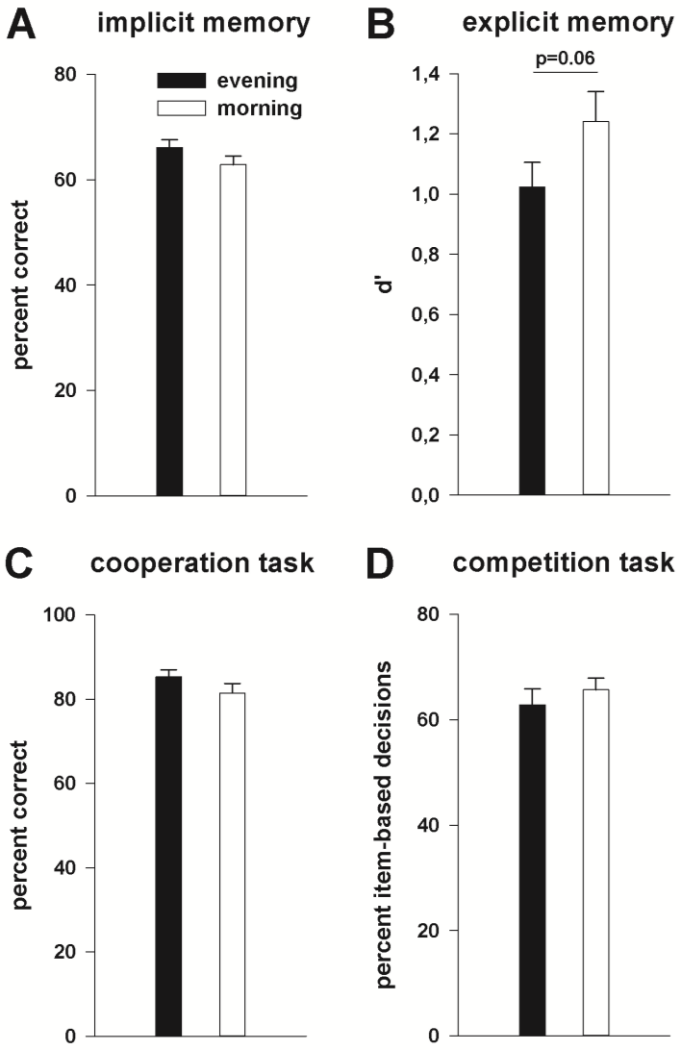


Supplementary Figure S3. Performance during retrieval of the classification task in the sleep and wake groups of the fMRI experiment. The sleep group performed significantly better on implicit rule memory (A). Performance on explicit item recognition and in the cooperative memory task was also numerically better after sleep, but significance was not reached, perhaps due to the small sample size (B, C). There was no difference in which memory system was used preferentially in the competitive memory task (D).

CIRCADIAN CONTROL EXPERIMENT

In the circadian control, we did not find significant differences between the morning and evening groups in implicit rule memory (evening: 66.1% ± 1.5%, morning: 62.9% ± 1.6%; $F_{1,46}$=1.7, p=0.19, see Suppl. Fig. S4), in the cooperative task (evening: 85.3% ± 1.7%, morning: 81.4% ± 2.3%; $F_{1,46}$=1.4, p=0.25), and in the competition between item-based and rule-based recall (item-based responses – evening: 62.8% ± 3.0%, morning: 65.7% ± 2.2%; $F_{1,46}$=0.9, p=0.35). However, participants

tended to be better at recognizing the individual items in the morning than in the evening (d' – evening: 1.02 ± 0.08, morning: 1.24 ± 0.10; $F_{1,46}$=3.7, p=0.06). This circadian effect may have masked potential effects of sleep on explicit memory. Complexity of rule reports did not differ between the morning and evening group (evening – complex: n=13 [54.2%], simple: n=11 [45.8%]; morning – complex: n=11 [44.0%], simple: n=14 [56.0%]; χ^2=0.5, p=0.48).



Supplementary Figure S4. Performance during immediate retrieval of the classification task in morning and evening learning groups. We found no differences in performance between groups who learned in the morning and in the evening on implicit rule memory (A), in the cooperative memory task (C) and in the competitive task (D). Participants who had learned in the morning tended to show better explicit recognition than participants who were trained on the task in the evening (B).

STRUCTURAL EQUATION MODELING

We further examined the relationships between different aspects of memory performance using path analysis in structural equation models. Training performance and all four measures of test performance were entered into this analysis. We modeled all plausible associations in a multi-group analysis, where parameters were free to vary across the sleep, wake, and control groups. We assumed that the level of initial learning affects the strength of explicit and implicit memory as well as of the win-or-lose task, which relied on the cooperation between explicit and implicit memory. In the competitive task, decisions could be made following either an item-based, explicit strategy or a rule-based, implicit strategy. Therefore, both the cooperative and the competitive task were assumed to be influenced by explicit and implicit memory. In addition, we were interested in the relation between implicit and explicit memory. Together, this led to a well-fitted model ($p=0.46$, $\chi^2=3.3$, $df=3$, indicating no significant deviation of the assumed model from the structure of the empirical data; RMSEA=0.03, CFI>0.99, SRMR=0.01; see Fig. 3 in main text). Modification indices showed no correlated residuals and suggested no additional paths that were not specified in our model.

The paths between the different observed variables show very similar weights for the wake and the immediate-testing control group. Thus, in a second step, we tested whether the wake group and the control group show the same underlying structure of memory, and whether the underlying structure in the sleep group deviates from the structure shown by the control group in two nested-models analyses. We restricted parameters by requiring all paths between the paired groups to be equal. While this restricted model can be accepted for the wake group (wake group and control group are not significantly different: $p=0.70$, $\chi^2_{diff}=6.4$, $df_{diff}=9$), this assumption does not hold for the sleep group ($p=0.048$, $\chi^2_{diff}=17.0$, $df_{diff}=9$). Because assuming an identical structure of memory for the wake and control groups is parsimonious and fits the data well ($p=0.69$, $\chi^2=9.8$, $df=12$; RMSEA<0.01, CFI=1.00, SRMR=0.01; modification indices showed no correlated residuals), all further analyses were conducted on this restricted model.

The main difference between the sleep group and the wake and immediate control groups lies in the relation between explicit and implicit memory. Whereas we observe a strong negative partial correlation between explicit and implicit memory

in the wake group (r=-0.55, p<0.001) and in the control group (r=-0.54, p < 0.001; values for the restricted model), the partial correlation in the sleep group is positive (r=0.15, p=0.38). This indicates that over sleep, but not over wakefulness, these two types of memories stop being in competition with each other and become cooperative. Indeed, nested-model comparisons show, that assuming no correlation between explicit and implicit memory in the wake and control group does not fit the data (p<0.001, χ^2_{diff}=23.0, df_{diff}=1).

Furthermore, we observe higher correlations in the wake and immediate control groups than in the sleep group between training performance and explicit and implicit memory tests (explicit – sleep: r=0.24, wake: r=0.50, control: r=0.45; implicit – sleep: r=0.18, wake: r=0.48, control: r=0.42; values for the restricted model). Nested models analyses show that we can assume both explicit and implicit memory to be independent of training performance in the sleep group (p=0.21, χ^2_{diff}=3.1, df_{diff}=2), whereas neither explicit (p<0.001, χ^2_{diff}=18.5, df_{diff}=1) nor implicit memory (p<0.001, χ^2_{diff}=17.7, df_{diff}=1) is independent of initial performance in the wake and control groups.

FUNCTIONAL IMAGING RESULTS

After 12 hours in the sleep condition, implicit rule memory activates the hippocampus, which is usually associated with explicit memory recall, more strongly than after wakefulness (p_{SVC}=0.03, t_{76}=3.7; see Table S1). In addition, we see stronger activation in the caudate nucleus in the same contrast (p_{SVC}=0.01, t_{76}=3.6; see Table S1).

Table S1. Implicit rule memory. Regions with higher activation in the sleep vs. wake group when making correct decisions (sleep – wake × correct – incorrect)

Region	*x*	*y*	*z*	*Voxels*	$T_{1,76}$	p_{peak}	p_{SVC}
R Hippocampus	32	-16	-17	54	3.72	0.0002	0.033
L Caudate Nucleus	-12	11	3	52	3.62	0.0003	0.012
R Pallidum	12	2	-4	17	3.51	0.0004	
L Thalamus	-17	-6	7	10	3.39	0.0006	
L Middle Temporal Gyrus	-56	-40	1	42	3.32	0.0007	
	-56	-37	9		3.30	0.0007	

ROIs for SVC correction: bilateral hippocampus, bilateral caudate nucleus (head)

During explicit item recognition, on the other hand, the caudate nucleus, which usually contributes to implicit memory recall, is more strongly activated when participants slept during the consolidation interval (p_{SVC}=0.02, t_{76}=3.7; see Table S2). In contrast, participants who stayed awake show greater activation in the hippocampus during this explicit memory task (p_{SVC}=0.01, t_{76}=4.2; see Table S3 and Suppl. Fig. S5).

Table S2. Explicit item recognition. Regions with higher activation in the sleep vs. wake group when making correct decisions (sleep – wake × correct – incorrect)

Region	*x*	*y*	*z*	*Voxels*	$T_{1,76}$	p_{peak}	P_{SVC}
R Superior Medial Gyrus	11	28	57	206	4.33	<0.0001	
L Inferior Frontal Gyrus (pars orbitalis)	-39	46	-14	160	4.12	<0.0001	
L Middle Frontal Gyrus	-38	17	49	391	4.07	0.0001	
L Caudate Nucleus	-14	10	7	69	3.71	0.0002	0.023
L Superior Medial Gyrus	-6	31	48	68	3.55	0.0003	
R Caudate Nucleus	15	-3	16	17	3.45	0.0005	
L Angular Gyrus (IPC)	-51	-58	34	13	3.32	0.0007	

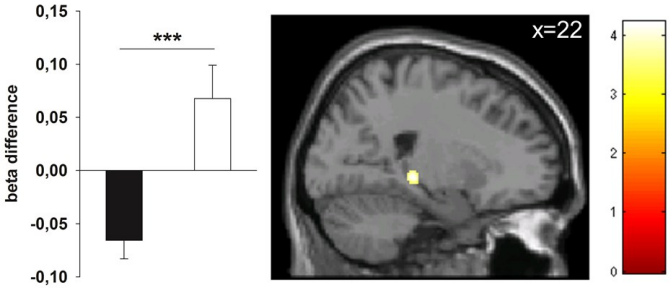
ROI for SVC correction: bilateral caudate nucleus (head)

Table S3. Explicit item recognition. Regions with higher activation in the wake vs. sleep group when making correct decisions (wake – sleep × correct – incorrect)

Region	*x*	*y*	*z*	*Voxels*	$T_{1,76}$	p_{peak}	p_{SVC}
R Hippocampus	21	-30	-2	138	4.22	<0.0001	0.009
R Lingual Gyrus	14	-45	3	75	3.54	0.0003	
R Temporal Pole	41	7	-17	16	3.37	0.0006	

ROI for SVC correction: bilateral hippocampus

Together, whereas over wakefulness hippocampal memory traces for explicit memory are strengthened, over sleep, activity patterns indicate an interaction between different memory systems and systems memory consolidation. After sleep, both implicit and explicit memory recall involve activity in the brain structure of the other memory system, respectively.



Supplementary Figure S5. Explicit memory recall after 12 hours wakefulness activates the hippocampus more strongly than after sleep for correct versus wrong decisions. Black bars represent beta values in the hippocampal ROI in the sleep group, white bars show beta values in the wake group.

Furthermore, in the cooperative win-or-lose task, participants who slept activate structures associated with both implicit and explicit memory more strongly. We observe higher activation after sleep than wakefulness in both the hippocampus (p_{SVC}=0.03, t_{76}=3.8) and the caudate nucleus (p_{SVC}=0.03, t_{76}=3.3; see Table S4).

Table S4. Cooperative memory task. Regions with higher activation in the sleep vs. wake group when making correct decisions (sleep – wake × correct – incorrect)

Region	*x*	*y*	*z*	*Voxels*	$T_{1,76}$	p_{peak}	p_{SVC}
R Postcentral Gyrus	51	-16	60	115	4.04	0.0001	
L Hippocampus	-30	-28	-10	63	3.76	0.0002	0.029
L Caudate Nucleus	-11	20	4	10	3.26	0.0008	0.032

ROIs for SVC correction: bilateral hippocampus, bilateral caudate nucleus (head)

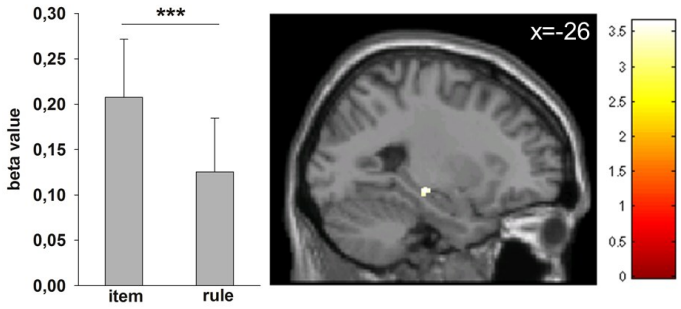
Increased overall activity during this task could be derived either from both tasks contributing to different items in a complementary manner, or from cooperative activity during recall of the same items. To test whether both systems show correlated activity for the same items, we used a psychophysiological interaction analysis (PPI) with the caudate activation found in the cooperative task after sleep as seed region. This analysis revealed that the caudate and the hippocampus and parahippocampus are concurrently activated during correct decisions after sleep (p_{SVC}=0.02, t_{76}=4.5; see Table S5). Cooperation of memory systems during recall in this task therefore does not seem to be an either/or activity, but a true cooperation between both systems during recall of each item.

Table S5. Cooperative memory task. Psychophysiological interaction (PPI) with caudate as seed region.

Region	*x*	*y*	*z*	*Voxels*	$T_{1,37}$	p_{peak}	p_{SVC}
R Hippocampus and Parahippocampal Gyrus	29	-42	-10	151	4.45	<0.0001	0.021
Bilateral Caudate Nucleus	-5	7	7	710	4.30	0.0001	0.004
	-6	-3	10		4.30	0.0001	
	6	14	6		3.82	0.0002	0.012
L Middle Cingulate Cortex	-8	-15	33	26	3.84	0.0002	
L Posterior Cingulate Cortex	-11	-49	28	56	3.74	0.0003	
L Putamen	-32	-12	-4	37	3.65	0.0004	
R Middle Cingulate Cortex	2	-18	48	71	3.62	0.0004	
L Middle Cingulate Cortex	-8	-39	46	56	3.51	0.0006	
L Precentral Gyrus	-32	-15	51	11	3.49	0.0006	

ROIs for SVC correction: bilateral hippocampus and parahippocampal gyrus, bilateral caudate nucleus (head)

We did not observe behavioral differences between the sleep and wake groups in the competition between item-based and rule-based decisions. During item-based decisions, we observe more hippocampal activation in all participants than during rule-based decisions (p_{SVC}=0.04, t_{76}=3.7; see Table S6 and Suppl. Fig. S6), which confirms that explicit learning strategies recruit the hippocampal memory system during our decision classification task.



Supplementary Figure S6. In the competitive memory task, the hippocampus shows higher activation during item-based compared with rule-based responses. Bars show mean beta values for item-based and rule-based decisions in the hippocampal ROI. *** p < 0.001

Table S6. Competitive memory task. Regions showing higher activation during item-based recall vs. rule-based recall.

Region	*x*	*y*	*z*	*Voxels*	$T_{1,76}$	p_{peak}	p_{SVC}
L Hippocampus	-26	-24	-10	30	3.65	0.0002	0.041
R Fusiform Gyrus	17	-39	-16	104	3.59	0.0003	
L Temporal Pole	-44	2	-23	27	3.36	0.0006	

ROI for SVC correction: bilateral hippocampus

SUPPLEMENTARY DISCUSSION

The task design was chosen based on a large literature of studies on rule-based classification learning (*2*). It has been shown that various strategies are used to solve classification tasks (*3*). We specifically enforced multiple systems use by providing strong cues for different learning strategies during task training. For one, we spurred implicit information-integration learning by basing the hidden rule on a conjunction of features, which is in its complexity impossible to verbalize, but has to be inferred from integrating information across many stimuli and feature dimensions. This kind of implicit learning has been shown to depend critically on the striatum (*4*). Feedback-driven training is thought to further reinforce these implicit aspects of memory and striatal involvement (*5*). Furthermore, we ensured that explicit strategies can also support task acquisition. During exemplar learning, every exemplar is stored in memory, along with its category label. This kind of explicit encoding strategy depends critically on the hippocampus (*6*). It has been shown that classification tasks can activate both the medial temporal lobe and the striatum depending on boundary conditions. The contribution of memory systems to a specific task depends on training mode and duration. It can also shift over time, perhaps reflecting strategy use (*3, 7, 8*). Our design made it possible to examine both the implicit and explicit components that are established during training separately, but also how they interact when solving ambiguous tasks, which entail cooperation or competition between different memory traces. Using fMRI during the test session enabled us to examine memory systems interactions not only on a behavioral, but also on a neural systems level. We show that sleep induces memory systems interactions and supports optimal behavioral output, by enforcing memory trace cooperation.

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DISCUSSION

SUMMARY OF FINDINGS

We investigated the effects of sleep on memory consolidation in multiple memory systems. In studies 1 and 2 we examined behavioral effects of sleep on both procedural and declarative memory tasks separately. We find that sleep benefits performance in all kinds of memories that were tested. Our results replicate earlier findings that less forgetting of declarative memory occurs over sleep, and that procedural memory can even improve during this time.

To show that memory performance after sleep is better than after wakefulness, however, does not mean that sleep facilitates the consolidation process per se, in the sense that it leads to a stronger and more resistant memory trace. It has also been discussed whether a reduction of interfering information during sleep mediates its beneficial effect on performance. To test effects of reduced interference on memory consolidation, we compared two conditions in study 1. In one condition, participants meditated after learning and in this way shielded their minds against intrusive internal and external inputs. In the other condition, participants were actively engaged in conversation in a busy environment, thus experiencing heightened interference. We find that a mere reduction of interference during the time following learning does not improve memory performance. Because this passive reduction of interference cannot account for the beneficial effects of sleep on declarative memory, our findings further corroborate the view that sleep actively facilitates the consolidation process.

Another idea why memory performance may be better when measured after sleep is that memory decay occurs at a slower rate during this time. In study 2, we find evidence that this account cannot explain sleep effects on memory, either. We tested effects of sleep vs. sleep deprivation on different kinds of declarative and procedural memory; both on the first day after the experimental intervention and after longer delays, so that initially sleep-deprived subjects had at least one night of recovery sleep. We find that declarative memory shows a benefit of sleep only on the first day after sleep or sleep-deprivation. However, these short-term effects of sleep are not due to increased fatigue in the wake control condition, because sleep deprivation

before recall did not impair memory performance. Instead, the hippocampus seems to act as a short-term memory buffer, so that a delayed active consolidation can take place during the first night of recovery sleep. This buffering also occurs for a procedural memory task that has been shown to activate the hippocampus. There, we observe visible improvements in performance after the first night of sleep after training, regardless of whether it occurred within the first hours after learning, or only on the next day. The total amount of sleep differed between conditions when participants were sleep deprived during the first night and when they were allowed to sleep normally during all nights. If decay processes occurred at a slower rate during sleep, we should have observed a lasting benefit of sleep on memory performance in all memory tasks. Thus, our findings provide strong evidence that sleep does not passively protect new memories from interference, nor simply slow their biological decay. They rather indicate that sleep holds an active role in memory consolidation.

Interestingly, consolidation of hippocampal-dependent and hippocampal-independent motor memory followed a qualitatively different time-course in the same study. Non-hippocampal memory shows long-lasting deficits if participants are sleep deprived after learning. When sleep followed learning, on the other hand, memory performance improved considerably. No such improvements occurred over wakefulness. Contrary to procedural memory that shows hippocampal contributions, non-hippocampal memory performance was not rescued by recovery sleep. These differences in the time-course of consolidation point towards different underlying sleep-dependent consolidation mechanisms. We suggest that whereas hippocampal-dependent memory benefits from reactivation-dependent processes during sleep, consolidation of non-hippocampal procedural tasks may rely on processes related to synaptic consolidation only.

From studies 1 and 2 we can assume that sleep actively benefits consolidation of both procedural and declarative memory. Furthermore, we found indications for two distinct sleep-related consolidation processes. This led us to investigate the mechanisms underlying behavioral effects of sleep on memory consolidation more closely. In study 3, we tested whether learning material is actively reprocessed during sleep after encoding of a hippocampal-dependent memory task. With the help of pattern classification algorithms, we traced neural correlates of memory

reprocessing in the human sleep EEG. We show that electrical brain activity during sleep holds information about the types of visual stimuli that were learned earlier. This speaks for an active reprocessing of learning-related information during sleep. In NREM sleep, slow waves and sleep spindles contain the highest amount of information about previous learning material. This fits well with previous findings that power in these frequency bands correlates with memory performance. Notably, our results show that reprocessing of daytime experience occurs during all sleep stages, including REM sleep. Moreover, it occurs only during specific times in the night. The existence of these windows of memory reprocessing shows that we do not only detect ongoing task-related activity, but that reprocessing is a process which is specifically activated during certain times in the night. These periods coincide with the timing of synaptic plasticity processes after learning. It is thus tempting to speculate whether memory reactivation is timed to occur during such windows of high synaptic plasticity.

In study 3, we have shown that declarative memory is actively reprocessed during sleep. The results of study 2 indicate that procedural tasks, which involve the hippocampus, rely on the same sleep-dependent consolidation mechanisms as declarative memory. In study 4, we directly investigated whether memory trace reactivation also mediates behavioral effects of sleep on such a procedural memory task. Participants were trained on a serial reaction time task, in which tones were associated with finger movements. This task had previously been shown to elicit hippocampal activity during learning. To externally drive the reactivation process, we presented the same sounds that were associated with finger movements in the learning session again during post-learning sleep. Presenting these cues boosts finger-tapping performance in a later memory test. The effects of cueing on performance are highly specific. Similar to what has been shown for declarative memory, they occur only after cue presentation during sleep, but not when cues were presented during wakefulness, and they pertain only to those finger transitions of a longer sequence that had been targeted during sleep. Introducing such additional external reactivation accelerates procedural memory consolidation. Thus, like for declarative memory, it seems to be the amount of reactivation that occurs during a given sleep period which determines the size of sleep effects. This is strong evidence that procedural memory, which has been shown to activate the

hippocampus in fMRI, relies on the same sleep-dependent consolidation mechanisms as hippocampal-dependent declarative memory.

In the previous studies, we have shown that sleep benefits memory consolidation in different memory systems, separately. However, in many situations, traces are laid down in more than one memory system. For instance, we implicitly develop speed and automaticity when learning a piano piece, but also gain explicit declarative knowledge about the structure of its composition. In study 5, we investigated how consolidation during sleep affects such separate memory traces for a single learning experience. In a task that allowed both implicit and explicit memory to develop during training, we find that sleep-dependent consolidation changes the relation between these separate representations. At the end of training, memory for implicit and explicit aspects of the task is negatively correlated. The structure of correlations between performance in different tasks reveals that sleep renders this initial competition between the two traces cooperative. Such a change does not occur over wakefulness. We observe this effect not only on the behavioral level, but also in memory systems activation, as revealed by fMRI. After sleep, the hippocampus, which is usually associated with explicit memory recall, contributes more strongly to implicit memory tasks, whereas the caudate nucleus, which is usually associated with implicit memory recall, in turn contributes more strongly to explicit memory retrieval. Thus, consolidation during sleep integrates implicit and explicit aspects of memory. Additionally, functional connectivity between the two regions increases over sleep. This newly emerging cooperation between different memory systems benefits behavioral performance. Our results show how flexibly sleep changes our memory consolidation. It not only strengthens memory, but additionally leads to a reorganization of the neural circuits that contribute to it. In this way, sleep can also change the quality of a memory trace.

SLEEP-DEPENDENT CONSOLIDATION IN DIFFERENT MEMORY SYSTEMS

ALL KINDS OF DECLARATIVE MEMORY BENEFIT EQUALLY FROM SLEEP

A number of studies report discrepant results on the effect of sleep on different types of memory. This raises the question whether all forms of declarative memory benefit equally from sleep. It has been suggested that the strength of initial encoding determines the size of subsequent sleep effects. There is dissent, however, on the level of initial performance that entails the strongest effects of sleep. Different studies find weak (Drosopoulos et al., 2007) or strong memory traces (Tucker & Fishbein, 2008) to be most susceptible to sleep. It is also not clear, whether memory for all types of learning material benefits from sleep. Payne et al. (2012) found that sleep only supports retention of semantically unrelated word pairs. In contrast, we found in study 2 that sleep only aids memory for semantically related word pairs. In other studies, effects have been found on both semantically related and semantically unrelated material. It is unclear why these discrepant results occur.

In study 1, we systematically addressed whether specific types of declarative memory are preferentially consolidated during sleep. We conducted three separate experiments in which we examined the effect of sleep on non-verbal and verbal memory, memory for both single and associated items, memory retrieved by recall and recognition procedures, and in free as well as in cued recall. We show that sleep benefits all of these forms of declarative memory. We tested three independent experimental samples of up to 20 subjects. When these groups are considered individually, not all memory tests benefit significantly from sleep. However, almost all tests show consistent and significant benefits of sleep, when the total sample, collapsed over all three experiments, is considered. Moreover, when the individual tests are combined into a total score for declarative memory performance, we find a consistent effect in all three experiments. In experiments testing only small samples of 20 participants and less it may be difficult to detect sleep-dependent effects on all kinds of declarative memory because the average effect sizes we observe are comparatively small ($d = 0.2$). It is therefore advisable to test a sufficiently large number of participants ($n > 40$-50) in order to reliably answer research questions concerning effects of sleep on memory performance. Discrepant results reported in the literature might be due to comparatively small sample sizes. Our results show that sleep benefits declarative memory performance in a broad range of memory

tasks using many different tests. The question, however, remains, why sleep has this beneficial effect.

REDUCTION OF INTERFERENCE CANNOT EXPLAIN EFFECTS OF SLEEP ON MEMORY

When we are awake, new information can overwrite recent memory traces. Because during sleep, the brain is in an off-line state and protected from new input, memory is protected from this kind of interference. This is why, early on, the question has been raised whether the beneficial effect of sleep on memory can be attributed to this passive protection against interference (McGeoch, 1932). For procedural skill learning, it has already been shown that a reduction of interference during an interval of wakefulness by either immobilizing the typing hand in a finger sequence tapping task (Walker et al., 2002), or by complete absence of interfering visual input during consolidation of a visual discrimination task (Mednick et al., 2002), does not have the same beneficial effects on memory as sleep.

For declarative memory, an interference-reduced wake control condition is difficult to achieve because the thoughts of the participants cannot be controlled. They may actively rehearse the learning material or engage in other cognitive activity. This activity cannot be objectively quantified. Therefore, we chose an experimental design in which participants focus their thoughts on a single point, as it is done during meditation. This precludes other interfering input and keeps them from rehearsing the material. We tested expert meditators in a sleep condition, an active wake condition, and an interference-reduced wake condition, during which they meditated. Our results demonstrate that reduced interference cannot explain the beneficial effects of sleep on declarative memory performance. Declarative memory is better retained over sleep than over wakefulness, yet there was no difference in performance between the active and the reduced-interference wake conditions (study 1). Thus, the effect of sleep is not passively mediated by a lack of input, which supports the view that sleep actively benefits memory consolidation.

SLOWER MEMORY TRACE DECAY CANNOT EXPLAIN EFFECTS OF SLEEP ON MEMORY

Besides interference, it has also been discussed whether a reduced metabolism during sleep causes slower memory decay (Thorndike, 1913). This hypothesis predicts that less forgetting should occur over intervals which contain longer periods of sleep. In study 2, we find evidence that contradicts this notion. We tested

how sleep affects different kinds of procedural and declarative memory. In a series of experiments, participants learned different memory tests. They were then either sleep deprived during the night after learning or were allowed to sleep normally. We tested memory both on the first day after the experimental intervention and after longer delays, so that initially sleep-deprived subjects had at least one night of recovery sleep. At all times of testing, the total amount of sleep thus differed between conditions when participants were sleep deprived during the first night after learning, and when they were allowed to sleep normally during all nights. All declarative memory tests show a benefit of sleep only on the first day after learning. We observed no long-term effects of sleep on memory. However, these short-term effects of sleep are not due to increased fatigue in the wake control condition because sleep deprivation before recall does not impair performance.

The same pattern of results also occurs for a procedural memory task that previously been shown to activate the hippocampus during learning and thus shares a neural basis with declarative memory (Squire & Zola-Morgan, 1991; Schendan et al., 2003). In this task, participants who sleep during the first night after learning perform better in a morning recall test than at the end of training. Participants who were sleep deprived show no such improvement. However, the increase in performance is only delayed in time. In subjects who were initially sleep deprived, performance receives a delayed boost over the first night of recovery sleep. Since the total amount of sleep differed between the sleep and sleep deprivation conditions at all times of testing, we should have observed a lasting benefit of sleep during the first night, if this benefit is in fact due to a lower rate of memory decay during sleep. Moreover, slower memory decay can also not explain that we observe an improvement in performance in the procedural task. Thus, our findings are strong evidence that sleep actively facilitates the process of memory consolidation.

A DISSOCIATION BETWEEN SLEEP-RELATED CONSOLIDATION PROCESSES FOR HIPPOCAMPAL AND NON-HIPPOCAMPAL MEMORY

In study 2, we show that sleep benefits consolidation of both procedural and declarative memory. Neither reduced interference during the retention interval nor the idea that memory traces decay at a slower rate during sleep can satisfactorily explain the observed effects of sleep. Interestingly, we additionally find evidence for at least two distinct sleep-related consolidation processes. The time-course of

consolidation of hippocampal-independent motor learning differs from the time-course of consolidation of hippocampal memory tasks. In contrast to declarative and procedural memory tasks that have previously been shown to activate the hippocampus, hippocampal-independent motor adaptation learning shows long-lasting benefits of sleep also four days after training. Participants who slept during the first night after learning perform even better during the recall test on the next morning than at the end of training. Participants who were sleep deprived show no such improvement. Here, recovery sleep did not result in delayed improvements of memory performance. Thus, motor adaptation requires sleep to follow shortly after learning. If no sleep occurs during the first day after learning, effects of training are lost.

In contrast, memory tasks that activate the hippocampus during learning show a significant behavioral effect of sleep only on the first day after learning. We no longer observe behavioral differences between the sleep and wake condition after two, three, four or six days. Importantly, these short-term effects of sleep are not due to increased fatigue in the wake control condition. Sleep deprivation before recall does not impair performance. The lack of long-term effect thus indicates that active consolidation takes place during the first night of delayed sleep. These differences in the time-courses of consolidation show that at least two distinct sleep-related consolidation processes exist.

It is noteworthy that also procedural finger sequence tapping did not show significant long-term effects of sleep, but performance in the wake group received a delayed boost after the first night of recovery sleep, similar to declarative memory. Traditionally, the distinction between memory systems is drawn along the line of procedural as opposed to declarative memory. Declarative memory is thought to depend on medial temporal lobe structures including the hippocampus, whereas procedural memory is assumed to rely on the striatum and neocortex (Squire & Zola-Morgan, 1991). However, recent neuroimaging studies have shown that the hippocampus is also activated during procedural finger sequence tapping tasks (Schendan et al., 2003; Walker et al., 2003). Thus, hippocampal activation could determine the sleep-dependent consolidation mechanism, regardless of the procedural/declarative distinction.

It has not only been shown that memory traces become reactivated in the hippocampus during sleep (Wilson & McNaughton, 1994; Nadasdy et al., 1999), but that the hippocampus can in turn trigger reactivation in other cortical areas (Ji & Wilson, 2007; Lansink et al., 2009; Peyrache et al., 2009). We suggest that consolidation of hippocampal memory tasks relies on these processes of memory trace reactivation. The hippocampus is a structure that shows rapid plasticity (Frank et al., 2006). It can function as an intermediate-term buffer for new information (Rolls & Treves, 1994; Treves & Rolls, 1994). We propose that the hippocampus buffers memory during periods of wakefulness and initiates an active systems consolidation process during the first sleep period following learning, even if it is more than 12 hours delayed.

In contrast, consolidation of non-hippocampal procedural learning must rely on a different consolidation mechanism. Although the exact mechanism remains unknown, it can be speculated that it depends on synaptic consolidation only (Bourtchuladze et al., 1994; Hernandez & Abel, 2011; Chauvette et al., 2012). It has been suggested that memory encoding produces so-called synaptic tags at synapses involved in learning (Frey & Morris, 1997; Martin et al., 1997; Martin & Kosik, 2002). These tags may help trafficking of newly translated plasticity related gene products which stabilize synapses. It has been shown that sleep facilitates this gene transcription and protein synthesis (Abel et al., 2013). If sleep does not follow shortly after learning, tags for sleep-dependent synaptic plasticity processes may be lost and no improvement in memory can result.

REACTIVATION-DEPENDENT MEMORY CONSOLIDATION DURING SLEEP

ELECTRICAL BRAIN ACTIVITY DURING SLEEP CONTAINS INFORMATION ABOUT DAYTIME EXPERIENCES

In study 2, we found evidence for two distinct sleep-related consolidation processes. This led us to investigate the mechanisms underlying effects of sleep on memory consolidation more closely. We hypothesized that memory trace reactivation mediates behavioral effects of sleep on performance in hippocampal-dependent memory tasks. In study 3 we looked for neural signatures of such memory trace reactivation in human EEG data. We trained linear support vector machine (SVM) classifiers on sleep EEG data that was recorded after participants learned pictures of

either faces or houses. If the learning material is reactivated during post-learning sleep, a classifier should be able to decide, based on electrical brain activity, which kind of material had been learned before the EEG data was recorded. Indeed, we find that classifiers can distinguish what participants had learned before going to bed with a correct classification rate exceeding chance level. The fact that sleep EEG holds information about visual stimuli that have been studied during the day indicates that learning material is reprocessed during the following sleep period. Thus, hippocampal-dependent memory is indeed recapitulated during sleep.

Interestingly, we find memory reprocessing during all sleep stages: light sleep, slow-wave sleep (SWS) and REM sleep. There are various ideas on how different sleep stages contribute to memory consolidation. One current view stresses the role of SWS in consolidation of declarative memory (Diekelmann & Born, 2010; Inostroza & Born, 2013). Several findings support this idea. Declarative memory benefits most strongly from sleep in the first half of the night, when slow wave activity is highest (Plihal & Born, 1997). Furthermore, cueing reactivation during SWS aids memory consolidation (Rasch et al., 2007; Rudoy et al., 2009), and increasing slow wave activity by transcranial direct current stimulation or by tone stimulation boosts memory performance (Marshall et al., 2006; Ngo et al., 2013). Recently, however, it has been put forward that also light sleep may play an important role in memory consolidation (Genzel et al., 2014). The finding that an ultra-short nap period of only 6 minutes can improve memory performance supports this view (Lahl et al., 2008). The role of REM sleep in memory consolidation has long been discussed, as well (Hennevin et al., 1995; Smith, 1995), and has lately gained renewed attention (Walker & van der Helm, 2009). Ongoing task-related activity has been shown during periods of REM sleep (Maquet et al., 2000). The amount of such re-expression of learning-related activity during REM sleep correlates with later memory performance (Peigneux et al., 2003). This supports a role of REM sleep not only in memory consolidation, but also in reprocessing of previously learned material. We show that this reprocessing of learning material occurs during all sleep stages that have previously been associated with sleep-dependent memory consolidation. Thus, our findings combine recent and previous views which stress the importance of either light NREM sleep, SWS, or REM sleep for memory consolidation (Hennevin et al., 1995; Ficca & Salzarulo, 2004; Diekelmann & Born, 2010; Genzel et al., 2014).

Because brain activity and neurotransmitter levels differ greatly between NREM and REM sleep, it has been suggested that these stages serve different functions in the consolidation process (Diekelmann & Born, 2010). There are two main suggestions about possible distinct roles of NREM sleep and REM sleep. The sequential hypothesis of consolidation during sleep assumes that a succession of NREM and REM sleep is critical for memory function. In this model, both sleep stages are thought to have different roles in the processing of memories, which are interlinked. Thus, they have to occur sequentially for successful consolidation to take place (Ambrosini & Giuditta, 2001). In contrast, the dual-process hypothesis suggests that the different processes occurring during REM sleep and NREM sleep pertain to different aspects or forms of memory, and are thus independent of each other (Ackermann & Rasch, 2014). We find that memory reprocessing occurs in close temporal proximity over different sleep stages. The same memory task induced learning-related activity in both NREM and REM sleep. It is therefore likely that these sleep stages cooperate or have complementary functions in the consolidation process. Our findings thus rather support the sequential hypothesis, which assumes an interaction between NREM and REM sleep during consolidation. We can, however, not answer whether these stages have to occur sequentially.

Exploring which features of the data from NREM and REM sleep are most predictive for the decision whether faces or houses have been learned before sleep can give a better understanding of the underlying neuronal processes. We find that different frequencies contribute to correct classification in REM and NREM sleep. Moreover, the topology of highly predictive channels for individual frequency bands differs between the two sleep stages. These results indicate that also the neuronal basis of reactivation differs between REM and NREM sleep.

Previous findings in animals already suggest that material-specific information can be found in NREM sleep oscillations. Cortical slow waves and sleep spindles initiate sharp wave ripple activity in the hippocampus (Sirota et al., 2003; Ji & Wilson, 2007). Neuronal replay occurs predominantly during these sharp wave ripple events (Kudrimoti et al., 1999; Lee & Wilson, 2002; Peyrache et al., 2009). It has been suggested that the different groups of neurons which fire during slow waves and sleep spindles can select the content of replay via entorhinal inputs to the hippocampus (Sirota et al., 2003). Thus, neuronal firing giving rise to slow waves

and spindles already carries material-specific information. Sequential replay of neuronal firing as observed during learning initiates in the hippocampus. Sequence replay in the hippocampus then triggers reactivation of neuronal firing sequences in the neocortex (Ji & Wilson, 2007). This hippocampal output may engage the same neuronal groups that initiated hippocampal replay (Sirota et al., 2003). These findings indicate that firing in neocortical and hippocampal cell populations is closely linked and that material-specific information about previous learning can thus be found on the level of field potential oscillations.

Indeed, we find that slow wave and spindle oscillations carry the highest amount of information about the kind of the previous learning material in NREM sleep. In humans, these oscillations have been shown to correlate with behavioral effects of sleep on memory. Spindle power increases after declarative learning and this increase in spindle power correlates with subsequent memory performance (Gais et al., 2002; Schabus et al., 2004). Increased slow wave and spindle activity after cue presentation in behavioral designs, which externally target the reactivation process, is associated with better memory performance (Antony et al., 2012; Schreiner & Rasch, 2014). In fact, external reactivation of memories during NREM sleep may be especially effective if the associated cue presentation elicits spindle-coupled slow waves (Schreiner & Rasch, 2014). This fits well with our results that both slow wave and spindle oscillations carry information about previous learning material. Together, these findings indicate that slow waves and spindles, in particular, may reflect reactivation processes during NREM sleep.

In REM sleep, slow frequencies below 4 Hz also contribute to classification, but informative channels show a different topography from that in NREM sleep. This suggests that also the neuronal bases giving rise to the activity differ from that in NREM sleep. Furthermore, our data indicate a role of frontal theta and occipital alpha activity in memory reprocessing during REM sleep. Increases in frontal theta power have already been linked to successful memory encoding and retrieval (Klimesch, 1999; Nyhus & Curran, 2010). Furthermore, theta is thought to be responsible for controlling, maintaining and storing memory content during wakefulness (Lisman & Jensen, 2013). Schreiner and Rasch (2014) showed increased frontal theta power after reactivating a verbal learning task by external cueing during sleep. This finding fits well with our results. Whether frontal theta

related to memory reprocessing in sleep has similar functions as during wakefulness, remains to be investigated. We find that apart from theta, also alpha oscillations over occipital regions were involved in memory reprocessing. Occipital alpha may carry information about the visual properties of the studied objects. Future research should therefore target the role of REM sleep theta and alpha oscillations in memory consolidation.

Although reprocessing of learned material occurs in all sleep stages, learning-related activity in the sleep EEG is found only during specific times of the night. This indicates that reprocessing is a cyclic in nature. We observe two time windows, three and six hours after learning, during which memory reprocessing increases in all sleep stages. The timing of these intervals during which memory reprocessing takes place might be determined by the timing of synaptic plasticity processes after learning (Igaz et al., 2002; Davis, 2011; Dubnau & Chiang, 2013). Specific windows, during which the beneficial effect of sleep on memory consolidation is highest, have also been discovered previously (Smith, 1995). It will be interesting to pursue the factors governing these temporal dynamics of memory trace reprocessing.

PROCEDURAL AND DECLARATIVE MEMORY BENEFIT FROM REACTIVATION DURING SLEEP

One way in which sleep actively consolidates memories is by memory trace reactivation. We found evidence for such memory reprocessing in a declarative memory task in study 3. Presenting cues that have been associated with visuo-spatial tasks again during the following sleep period can boost declarative memory performance in humans (Rasch et al., 2007; Rudoy et al., 2009). In study 4, we tested whether reactivation mediates behavioral effects of sleep on procedural memory in a similar way as for declarative memory. It has been shown that external cueing during sleep benefits consolidation of a sequential motor task (Antony et al., 2012). We also find that re-exposing participants to part of a tone sequence during sleep, which was studied in a finger-tapping task, improves performance. Furthermore, we show that this effect is highly specific for the finger transitions that were cued during sleep. A beneficial effect of cueing occurs only when cues are presented during consolidation in sleep, but not when presented during quiet or active wakefulness. These results mirror findings on reactivation of declarative memory, for which effects of cueing are also highly specific and can be traced to individual study items (Rudoy et al., 2009). Furthermore, such targeted reactivation of

declarative memory is only effective during sleep, but not during wakefulness (Rasch et al., 2007).

For declarative memory, reactivation can accelerate the naturally occurring consolidation process (Diekelmann et al., 2012). We show in study 4 that driving the internal reactivation process by presenting cues of the learning task likewise accelerates consolidation of a procedural finger-tapping task. Longer sleep times or externally driving the internal reactivation process during shorter periods of sleep result in greater sleep effects. It thus seems to be the amount of reactivation occurring during sleep that determines the size of sleep-dependent benefits for both declarative memory and procedural sequential motor tasks.

HIPPOCAMPAL CONTRIBUTIONS TO REACTIVATION-DEPENDENT CONSOLIDATION

The striking similarities between reactivation-dependent consolidation of declarative memory and procedural motor sequence learning make it likely that both share a common neural basis. In fact, we show in study 2 that long-term consolidation of motor sequence learning follows the same time-course as consolidation of declarative memory tasks. These dynamics are qualitatively different from sleep-related long-term consolidation of a motor adaptation task. In contrast to motor adaptation learning, declarative memory tasks and motor sequence learning show a hippocampal contribution during learning (Squire & Zola-Morgan, 1991; Corkin, 2002; Schendan et al., 2003; Walker et al., 2003). It can be speculated that these hippocampal contributions to motor sequence as well as declarative memory make both susceptible to targeted memory reactivation during sleep. Across systems reactivation between the hippocampus and the striatum can explain how procedural memory tasks, like the one we tested in study 4, can benefit from reactivation-related memory consolidation (Pennartz et al., 2004; Lansink et al., 2009). Our results show that consolidation of memory with a hippocampal contribution, even if a motor skill is acquired, relies on reactivation-dependent consolidation during sleep. Tasks with no hippocampal contribution, on the other hand, do not depend on the same sleep-related consolidation mechanisms (see study 2). Whether consolidation of tasks such as motor adaptation learning relies on purely neocortical reactivation processes or synaptic consolidation only, is currently unknown. The hippocampus, however, clearly seems to play an important role in reactivation-dependent memory consolidation.

SLEEP INTEGRATES SEPARATE ASPECTS OF A MEMORY REPRESENTATION

So far, our experiments have shown that sleep benefits performance on a wide range of memory tasks. We find sleep effects on both hippocampal and non-hippocampal memory. Even though the processes that mediate these effects may be qualitatively different, consolidation of both types of memory clearly benefits from sleep. We thus observed sleep effects on consolidation separately in different memory systems. However, in many situations, memory traces are formed in more than one memory system at a time. This occurs, for instance, when we learn a foreign language. We explicitly memorize the vocabulary and grammatical rules that govern its use. However, we also develop an implicit feeling for correct grammatical structures. Sleep effects have been found both on explicit memory for foreign vocabulary and implicit memory for grammatical rules (Gais et al., 2006; Nieuwenhuis et al., 2013). In study 5, we tested whether sleep can benefit explicit and implicit representations of the same learning task simultaneously. It has been shown that implicit and explicit memory systems can interact during task acquisition (Poldrack et al., 2001). We examined whether these memory systems interactions continue during offline processing in sleep.

We devised a rule-learning task that allows forming both implicit and explicit memory during training. We then tested how these different representations develop over sleep and wakefulness. Participants who slept were better at explicitly recognizing items they had seen during training. They were also better at applying the implicit rule underlying the task in previously unknown situations. We thus show that sleep benefits the consolidation of both implicit and explicit aspects of memory in parallel. Moreover, we find that the interactions between these representations change over sleep. Implicit and explicit memory is negatively correlated at the end of training. Over sleep, this association turns positive. This relational structure of the memory representations changes only over sleep. No such change can be observed after wakefulness. Interestingly, participants who slept also perform better on a task that allows memory systems cooperation. This indicates that sleep induces cooperation between implicit and explicit aspects of memory.

By rendering initial competition between implicit and explicit memory traces cooperative, sleep changes the quality of memory systems interactions. Qualitative changes of memory over sleep have been observed before. Some studies indicate

that sleep may lead to a more semantic representation of declarative memory (Payne et al., 2009; Diekelmann et al., 2010). Similar to our results, sleep has also been shown to benefit abstraction of implicit regularities in rule learning tasks (Gomez et al., 2006; Ellenbogen et al., 2007; Hupbach et al., 2009; Durrant et al., 2011; Durrant et al., 2013; Nieuwenhuis et al., 2013). Thus, sleep can support the emergence of generalized knowledge in both implicit and explicit memory tasks. For the first time, we now show that sleep also changes the interactions between implicit and explicit knowledge acquired during learning.

It has been found that sleep can render implicit memory, like motor skills, more explicit, so that participants become aware of the sequential structure of the motor task (Wilhelm et al., 2013). If sleep integrates implicit and explicit aspects of memory, we should observe a similar emergence of explicit knowledge about the rules underlying our memory task. Participants who slept indeed reported more complex and fitting descriptions of these rules. This further highlights that sleep changes not only the strength, but also the quality of memory representations.

The change in behavior over sleep is reflected in a concurrent change of brain activity. After sleep, implicit memory recall activates the hippocampus more strongly, which is usually activated in explicit memory recall, whereas retrieval of explicit memory recruits the caudate nucleus, which is usually activated during implicit memory tasks. In a cooperative task, both implicit and explicit memory associated structures contribute to correct decisions after sleep. Importantly, these structures are not only concurrently active, but also show correlated stimulus-induced responses. This demonstrates that the systems cooperate while solving the task. Thus, what we observe is not a transfer of memory from one to the other system, but stronger relative systems contribution and mutual information transfer between systems. It has been suggested previously that sleep can render competition between memory systems cooperative (Albouy et al., 2008). We extend these findings considerably by showing that sleep integrates implicit and explicit aspects of memory on the behavioral and the brain systems level to enable optimal performance.

CONCLUSIONS

The studies presented in this thesis investigated the effect of sleep on consolidation in multiple memory systems. We showed that sleep actively consolidates different kinds of memories. Sleep consistently benefits consolidation of both declarative and procedural memory. The mechanisms that underlie these beneficial effects, however, are not the same for all types of memory. Our results indicate that at least two distinct sleep-related consolidation processes exist.

This is why we investigated the mechanisms underlying memory consolidation during sleep. Reactivation of neuronal firing patterns has already been observed in animals. We now detected memory reprocessing in human electrical brain activity during sleep. We show that reprocessing occurs during all sleep stages but peaks during windows of high synaptic plasticity after encoding. Thus, memory reactivation may be a cyclic process. The fact that different frequencies carry learning-related information during NREM and REM sleep indicates that these sleep stages may serve different functions in the consolidation process. However, the same memory task induces learning-related activity in both NREM and REM sleep and reprocessing occurs in close temporal proximity across these sleep stages. We therefore suggest that REM and NREM sleep have complementary functions in memory consolidation.

We show that the process of memory trace reactivation can also underlie effects of sleep on procedural memory. A motor sequence task that activates the hippocampus relies on reactivation-related consolidation in a strikingly similar manner as hippocampal-dependent declarative memory. It seems that hippocampal involvement determines the nature and mechanisms of sleep-dependent consolidation. Thus, it is not the distinction between procedural and declarative memory that decides how consolidation occurs, but the neural systems involved during learning and their inherent processing modes. Consolidation of memory that shows hippocampal contributions during encoding, involves memory trace reactivation during post-learning sleep, regardless of whether declarative or procedural memory is formed. Non-hippocampal memory relies on different consolidation mechanisms. These might be related to molecular processes occurring during synaptic consolidation.

The fact that implicit procedural memory can benefit from hippocampally-induced reactivation indicates flexible interactions between different memory systems during sleep-dependent consolidation. In fact, we find strong evidence for such ongoing interactions in our last study. Sleep not only strengthens implicit and explicit representations of the same learning experience separately. It integrates these different aspects of the memory representation. This memory restructuring renders initial competition between explicit and implicit memory cooperative and enables optimal performance.

Together, our results demonstrate how flexibly sleep changes our memory representations. Sleep not only strengthens individual aspects of memory, it integrates them over multiple memory systems. In all the tasks we tested, this enhanced performance. Especially the finding that sleep can change memory systems interactions underlines the complexity of the processes taking place during this off-line state. Sleep holds an important part in our life. It not only strengthens, it also shapes our memories.

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EIDESSTATTLICHE VERSICHERUNG

Hiermit versichere ich an Eides statt, dass ich die vorliegende Dissertation „*Sleep-Dependent Consolidation in Multiple Memory Systems*“ selbstständig angefertigt habe, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.

I hereby confirm that the dissertation "*Sleep-Dependent Consolidation in Multiple Memory Systems"* is the result of my own work and that I have only used sources or materials listed and specified in the dissertation.

Die Autoren leisteten folgende Beiträge zu den Publikationen und Manuskripten:

1) Schönauer M, Pawlizki A, Köck C, Gais S. Exploring the effect of sleep and reduced interference on different forms of declarative memory. Sleep 2014;37:1995-2007. *AP and SG planned and designed the experiments. MS, CK, and AP collected the data. MS and AP analyzed the data. MS and SG wrote the manuscript.*
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Hiermit bestätige ich die angegebenen Beiträge zur Erstellung der Publikationen und Manuskripte.

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Prof. Dr. Steffen Gais